Right honorable Prime Minister Dr. Baburam Bhattarai and honorable State Minister of Agriculture and Cooperative Om Prakash Yadav with other distinguished guest during opening ceremony

Participants of VETCON'12 during opening ceremony

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PROCEEDINGS on 10th National Veterinary Conference of Nepal Veterinary Association (VETCON'12)

"Veterinarians for Safeguarding Animal, Human and Environment"

PROCEEDINGS

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Right honorable Prime Minister Dr. Baburam Bhattarai inaugurating VETCON'12

Honorable State Minister of Agriculture and Cooperative Om Prakash Yadav addressing the opening ceremony of VETCON'12

Participants of VETCON'12 standing during national anthem play during opening ceremony

Secretary of Ministry of Agriculture and Cooperative Nathu Prasad Chaudhary addressing the opening ceremony of VETCON'12

World Veterinary Association president Dr. Fouzi Kechrid addressing the opening ceremony of VETCON'12

Nepal Veterinary Council president Dr. Shubh N. Mahato addressing the opening ceremony of VETCON'12

Honorable National Planning Commission Vice-President Dipendra Bahadur Chhetri addressing the gathering of VETCON'12 participants during closing ceremony.

Handing over of Priyanshu & Kashturi fund to the widow of Late Dr. Surendra Sah by NVA president Dr. Adarsha Pradhan

Oath taking ceremony of newly elected officials of Nepal Veterinary Association on closing day of VETCON'12

Felicitation of NVA member by honorable Vice President of National Planning Commission, Dipendra Bahadur Chhetri

Blood donation program during VETCON'12

Dr. Krishna Prasad Paudel presenting a theme paper in VETCON'12

THEME PAPERS

ONE HEALTH (OH) PROGRAM FORMULATION TO CONTROL ANY EMERGING AND RE-EMERGING ZOONOTIC DISEASES IN NEPAL: PERSPECTIVES OF TUBERCULOSIS

D.D. Joshi[1], P. Heidmann[2], Y. P. Joshi[1], B. Bhattarai[1], T.N. Gaire[1], A. Silwal[1] and S. Dhakal[1]

ABSTRACT

One health approach is the interdisciplinary and cross-sectoral approaches to disease prevention, surveillance, monitoring, control and mitigation as well as to environmental conservation more broadly and in sustainable way. One health concept is important to formulate control strategies based on current knowledge for tuberculosis both in animals and human beings. This paper includes theview of works on bovine tuberculosis and discusses about its zoonotic risk based on available information on human tuberculosis. Research articles from Nepal reporting the evidence of tuberculosis in animals and zoonotic tuberculosis in human beings were used for this study. All the studies we found on cattle and buffalo tuberculosis conducted in Nepal were identified and their details, including the reported test based prevalence, were entered into the database and reviewed for pooled analysis. Studies covering tuberculosis in human-animal interface were also reviewed. Pooled analysis of data revealed test based tuberculosis prevalence of 6 % in cattle, 9 % in buffaloes and 9% overall prevalence in cattle and buffaloes. Association between human and animal tuberculosis was not statistically significant but number of extrapulmonary tuberculosis still suggest a possible role of animal source. Summary of these findings indicate a burden of tuberculosis in animal population and possible interaction with human beings. In absence of control program in animals, the proportion of infected animals is more likely to increase than decrease. The real burden of this disease is probably much higher than currently been known. Although human health is the first priority, an integrative approach to control disease human and animals will not only be cost effective but also more efficient. Therefore, one health concept will strengthen human and veterinary public health services, collaboration in regard to epidemiology training and disease management, a sound regional communication strategy, and a clear chain of command in terms of emerging diseases like tuberculosis, Japanese encephalitis, echinococcosis/hydatidosis, taeniasis/cysticercosis, brucellosis, anthrax and other emerging and re-emerging zoonotic diseases we may encounter in the future.

Keywords: One Health, tuberculosis, tuberculin, zoonoses

INTRODUCTION

One Health or "One Medicine" concept coined by Dr. Calvin Schwabe is a movement to promote a collaborative effort between different health related professionals and paraprofessionals including the physicians, veterinarians, and environment related experts (AVMA, 2012). It has been defined as

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"the collaborative effort of multiple disciplines-working locally, nationally and globally – to attain optimal health for people, animals and our environment" (OHITF, 2008) The one health initiative considers the health of people, animals, and the environment in which we all coexist is interconnected and that all these components are important for a better overall health. More than 535 prominent scientists, physicians and veterinarians worldwide have endorsed the initiative (AVMA, 2012).

"One World, One Health™" (OWOH) has been championed by the Wildlife Conservation Society (WCS) but with a strong global presence. At an international meeting convened by WCS in 2004, a group of experts stated twelve principles of OWOH affirming the interconnectedness of the health of people, domestic animals and wildlife, and the resilience of ecosystems, and proposed forward-looking, adaptive approaches to managing these interactions (Figure 1).

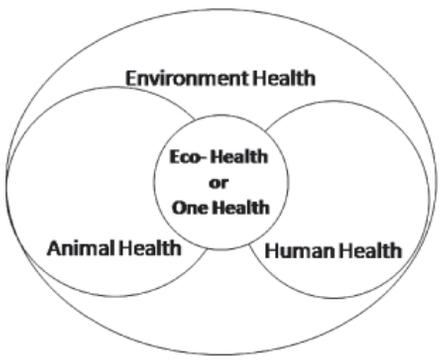

Fig. 1: One health concept (IDRC, 2011& WCS, 2011)

OWOH, or often, more simply, one health, was subsequently discussed and moved forward at a series of international meetings, primarily focused on animal and pandemic influenza, and emerging infectious zoonoses more generally, in Beijing, New Delhi, Egypt, Canada and Australia. A strategic framework was developed and written by the Food and Agriculture Organization of the United Nations, United Nations Children's Fund, World Health Organization, World Organization for Animal Health, the World Bank, and the United Nations System Influenza Coordination (OHOW, 2010).

One health approach is the interdisciplinary and cross-sectoral approaches to disease prevention, surveillance, monitoring, control and mitigation as well as to environmental conservation more broadly and in sustainable way. This approach could be used to develop the control strategies against serious zoonotic disease like tuberculosis which has caused significant impact on human health, family of the infected person and even a potential threat to the livestock population reared by infected individual in a country like Nepal where human-livestock-environment association in very close. This is of particular importance for 83%(CBS, 2011) of Nepalese population in rural areas where environmental resources and agriculture are the basis of livelihood.

Bovine tuberculosis is prevalent among cattle and buffaloes in Nepal (Joshi *et al.*, 1974; Morel, 1985; Joshi, 1986; DhakalandTiwari, 1993; NZFHRC, 2002).M. bovis can infect humans, primarily by the ingestion of unpasteurized dairy products but also in aerosols and through breaks in the skin.It is a disease which poses great public health significance. It is of significance to the international trade of animals and animal products as well (FAO, 1987).An estimated 7,000 people in Nepal die of TB every year; around 19 people die a day as per an official report. According to the FAO (1987) report, Bovine T.B. was detected as enzootic in Nepal.

One health concept is important to formulate control strategies based on current knowledge for tuberculosis both in animals and human beings. This paper includes views of works on bovine tuberculosis and discusses about its zoonotic risk based on available information on human tuberculosis.

MATERIALS AND METHODS

Research articles from Nepal reporting the evidence of tuberculosis in animals and zoonotic

tuberculosis in human beings were used for this study. All the studies we found on cattle and buffalo tuberculosis conducted in Nepal were identified and their details, including the reported test based prevalence, were entered into the database and reviewed for pooled analysis. To avoid publication bias, both published and unpublished studies were included. Most of the studies in Nepal are in the form of reports, thesis or other academic works and are less likely to be published in a peer reviewed journal. Therefore, inclusion of unpublished data was important to improve the scope of this study.

Animal studies reviewed for this paper except Joshi (1987) and Joshi *et al.* (1999) used single intradermal test. In this test, 0.1ml Purified Protein Derivative (PPD) of bovine tuberculin is injected in the neck or caudal fold and the reaction was read between 48-72 hours following injection, for maximum sensitivity and 96 hours for maximum specificity (Radostitis*et al.*, 2000). Reactions like swelling, redness etc. were noted. The measurement is made with VernierCalliper or through palpation (Chakraborty, 1997; Radostitis*et al.*, 2000). Summary estimate analysis was performed after extracting relevant data from each study.

In case of study in humans by Joshi (2003), sputum of the suspected individuals were collected based on clinical signs like persisting cough from two weeks or more, chest pain, fever and haemoptysis. The sputum was collected using specialized standard procedure and carried to DPHO, Kanchanpur for sputum smear microscopy. The number of bacilli was recorded according to recommendation of American Lung Association. All the positive cases (+slides) were carried to National Tuberculosis Centre for the verification and grading. The prevalence of tuberculosis was estimated from the animals of all sputum positive cases and 15% houses with sputum negatives cases for verification.

Study by Gaire (2009) was unique to estimate the prevalence of bovine tuberculosis in animals raised by tuberculosis infected persons who were undergoing Directly Observed Treatment for Shorts course (DOTS) at Kavre district, during the period of August to December 2009. For this DOTS center was visited to find out the tuberculosis infected person having the animals and immunological test was adopted in their animals for the test of bovine tuberculosis. A total of 50 animals reared by 25 tuberculosis (TB) infected persons were tested by using intradermal cervical tuberculin test.

RESULTS

Studies conducted in 16 different buffalo populations and 10 cattle populations reporting test for tuberculosis were available. The first report on buffalo TB in Nepal was from the government farm at Pokhara. Intradermal testing of 39 buffaloes revealed 23.08% prevalence in the farm (Joshi *et al.*, 1974). Similarly, 88 buffaloes in the same farm were tuberculin tested again in 1981 and 4 (4.55%) were found to be positive for TB (Joshi, 1986). Animals in Dhankuta were tested using the single intradermal comparative tuberculin test by Morel (1985). A total of 2% buffaloes were positive to the test. Details of such studies in buffaloes in different parts of Nepal have been presented in Table 1.

Table 1: Percentage of buff aloes testing positive for tuberculosis reported by various reports from Nepal

Authors	Location	N	Number		Test positive (%)	
			Positive	Doubtful	Including doubtful	Excluding doubtful
Joshi *et al.*, 1974	Pokhara	39	2	7	23.08	5.13
Joshi *et al.*, 1981	Pokhara	88	4	0	4.55	4.55
Morel, 1985	Pakhribas	NA	NA	0	2	2
Dhakal and Tiwari, 1993	IAAS	39	4	16	51.45	10.25
Dhakal and Tiwari, 1993	Mangalpur	107	18	16	31.77	16.82
Joshi *et al.*, 1999 (83-84)	Kathmandu	3232	452	0	13.99	13.99
Joshi *et al.*, 1999 (92-96)	Kathmandu	5500	350	0	6.36	6.36

Authors	Location	N	Number		Test positive (%)	
			Positive	Doubtful	Including doubtful	Excluding doubtful
NZFHRC, 2002	Godavari	3	1	0	33.33	33.33
NZFHRC, 2002	Kathmandu	6	0	0	0	0
NZFHRC, 2002	Kavre	20	11	0	55	55
Joshi, 2003	Kanchanpur	17	2	0	11.76	11.76
Bhattarai, 2003	Kanchanpur	17	1	6	41.17	5.88
Pun *et al.*, 2004	Kaski	654	35	0	5.35	5.35
Bhattarai, 2006	Chitwan	114	12	0	10.53	10.53
Gaire, 2009	Kavre	5	1	0	20.00	20.00
Silwal, 2011	Dhading	5	1	0	20.00	20.00
Overall data	Total	9846	894	39	9.48	9.08

All these studies used tuberculin testing as the basis of diagnosis except for Joshi *et al.* (1999) study which was conducted in two phases during 1983-1984 and later during 1992-1996 among the slaughter buffaloes of Kathmandu.

The first report of cattle tuberculosis was with one positive reactor bullock of 6 bullocks tested in the government farm in Pokhara(Joshi *et al.,* 1974). Subsequent studies in cattle and their results are presented in Table 2.

Table 2: Percentage of cattle testing positive for tuberculosisreported by various reports from Nepal

Authors	Location	N	Number		Test positive (%)	
			Positive	Doubtful	Including doubtful	Excluding doubtful
Joshi *et al.*, 1974	Pokhara	6	1	0	16.67	16.67
Morel, 1985	Pakhribas	NA	NA	0	1.00	1.00
NZFHRC, 2002	Godavari	97	11	0	11.34	11.34
NZFHRC, 2002	Kavre	9	0	0	0.00	0.00
Joshi, 2003	Kanchanpur	53	6	0	11.32	11.32
Bhattarai, 2003	Kanchanpur	53	7	2	16.98	13.21
Pun *et al.*, 2004	Kaski	800	32	0	4.00	4.00
Bhattarai, 2006	Chitwan	120	8	0	6.67	6.67
Gaire, 2009	Kavre	45	4	0	8.89	8.89
Silwal, 2011	Dhading	45	2	0	4.00	4.00
Overall data	Total	1228	71	2	5.94	5.78

Table 3 below summarizes the estimate of test positive percentage by pooling the total number of animals tested, total positive reactors and total doubtful reactors reported by the reports cited above.

Table 3: Estimated test positive percentage of cattle and buffaloes based on previous reports on cattle and buffalo tuberculosis in Nepal

Species	Percentage of test positive animals			
	Excluding doubtful reactors		Including doubtful reactors	
	Point estimate	95% CI	Point estimate	95% CI
Cattle	5.78	4.58 to 7.20	5.94	4.72 to 7.37
Buffalo (tuberculin)	8.26	6.75 to 9.99	12.298	10.47 to 14.33
Buffalo (lesions)	9.19	8.59 to 9.80	0	-
Buffalo overall	9.07	8.52 to 9.25	9.48	8.91 to 10.07

All cattle and buffaloes	8.71	8.20 to 9.25	9.08	8.56 to 9.63

Together with tuberculosis in bovines, Joshi (2003) also included human tuberculosis under his study. Out of 70 symptomatic (40 male and 30 female) individuals from 125 randomly selected household subjected to smear microscopy by Ziehl-Neelsens, pulmonary tuberculosis prevalence among total symptomatic individuals was found to be 9%. Out of 10, total smear positive cases infection in male and female was found to be 80% and 20% respectively. High prevalence of disease was revealed among the economically productive age group of 20-49 years. Prevalence of bovine tuberculosis was found to be higher in the houses with sputum positive human cases than that of the sputum negative ones but there was no statistically significant association (P>0.05). Since tuberculosis in both cases is high, there is strong probability of transmission from animal to human being; hence the community people of this area might be at greater risk of contracting the infection (Joshi, 2003).

Study by Gaire (2009) included 50 animals reared by 25 tuberculosis (TB) infected persons were tested by using intradermal cervical tuberculin test. A total of 5 animals (10%) were positive for tuberculin having the change in skin thickness. Similarly, among the 25 tuberculosis infected household, 13 (52%) had an extra pulmonary type of TB in family members and 12 (48%) had pulmonary type of TB. Animals were also higher infected (60%) in household where their owner had extra pulmonary type of TB raising suspicion on transmission between people and their animals. Overall prevalence of TB in human where their animal was infected with BTB in the study area was 20%. Likewise out of total 25 TB infected household, 40% had the habit of drinking raw milk. The relationship between raw milk ingestion by TB infected person and number of infected animal was markedly higher (50%). Out of 25 TB infected household maximum (44%) infected were old person and higher animals were (14.28%) associated with infections in same households.

Well-structured questionnaire sets were prepared for study of Gaire 2009 and Silwal2011 and surveyed to determine the knowledge and awareness level of livestock owners about tuberculosis and its zoonotic aspects. The farmers knowledge on zoonotic aspect of bovine tuberculosis was overall only 2% (Gaire, 2009).In the study conducted in Dhading district out of 20 farmers only 3 were aware about TB and its zoonotic importance (Silwal, 2011) indicating a serious need of education. We also found that some of the studies also extended beyond estimation of prevalence in animal population. Consent of the animal owner and human subjects both were taken in each of these studies. In addition to that, during the studies conducted by NZFHRC (NZFHRC, 2002; Silwal, 2011)concerned stakeholders including dairy farmers, animal health and human health private and government personnel, milk collector, dairy business people, dairy product consumers, local leaders were made aware about the tuberculosis and its transmission from dairy animals to human and how to control this in the community.

DISCUSSION

Tuberculosis cattle and buffaloes appear to be widely distributed in Nepal, and products from these animals are being consumed daily. The species of Mycobacteria identified from Nepalese animals are: bovis, thermo resistible, Nepal-1 and Nepal-4 from milk, and fortuitum from feces (Pun *et al.*, 2004), which are transmissible to humans. The reports reviewed above also indicate the widespread prevalence of tuberculosis all over Nepal.

Milk and meat from infected animals are the major source of zoonotic tuberculosis.The fact that meat and milk being the major contributor of human infection cannot be neglected in the countries like Nepal, where meat inspection of slaughter animals is not done and unpasteurized milk is a regular commodity of most of the households. With nominal share of other livestock, buffaloes and cattle are the sole source of milk in Nepal. Furtheralmost all households of 83% rural population in Nepal (CBS, 2011) keep cattle or buffaloes. However, only a part of urban milk produced is pasteurized. Thus most of the consumers are still in the risk of milk borne diseases including TB. Nepalese people mostly boil milk and the sample strains of Mycobacterium spp. were killed when

heated at 750C ensuring the safety for Nepalese rural milk consumers, who do not have facilities to check the temperature of the milk while boiling (Dhakal*et al.*, 2005). However, in absence of knowledge of possibility of transmission, cross contamination may still occur.

Buffaloes provide over 64% meat consumed in Nepal (MOAC, 2006). The presence of TB lesions in slaughtered buffaloes in Kathmandu (Joshi *et al.*, 1999) indicates possibility of human infection delicacies including kachila use raw meat. Similarly, people unaware about disease risk due to cross contamination also indicate possibility of human infection. With majority of the people being illiterate with lack of knowledge on zoonotic TB, there may exist a regular transmission cycle among the Nepalese people and their animals. Reports on human tuberculosis and its association with bovine also demonstrate a possible human-animal cycle. Though pulmonary cases dominate human TB cases, a fair amount of extra pulmonary cases as high as 30 to 39% clearly indicate a possible role played by animal carriers in the disease transmission dynamics in Nepal(Joshi *et al.*, 1999). Even in absence of studies to specifically point out the role of patients in transmission of disease to humans, these findings point out possible anthropozoonosis.

Studies establishing causal relationship between human animal cycle and possibilities of Mycobacteria harbouring in the environment are not available at the moment. Studies by Joshi (2003) and Gaire (2009) have envisaged the possible association indicating the need of one health approach to combat tuberculosis. The lack of significant association between human and animal tuberculosis is most likely due to the limited sample size. Nepal has been combating human tuberculosis since several years but the control is not as expected. This could partly be attributed to possibility of animal in the disease ecology. One health approach with due consideration to all determinants of disease might improve the control program with an added benefit to the livestock producers.

One of the major limitations of this study is heterogeneity in sample size and geographical area of the herds included in respective studies estimating prevalence. The geographical regions included in reviewed studies are not necessarily representative of entire Nepal and animal population tested does not necessarily represent a representative sample of geographical area or animal populations under study. All the studies available were included in this study and overall quality of findings may be different. Even in used intradermal tuberculin test but the interpretation of results vary slightly. While some authors advocate measurement of skin thickness for interpretation of test positivity (Chakrabarti, 1997), subjective method of palpation is more accurate and also permits a decision to be shaded by the nature of the lesions (Radostitis*et al.*, 2000). Type of tuberculin purified protein derivative (PPD) used for testing may also influence the intensity of reaction (Srivastav, 1995a).

In spite of these limitations, our focus was to elucidate the zoonotic aspect of TB based on the available studies in animals and human beings. Summary of these findings still indicate a burden of tuberculosis in animal population and possible interaction with human beings. Prevalence is dynamic and may differ over time and place. In absence of control program, the proportion of infected animals is more likely to increase than decrease. Therefore, our estimates are probably underrepresentation of the true status. Current burden of tuberculosis in animals and humans might be higher than reported here.

CONCLUSION

Diseases of animals often receive lesser priority in developing countries like Nepal. Although human health should receive priority, even primary healthcare for human beings is not accessible to the entire population. In this scenario, an integrative approach to control disease human and animals will not only be cost effective but also more efficient. Both human and animals are the part of disease ecology. Collective role of veterinarians, medical practitioners and environment experts can help to control diseases more effectively in local, national and global level. This supports the contention that focus towards control of such diseases solely in human or animal populations is not

adequate. Awareness programs in national level are of utmost importance but the organizations like NZFHRC should continue educating the stakeholders.

The one health (OH) concept tools will be strengthening human and veterinary public health services, the cooperation between human and animal health sectors in regard to epidemiology training and disease management, a sound regional communication strategy including inter-ministerial matters, and a clear chain of command in terms of emerging diseases like tuberculosis, Japanese encephalitis, echinococcosis/hydatidosis, taeniasis/cysticercosis, brucellosis, anthrax and other emerging and re-emerging zoonotic diseases to be established. Mycobacteria can survive in the environment and may pose a risk to humans and animals. We did not find such reports in Nepal but this aspect of disease ecology cannot be neglected.

The role of veterinarians and environment related professional should not be overlooked while formulating a disease control program.

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FOOD SECURITY AND ECONOMIC GROWTH THROUGH LIVESTOCK IN NEPAL

K. P. Paudel[1] and S. N. Mahato[1]

ABSTRACT

The Global Hunger Index (GHI)of Nepal for the year 2011 remained just below 20.0 (was 20.6 in the year 2008) and the hunger situation has been described as "serious" (GHI 10.0 – 19.9). There are sub-regions within the country where the situation is still "alarming" (GHI 20.0 – 29.9) or "extremely alarming" (GHI >30.0). This implies overall food security situation of the country has slightly improved recently, however this improvement is mainly attributed to significant reduction in human population growth and spells of good monsoon. There are little evidences for improved food security associated with increased production and productivity of staple food commodities. This paper reviews plans, programs and projects of government and non-government sectors aimed at food security and economic growth through livestock. The hunger and food systems have not improved to an anticipated level and our set targets in the short, medium and long-term plans are met partially only. This demands more critical understandings of poverty and hunger situations and food systems and the means rural people adopt to cope with food insecurity. Among rural communities food insecurity experienced as 'not enough' in quantity and quality, and associated psychological and social stress create vicious cycle of poverty and hunger resulting in reduced agricultural productivity. Meantime farmers dispose the accumulated livestock assets for purchasing staple food. The role of livestock is also immense in diversifying diet menu thus food quality at the family level. Moreover, its potential contribution to increasing family income, nutrition and thus to economic growth of the country is also significant. Studies on some successful cases of Heifer International Nepal reveals that livestock plays a key role in improving food security in terms of quantity, quality and continued accessibility and also gears for increasing family income. Evaluations reveal that project beneficiaries of Heifer Nepal have been able to increase their median annual income by about 55% from NRs 58,173 for the year 2005/06 to NRs 89,986 for the year 2009/10. Similarly, another study reveals Heifer Project beneficiaries have increased their yearly income that can be attributable to Heifer Nepal was estimated to be US$565. The Heifer recipients are among the poorest in the rural areas of the country, this income gain is substantial. Gains in terms of assets by participants are also significant. On average, each family increased its assets as a direct result of the Heifer projects by US$1,749. The average cost per household to HPI-Nepal to provide its services (including animal gifts and training) was estimated to be $371. Considering that the average yearly household income of the participants was estimated at $565, the expense of distributing animals is quickly outpaced by the income that the animal generates implying significant gearing effects. Combining household income and assets accumulated during the first five years after receiving the animal gifts and training shows a return of $10 on each dollar invested by HPI-Nepal through animal distribution. Similar findings are reported for Third Livestock Development Project and Community Livestock Development Project. Despite such positive outcomes, investment in agriculture as a whole and livestock sub-sector in particular is far less than what was planned in APP (1995 – 2015). Based on these evidences more extensive programs on livestock are justifiable to ensure food security and thus economic development of the country.

1 Heifer International Nepal

INTRODUCTION

Food security refers to "Secured access to sufficient and affordable nutritious food for a healthy and active life". Ensuring food security requires examining quantity, quality including dietary diversity, time, access and affordability for food. Generally, food security of a country is assessed based on Global Hunger Index (GHI) that considers:

- The percentage undernourished in the population (reflecting the share of the population with insufficient dietary energy intake)
- The prevalence of underweight in children under the age of five (indicating the proportion of children suffering from low weight for their age)
- The under-five mortality rate

The Global Hunger Index (GHI) of Nepal for the year 2011 remained just below 20.0 and the hunger situation has been described as "serious" (IFPRI 2011). There are sub-regions within the country where the situation is still "alarming" (GHI 20.0 – 29.9) or "extremely alarming" with (GHI >30.0 (WFP 2009). This implies overall food security situation of the country has slightly improved recently, however this improvement is mainly attributed to significant reduction in human population growth and spells of good monsoon.

Nutrition is an important component of food security. The population of Nepal, especially the most vulnerable - women and children, are affected by all the major micronutrient deficiencies. Malnutrition increases the risk of child mortality, hinders cognitive development of those who survive posing severe long-term impact on national economic development. More than 3.5 million people are estimated to be moderately to severely food insecure with wide regional variations within the country. The highest rates of hunger are found in the Mid and Far-Western Hill and Mountain regions. The major causes of this food insecurity are:

- Agricultural/livestock productivity remains low due to several factors including land fragmentation
- Seasonal food shortages are quite common forcing people to opt for off-farm employment – diverts focus from agriculture at the family level
- Use of crops and livestock with inherently poor genetic characteristics
- Ongoing problem of land degradation
- Water/irrigation is inadequate
- Dietary diversity is narrow and difference in intra family allocation exist leaving some member within family more vulnerable than others

Livestock in Food Security and Economic Growth

Nepal confronts various forms of nutritional problems ranging from deficits in energy intake and imbalances in consumption of specific macro and micronutrients. Nepal is among the ten countries of the world with the highest stunting prevalence, a measure of chronic under nutrition, and one of the top twenty countries with the largest number of stunted children (UNICEF, 2009). The role of livestock in food security should be viewed from two angles: as a source of income to purchase staple food ingredients and source of nutrients of animal origin specifically protein that has tremendous role in physical and mental development of human being. Provision of animal source proteins during early life is critical for an individual's physical and mental development including acquiring cognitive skills in the process of growth.

Some people consider livestock is an inefficient means of attaining food security as livestock consume substantial cereal grains posing threat to it. However, the facts are - grains are not indispensable for feeding ruminant livestock. Livestock can be raised even in degraded and marginal lands if managed properly to complement the food security efforts even in areas where integrated crop-livestock systems are not feasible.

Livestock has played important role to rescue farmers from adverse shocks (drought, discontinued work/ employment, illness, death etc.) in times when there is some degree of immediate food shortage. Livestock is the first asset to be mobilized in such difficult situations. There are evidences that increased output of livestock and income has played tremendous role in addressing seasonal food insecurity. Moreover, agricultural productivity has direct relationship with livestock productions systems and functions. In Nepal, agricultural productivity is higher in areas where crop-livestock integration is well established and improved livestock husbandry for higher productivity are practiced under stall-feeding management systems (ABPSD 2009). Our experience reveals that cereal system initiatives are not effective in isolation; we often have tendency to underestimate the overall contribution of livestock.

Livestock as a source of income

Animal products not only represent a source of high-quality food, but, equally important, they are as a source of income for many small farmers in developing countries including Nepal, for purchasing food as well as agricultural inputs, such as seed, fertilizers and pesticides. They contribute about 20% to total HH income (figure 1).

Table 1: Income by sources in rural households

Income source	Contribution to total HH income
Cereal and legumes	20
Horticultural crops	4
Livestock	20
Jobs in Nepal	22
Labour work	13
Jobs abroad	16
Special activity	5

Source: Paudel et al 2011

Livestock as a means of generating employment

Livestock farming is one of the sustainable employment options. The number of persons engaged in pre-production enterprises, production functions, collection and marketing of live animals and their produce, processing enterprises and selling in end market employs ample human resource. For example, in organized markets, the number of persons employed in goat enterprises in Nepal is estimated to be 2000 collectors, 133 traders, 882 meat retailers, 59 transporters and 937 private sector service providers. Production level employment is still a part time activity with just about 0.2 million HH engaged in goat farming having more than 10 goats/HH. A crude estimate reveals that in every 1,000 goats traded, there is an additional engagement of 150 - 200 farmers (increased in hour/day engagement), 2 collectors, and one butcher in the system. Similarly, there is an addition of two traders, and one transporter in the turnover of every 15,000 – 20,000 goats. So is the case in dairying - one hundred litres of milk traded, engages 7- 8 persons in the system. The point made here supports the fact that though the system might not be efficient, livestock enterprises are one of the important subsectors of employment with huge potential for commercialization.

Livestock as a source of energy and draft power

More than two-third of Nepalese crop cultivation is dependent on animal draft power. Moreover, role in transportation of goods including input supply and delivery of farm produce to the market is tremendous in Terai and high mountains.

Livestock as a source of fertilizer and soil conditioner

Nutrient recycling is an essential component of any sustainable farming system. The integration of

livestock and crops allows for efficient nutrient recycling. Animals use the crop residues, such as cereal straws, as well as maize and sorghum stovers and groundnut haulms as feed. The manure produced can be recycled directly as fertilizer. The chemical composition of manure varies, however, according to the animal species (poultry manure appears to be a more efficient fertilizer than cow manure) and also to the nature of their diet. Manure makes direct contribution sustaining plant nutrients in soil and provides important organic matter to the soil, maintaining its structure, water retention and drainage capacity. The value of manure is so well-recognized that some farmers keep livestock primarily for this purpose. The cultivation of legume fodders and trees, for example, in Nepalese farming systems, also contributes to the enrichment of soils through nitrogen fixation..

HEIFER EXPERIENCE

The association of Heifer International in Nepal can be traced back to 1957/1958 A.D. During that time, Nepal received, as a gift from Heifer, 10 Brown Swiss cows, 5 Hampshire pigs and 57 sheep of exotic breeds in year 1957 followed by two Jersey bulls and 4 Corriedale ram in 1958. More systematically, Heifer International activities commenced in Nepal with introduction of a goat project in Chitwan in year 1993 and a water buffalo project in Nuwakot in year 1995. The success of these two projects formed the foundation for establishing a country Office of Heifer Project International in Nepal (HPIIN) in year 1997. Since then, Heifer has been working in Nepal in partnership, mainly with local non-governmental organizations (NGOs), adopting a Value-based Holistic Community Development (VBHCD) approach called Heifer development model for transforming the existing rural community into a self-reliant one keeping intact its good social/ cultural values. While applying the VBHCD model, Heifer mainly uses livestock and technical trainings as tools for poverty reduction and acts as a catalyst for holistic transformation and development in accordance with the will and vision of the community.

Heifer's Overall impact

Heifer programs have succeeded in making significant improvements in terms of nutrition; income; assets; knowledge on basic care and management of animals; environmental care and management; children's access and retention in schools; gender equity; reducing caste discrimination; community empowerment; and adoption of Heifer values by other agencies at regional and national levels (Thomaz et al 2011).

Impact on human nutrition through increased/changed diet ingredients

The special study on income, assets, and nutrition (IAN) suggests substantial impacts have been produced by HPI-Nepal in those areas. In terms of nutrition, the Heifer projects helped participants migrate from a situation of having some shortage of carbohydrates for part of the year to an almost ideal amount of carbohydrate intake year-around. All of the families considered to be in a critical situation in terms of carbohydrate intake (mild shortage year-round) at baseline (26.7%) were helped by the projects to move to a situation where, at a minimum, they have access to carbohydrates two or three times per day for most of the year. Evaluation reports reveal results for supplements (vegetables and fruits) and protein intake are even more impressive. About 6 out of 7 (85.7%) and 4 out of 5 (81.3%) participant families reported an increase in the frequency with which they eat vegetables, fruits, and protein (mostly lentils, milk, and meat). All of the families considered being in a critical situation, e.g., experiencing a severe shortage of vegetables and fruits year-around (60.6%), moved to a reasonable situation where they have at least 2 servings of fruit or vegetables per day for most of the year. From the 52people who were considered to be in a critical situation of severe shortage of protein for most of the year before the Heifer projects started, 45 (86.5%) are now in a situation where they have at least 1unit of protein per person per day for most of the year.

Heifer project beneficiaries have adopted measures to improve family nutrition by changing diet composition and practicing routine health check-ups. The group of women expressed with full consensus that nutrition related issues are discussed in the monthly group meetings.

Implementation of decisions of the group meetings is realized by almost all of the members. As a result, initiation of kitchen gardening and incorporation of green vegetables in the daily diet is now a routine practice. Preparation of balanced food has been practiced by incorporating grain legumes, pulses, green vegetables, milk and occasionally meat as far as possible. Women members are aware of conventional practices of discrimination between boy and girl child and have now corrected the measures by providing food to boy and girl children without any discrimination. People have started to eating seasonal fruits that previously were otherwise wasted (mainly papaya in Terai) and so on. Some of the important measures adopted for mitigating malnutrition problems as stated by the group members are as follows:

- Most of the families have now a sizable kitchen garden (Table 11). The growth in number of households involved and mean area under vegetable cultivation we consider are appreciable transformations in terms of green vegetable production due to project effect. The consumption of green vegetable has increased substantially.
- Grain legumes consumption which was not a regular practice before has been adopted now
- All kinds of fruits have been treated as healthy food item; so the seasonal fruits are consumed wisely and not wasted
- Consumption of milk and meat both in quantity and frequency has been increased and they provide to both sons and daughters without any discrimination
- They seek regular health check-up from the nearby health service centres

An improvement in consumption pattern of food is evident in all most all the communities. Mean quantity of food commodities consumed annually has increased for most of the food items, predominantly of wheat, spring maize, pulses, legumes, potato, fresh vegetable (both summer and winter), fruits, and milk and to a some extent of meat consumption.

A crude assessment of the impact of project intervention on family nutrition and health assessed by analysing the changes in mean quantity of food consumed by a family between the years 2005/06 and 2009/10 reveals an increment in consumption of quantity of food in all most all commodities, most prominent being in wheat, spring maize, pulses, legumes, potato, fresh vegetable, fruits and to some extent in milk and meat. These figures do represent the consumption of commodities that were produced in own farm and exclude the quantity purchased. Families with insufficient farm production to feed for twelve months purchase additional food from their cash income which has been accounted in the total family income.

Mean quantities of milk and meat has also increased. However, the consumption even for after project scenario (187 litre of milk and 15 Kg of meat by a family of 5 persons) is still far less than the national annual per capita consumption of 52 litre and 9.0 kg (ABPSD, 2009). It appears that in majority of the family livestock produce including live animals are sold to earn cash for purchasing the basic staple food commodities. This reflects the situation of a typical rural farm family.

Increased income

More than 9 out of 10 (91.5%) farm families have been able to increase their income as a direct result of their participation in the Heifer projects. The evaluation reveals an average yearly income gain by a household that can be attributable to Heifer Nepal was estimated to be US$565. The average asset accumulation for families interviewed in this evaluation was $1,749. Heifer recipients are among the poorest in the rural areas of the country, this income gain is substantial. Gains in terms of assets by participants were also significant. On average, each family increased its assets as a direct result of the Heifer projects by US$1,749.The average cost per household to HPI-Nepal to provide its services (including animal gifts and training) was estimated to be $371. Considering that the average yearly household income of the participants was estimated at $565, the expense of distributing animals is quickly outpaced by the income that the animal generates. Combining household income and assets accumulated during the first five years after receiving the animal gifts and training shows a return of $10 on each dollar invested by HPI-Nepal through animal

distribution. While the gift of animals has contributed greatly to a household's ability to improve income and assets, the HPI-Nepal trainings have had the largest influence on change.

Some of the most commonly mentioned training topics that have had a large impact include training on nutrition, kitchen gardening, money management, proper care for animals and the environment, importance of gender equity, and how to work together as a group. These trainings, along with the structure of the program, also have helped to reduce caste discrimination through holding events where members of different castes eat together, sit together, and visit one another's homes.

The Heifer contribution to increase the income of participant families is very significant. About 9 out of 10 (91.5%) of the 94 families interviewed indicated that they have increased their income as a direct result of their participation in a project. Selling goats, milk, products of vegetable gardens and increased crop yields, and a few entrepreneurial activities (e.g., handicrafts or small shops) were the most commonly cited means used by participants to increase their income.

Table 2: Household Income of the SHG members under umbrella Project Nos. 16 and 17 (NRS at 2005/06 Price; n=198)

Parameters	Income from Job abroad included		Income from Job abroad excluded	
	Before Project	After Project	Before Project	After Project
	Total Income	Total Income	Total Income	Total Income
Mean	102,128	149,149	85,234	126,674
Median	65,123	115,891	58,173	89,986
Change in median income	-	78%	-	55%
Remittance cases	29	50	-	-
Families above PL	38	63 (25)	29	51 (22)

Source: Paudel et al 2011. The figures in parentheses in the row 'families above PL' denote HPI project gain.

Livestock gears income increase

An increasing trend in mean herd/flock size appears: goats, buffalo, pigs and cattle. This change in herd structure implies that a considerable capital in the form of livestock asset is accumulated as an additional project benefit. On the other hand the larger impact is in expansion of ownership pattern of livestock. The number of households keeping various livestock have increased tremendously in the last five years. Among the sample households of 198, the number of families keeping buffaloes, goat, pigs and poultry has increased by 22, 46, 2 and 23 respectively during the years between 2005 to 2010.

Livestock in the form of savings to ensure food security during scarcity

Study reveals that farmers strategically accumulate livestock as an asset if they have surplus income and dispose it in need (Table 2).

Table 3: Frequency of beneficiaries with range of accumulated assets and from increased income in Heifer projects

Description	Frequency of respondents (n = 175)	Percentage
Land purchase	35	20
Built new/renovated house	28	16
Purchase of livestock	Buffalo 101	26
	Goat 144	38
Purchased gold/Jewellery	28	16
Purchased cookeries and utensils	13	7
Built/renovated cattle sheds	3	2
Purchased sewing machine	10	6
Bought chaff cutter	1	1
Purchased Water Pump set	1	1

Examples of successful project

Two major projects are referred in this study. Third Livestock Development Project (TLDP) and Community Livestock Development Project (CLDP) are described for the reason the projects were based on empowering community to enable them raise livestock, a similar approach of holistic community development through empowerment that heifer adopts. Both the projects were funded by Asian Development Bank (ADB 2006, ADB 2010).

TLDP: The Project's impact was highly effective in improving productivity of livestock, income, social empowerment of poor households and women, and preservation of the natural environment. The capacity of the private sector to process and market milk and meat increased significantly. The impact of the Project on rural inhabitants' livelihoods would be much higher during the remaining economic life of the Project, as incremental benefits from investment in capacity building at local levels are greater in the medium and longer-term.

The financial and economic analysis of Third Livestock Development Project (TLDP) reveals that overall financial internal rate of return (FIRR) for the Project was 24.6%, compared with the appraisal target of 17.8%. FIRR for the cow farm model was 17.4%, which was slightly lower than the appraisal target of 19.7% because of higher investment costs and lower productivity. FIRR for goat raising was 17% and the for pig raising was 18%. The overall economic internal rate of return (EIRR) on the basis of cost and benefit streams for the Project was 23.4%, higher than the appraisal target of 17.8% The main reasons the EIRR exceeded expectations were higher than expected overall milk and meat production and total project cost 20% below appraisal estimates. About 56,500 farm families benefited from improved livestock productivity, about 3%higher than the target. These families' annual average income has increased by about 35% compared with the baseline level. The Project has further benefits of (i) improvement in agriculture production due to increased supply of farmyard manure, (ii) import substitution of concentrates from India by increased production of forage, (iii) improved access to milk and meat markets,(iv) improved skills, and (v) secondary and tertiary benefits to inputs suppliers and traders, resulting from raising productive livestock and livestock production.

Community Livestock Development Project

The project directly benefited 207,864 households through increased livestock productivity and processing and marketing activities, compared with the target of 164,000 households. The project increased real per capita income by 88 %(NRs2,574) in 2010, compared with the target of 50% (NRs1,464), at 1996 constant prices. A total of 6,245 person-years of incremental employment were generated in the livestock production, processing, and marketing enterprises that were supported or strengthened by the project, which is much higher than the project target of 5,100 person-years. An additional 13,597 person-years of full-time job were created in the project-supported livestock farms, for which there was no target at project design. These are only initial impact values, as impacts will continue to be realized during the project's economic life. Thus, an impact assessment after5 year will provide the best measures. The project was found efficient in achieving its outcome and outputs on the basis of financial and economic analyses by following a similar methodology to that used at appraisal.

The project was effective in improving nutritional intake of girls and boys under 6 years of

age that increased by 19.1%, compared with the target of 20%. This was based on an estimated 11% increase in milk consumption, 50% increase in meat consumption, and a substantial increase in vegetable consumption through kitchen gardening. The project increased cow milk production by 140% and that of buffalo by 57%, compared with the target of 50%.off-take of goat increased by 28% in project districts, against the target of 30%. The project's focus on women and disadvantaged groups through support for goat raising, forage cultivation, and microfinance services was the main reason for achieving much higher female participation, 62% against the target of 35%. The project also developed 2,856 livestock production, processing, and marketing enterprises, against the

target of 1,050. The project promoted gender equality and social inclusion, particularly in reaching poor women and men, the landless, and households headed by women. Women represented 62% of the beneficiaries of the goat program, and representation of disadvantaged castes amounted to33% and that of ethnic groups to 28%. The increased income mainly went to women who usedit for children's education, family healthcare, and purchase of food.

LESSONS AND RECOMMENDATIONS

- Programs on livestock development should be planned with provisions for empowerment and family focus to food security. A poverty mapping tool needs to be used to determine target families
- Social capital formation becomes prerequisite to economic development through livestock for its sustainability,
- Crop- livestock integrated system need to be promoted - livestock are the means that help farmer recover from shocks/vulnerabilities
- Livestock programs should follow a business-oriented value chain approach for increasing productivity and production and ultimately the income
- Promotion of public private partnership - we need to explore new avenues of Government and non-government partnership as well.

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ORGANIC LIVESTOCK PRODUCTION: STANDARDS, PROCEDURE AND APPROACHES FOR NEPALESE FARMERS

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ABSTRACT

The traditional livestock production system in Nepal has always been managed under organic production system in which the use of antibiotics and other chemical products was non-existent. However; with the advancement of veterinary services, inputs and increased drive for commercialization and intensification of livestock production system, the use/abuse of these products (veterinary drugs, anthelmintics, insecticides etc) has increased significantly in some production pockets causing a serious concern to consumers and environment. The deleterious effects of many such chemicals to human health and environment have stimulated the concept and processes of organic livestock production throughout the world and standards have been set up for organic livestock production system and products in many countries. Nepalese farmers, though, have been practicing organic production system as their traditional practice; have not yet been identified and certified as organic producers to get the benefits of organic produce. Some of the classical organic products of our country are mutton, chevon and fiber produced from migratory sheep and goats, chicken meat from backyard poultry, yak cheese, milk and milk products (ghee, chhurpi) and dry meat from remote hills and mountains of the country. As the possibilities of commercialization in remote regions are difficult, farmers could not get the justifiable economic return for their produce. Hence, initiating the certification system for organic livestock production and produce would benefit the large number of producers involved in livestock production in the remote regions of the country; if the system of certification and marketing for organic livestock produce could be established in the local and international markets. It would also contribute to sustain the existing livestock production system in these regions, which otherwise, would be unable to survive under the rigors of current socioeconomic environment. It would be particularly suitable to the hills and mountains of Nepal, where the difficult terrain is an obstacle to develop infrastructure, market, efficient service and input delivery system which are essential for commercialization of livestock enterprise. This paper highlights the required standards, procedure and approaches for organic livestock production and discusses its potentials for increasing the income of livestock farmers in the country.

INTRODUCTION

International Federation of Organic Agriculture Movements (IFOAM), an international umbrella organization established in 1972 for organic farming organizations defines organic farming as a production system that sustains the health of soils, ecosystems and people. It relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs with adverse effects. Organic agriculture combines tradition, innovation and science to benefit the shared environment and promote fair relationships and a good quality of life for all involved". Thus, organic agriculture is the agricultural production system which relies on techniques such as crop rotation, green manure, compost and biological pest control to maintain soil productivity and control pests on a farm. Organic farming excludes or strictly limits the use of manufactured fertilizers, pesticides (which include herbicides, insecticides and fungicides), plant growth regulators such as hormones,

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livestock antibiotics, food additives, and genetically modified organisms.

Organic Livestock means the livestock production system, in which, the management system is based on appropriate and harmonious relationships of land, plants and livestock and responds to the physiological and behavioral needs of animals to minimize stress, promote animal health and prevent diseases by good animal husbandry practices and avoid the use of veterinary drugs (any substance applied to any food producing animals and its products, whether used for therapeutic or diagnostic purposes or for modification of physiological functions and behaviors) and chemical substances.

GENERAL PRINCIPLES FOR ORGANIC LIVESTOCK PRODUCTION

The Organic Livestock Production shall be in compliance with the following principles:

- The areas dedicated for organic livestock production shall be managed and maintained according to the organic agriculture principles on production, processing, labeling and marketing.
- The organic livestock production shall improve and maintain soil fertility, enhance bio-diversity and ecology, and diversify the farming system.
- In organic Livestock production, herbivores animals should have access to pasture for grazing and other animal species should have access to open-air exercise areas appropriate to their health, weather conditions, and geography, or to the traditional farming systems with access to pasture, providing an appropriate welfare for the animals.
- Stock densities for livestock shall be appropriate to animal species, feeding, stock health, nutrients balance, and environmental impact.
- Livestock breeding shall be natural breeding to minimize stress and prevent diseases.
- Avoid the use of chemicals or veterinary drugs and livestock by products except milk as raw materials for feedstuff, and maintain animal health and welfare.

THE REQUIREMENTS FOR ORGANIC LIVESTOCK PRODUCTION ARE AS BELOW (Table 1)

Table 1: Organic livestock production requirements

Items	Requirements
Livestock sources	• Stock born of or from parents that are managed according to the organic production system. • To change the breed for supporting the demand of market, new improved breeding stock born from natural methods can be obtained.
Feedstuffs	• Shall be produced according to the requirements of the Organic Agriculture. • Shall not contain genetically modified organisms (GMOs) and products. • Raw materials or substances shall be permitted according to national legislation on animal feeding and shall not be against the organic livestock principles. • A substantial proportion of dry matter in the daily rations of herbivores shall be composed of roughage, fresh or dried fodder, or silage but feeding with silage alone throughout the life span is not permitted. • Fresh water is accessible and available for livestock at all times. • Feeding materials used as mineral, vitamin or pro-vitamin can only be used if they are of natural origin. If shortage, or in exceptional circumstances, synthetic substances may be used with full details of their origin and production processes. • Feeding materials of plant origin from uncertified organic sources can only be used, under the conditions that they shall not be produced or prepared by using any chemical treatments. • Uses of synthetic nitrogen or non-protein nitrogen compounds are prohibited. • The feeding of mammalian material to ruminants is not permitted, except milk. • Binders, anti-caking agents, emulsifiers, stabilizers, thickeners, surfactants, coagulants, antioxidants, coloring agents, flavor agents, and appetite stimulants can be used BUT only from natural sources. • Antibiotics, coccidiostats, medicines, growth promoters or any other substance intended to stimulate growth or production are prohibited.

Items	Requirements
Livestock health management	• The animal health management shall be applied according to animal species and breeds. • Medical herbs, indigenous technologies shall be applied for animal treatment. • If the management practices or permitted drugs cannot control the diseases, the use of other veterinary drugs can be applied if necessary with clear withdrawal period under supervision of a qualified veterinarian. • Where the epidemic or suspicious disease, health problem occur and existing permitted treatment or management practice cannot control or treat the diseases, in cases required by law, vaccination of livestock, use of parasiticides, or therapeutic use of necessary veterinary drugs are permitted and withdrawal period extended two folds of the manufacturer's instruction is strictly observed. • Use of chemical allopathic veterinary drugs for disease prevention is prohibited and hormonal treatment shall only be used under veterinary supervision. • Growth stimulants or substances used for the purpose of stimulating growth or production rate are prohibited. • Any surgical operation shall be practiced for animal safety and welfare. • All appropriate medications must be used to restore an animal to health when methods acceptable to organic production fail. Livestock treated with a prohibited substance must be clearly identified and shall not be sold, labeled, or represented as organically produced.
Livestock management	• Have an open-air exercise area for animal. • Natural Breeding shall be applied, if necessary, artificial insemination technique may be allowed. • The embryo transfer techniques and the use of hormonal reproductive treatments and use of genetic engineering technique for altering genetic of the animal are prohibited. • Livestock shall be temporarily confined in the housing during certain periods, for their health and safety or to prevent from destroying the water resources, environment, plant and soil. • The free range stocking density shall be suitable to prevent degradation of the soil fertility and over-grazing of vegetation. • All mammals shall have access to an open-air exercise area with exception of parent stock or the livestock of final fattening phase. • The housing of calves in individual boxes and the tethering of livestock are not permitted without the approval of the certification body of organic livestock production system. • Continuous total confinement of any animal indoors is prohibited. Continuous total confinement of ruminants in yards, feeding pads, and feedlots is prohibited. • Poultry shall be reared in open-range conditions and have free access to open-air exercise area. The keeping of poultry in cages is not permitted. • Housing for poultry shall have the strong construction covered with litter material as straw, wood shavings or grass. Artificial light used for product stimulation is prohibited.
Environmental management	• Waste from farm areas shall be appropriately managed to avoid soil and water degradation, nitrates and pathogenic bacteria contamination of soil and water and optimize recycling of nutrients. • Manure compost application rates shall be appropriate to avoid pollution.
Recording	• Livestock management, disease prevention and treatments shall be recorded

Existing livestock production system can be converted to organic production system if organic practices are adopted, however, the production system can only be certified organic following the a certain conversion period for specified production objectives (Table 2).

Table 2: Conversion period for organic livestock production

Species	Production objective	Conversion period
Cattle and buffaloes	Meat products	12 months
	Calves for meat production	6 months, shall be introduced in as soon as they are weaned and less than 6 months old
	Milk production	The conversion period is 90 days, and 6 months and thereafter that milking product can be certified as organic.

Species	Production objective	Conversion period
Ovine and Caprine	Meat products	6 months
	Milk products	The conversion period is 90 days, and 6 months and thereafter that milking product can be certified as organic.
Porcine	Meat products	4 months
Poultry	Meat Products	whole of life span
	Eggs	6 weeks

Note: At the beginning of the conversion period, feedstuff shall be composed of at least 70% of organic sources for ruminants and 65% for non-ruminants, calculated on a dry matter basis.

THE NATIONAL CONTEXT

The recent statistics on livestock in Nepal (Table 3) shows that except for sheep population, the population of other domestic livestock species has increased during the last decade and is in increasing trend at present as well (Pathak, 2011).

Table 3: Livestock population in Nepal

Livestock species	Population (million)	Average annual growth rate (1997-2011)	Percent of improved breeds in the population
Cattle	7.19	0.49	10
Buffaloes	4.83	2.29	26
Sheep	0.80	-0.93	5
Goats	8.84	2.36	6
Pigs	1.06	4.02	34
Poultry	25.76	4.94	55

Although, the accurate figure on the national livestock population catered by veterinary service is not available, it has estimated that national veterinary service is only able to reach about 17.5-23 percent of national livestock population (Pathak, 2011). However, despite this low coverage, annual import cost of veterinary vaccines and medicines is more than 4 billion NPR (Pathak, 2011), which reflects the potential of market expansion if service coverage is expanded. The critical drawback of this expansion is the increased proportion of misuse and abuse of drugs, which affects the overall environment (human health, animal health, high level of chemical residues in animal products and development of resistance in microorganism and parasites). The regulatory mechanism is very poor and drug usage is sale oriented.

On the other hand, except for the herds commercially managed and located near the urban and peri-urban areas and road corridors, the majority of small and large ruminant population in Nepal is managed under organic management system in which access to modern veterinary care is limited or nonexistent; the degree of which is dependent upon remoteness, access and nearness to the service centers or agro-vets. It can be said that the animals reared in all mountainous districts, most of the hill districts and rural areas of Terai districts are managed under organic management at present or could be readily converted to it. Some classical production system and products like yak cheese, transhumant sheep and goats, buffaloes raised in high hills and mountains, ghee produced from remote villages are typical examples of the products that could get organic certification immediately. Similarly, the animal population uncovered by regular veterinary care could also be converted to organic production system following the adoption of specified conversion period. Hence, from the trade and business point of view, the national livestock population could be categorized in to two production systems, viz: intensive commercial production system and extensive organic production system. Thus, farmers adopting either of the system could get the benefit of their produce as the premium price of the organic produce could match up the market price obtained from the intensive production system.

REQUIRED INITIATIVES

The following initiatives are needed to be undertaken to establish the organic livestock production system in the country:

Need to identify and specify the production system

The national livestock population in Nepal could broadly be categorized in two categories: those having access to modern veterinary medicines and care and those not having access to it. Currently, the modern poultry production system, some newly established dairies could broadly be included in the commercial production system and the remaining livestock population reared without the access to the modern veterinary care under the traditional management could be regarded under the organic production system. It can thus be estimated that the livestock population which is not under "improved" status is largely reared under the subsistence management is basically the population managed under the organic production system. Hence, if the system of production management could be classified, we could have two production system in the country, i.e. Commercial production system with regulated use of antibiotics and other drugs and Organic production system, which is the existing traditional production system. The need is to identify and register the system and declare it through proper regulatory system.

Certification and declaration of organic products

Once, the production system is properly identified and registered, it would be important to certify it through the national and international organic certification system, so that the livestock products produced through this system could get the premium price of the organic produce. Some of the classical products of Nepal could be "Organic Yak Cheese"; "Organic mutton and Chevon from High land Himalayas"; "Organic Ghee"; "Organic Buffalo meat from Himalayan mountains"; "Organic Himalayan Honey"; "Organic chicken and eggs from backyard poultry". All these products could get high value in the national and international market if the certification and declaration system for organic production system could be developed in the country.

Identification of local and international market

To promote the organic livestock products in the market, it is important to explore and exploit the niche market for organic products locally and internationally. A vast affluent consumer market is available in our neighboring countries, where these organic products will have high aesthetic value to get the premium price. In addition, gulf countries and countries of emerging economies in the asia-pacific region could be the other potential market, if the processing is done according to their religious requirements. Excessive and unregulated use of antibiotics and other chemicals in the animals and animal products have made the conscious consumers very wary of the ill effects of these chemicals, which will act as a catalyst to organize organic consumers locally and internationally.

Organizational development and linking organic producers to the market

The most important aspect of organic livestock production is to organize the producers in to "Organic production groups" for a particular product and link these groups to the market. A strong network of producers will have to be developed to carry out the production and marketing of the products. Organic producer groups could be further strengthened by organizing them in to cooperatives, which could undertake collection, branding and marketing of the products in the local and international market. Government should facilitate the process of market identification and trade promotion by providing incentives and other facilities at various levels of production and marketing.

Effective monitoring and regulatory mechanism for organic standards maintenance and quality control:

Effective monitoring of the production system and maintenance of the quality standards would be important to be able to get access, compete and remain in business in the national and

international market. To maintain the standards of the organic products, "Organic Inspectors" should be employed by the regulatory authorities (Government) for different production regions and commodities, who should monitor the production system and certify for its organic produce. Infrequent checks on residues by standard laboratory procedure (Internationally accepted) should be made randomly on batches of products to produce as evidence of the quality. It is important that the trust of the consumers should be maintained on the products by maintaining the quality standards, which if lost is difficult to regain.

CONSTRAINTS AND LIMITATIONS:

Though, the livestock production system which is managed in the remote areas of the country away from the veterinary centers is basically the organic production in the country, yet there are considerable problems which will have to be addressed to market the organic livestock products within and outside of the country. The first constraint is the lack of authentic data on existing production system and its description, the population within each system and the area where each system is practiced. The second prerequisite would be to establish the regulatory bodies and mechanism within the country which will deal with all regulatory mechanism for organic production and declare the produce as organic. The third and the most important aspect would be identify the market and marketing network within and outside of the country for these specific organic products which could fetch the premium price for these products. The well-established marketing channel and network and income from the products would be the catalyst for the producers. Hence, it would be important to address these constraints if the organic production system in the country is to be promoted.

THE WAY FORWARD

The following steps would have to be taken to have efficient organic livestock production and marketing:

1. Identify and certify the organic production system
2. Identify niche organic livestock product for the organic production and marketing
3. Establish the organic certification system and mechanism within the country
4. Obtain international recognition for the organic production and certification system
5. Organize organic production groups and establish strong networking between the groups
6. Identify market for organic products nationally and internationally
7. Publicity and media campaign nationally and internationally.

Establish markets for organic products and develop market network nationally and internationally.

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FOOD OF ANIMAL ORIGIN PRODUCTION CHAIN, VETERINARIAN AND VETERINARY SERVICES IN THE FUTURE IN NEPAL

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ABSTRACT

The globalization of trade has made food safety an international issue, as food contaminated in a producing country may cause food-borne outbreaks in an importing country. Food safety and quality have become increasingly important after Nepal's membership to WTO and in recent years, not only in terms of protecting the health of the consumer, but also to meet requirements for international trade. Veterinary activities at the farm level, aiming at protection of public health from food-borne hazards, are implemented mainly by Veterinary inspectors, and to a lesser degree by private veterinarians and those of food safety agencies like DLS and DFTQC. This article discusses major hazards along the food chain of animal origin and the roles and responsibility of Veterinarians and veterinary services in the changing context of WTO/SPS and major emphasis is given to the primary production as it is the first step of food chain. The role of the Veterinary Services with the change of Acts (Animal health and Livestock services Act and its regulation and Animal slaughterhouse and meat inspection Act and its regulation) should be confined to assessment of the HACCP food safety programs and their auditing to verify that they are correctly implemented by the industry. It should be noted that the management of food establishments now has the primary responsibility for the production of safe food. It is concluded that present developments in food safety issues at international level, create new responsibilities to veterinarians and the Department of Livestock Services in Nepal.

INTRODUCTION

Agriculture is the major sector of Nepalese economy. It provides employment opportunities to 66 percent of the total population and contributes about 36 percent in the GDP. The livestock contribution to Agriculture Gross Domestic Product (AGDP) is about 29%. The livestock sector plays an essential role in agricultural and economic development as well as food security. The global livestock output grew at a rate of 2.4% in 1998: increase by over 70% in the next 30 years(FAO, 2000), and world demand and consumption of livestock products is expected to nearly double in the next 20 years(Delgado, C et al, 1999). Most of this increase is expected to take place in developing countries associated with greater population growth and emerging economies; particularly in Asia. The sustainable development of Livestock sector with quality and food safety objectives is the key for the development of national economy.

Nepal has a population of about 26 million (Population census, preliminary report, 2011) with growth of 1.42% where 70% of people are non-vegetarian. The per capita meat requirement is 14kg /annum but the availability is only 9.5 kg.The total meat production in 2009/2010 is 248573 metric

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ton where buffalo, Goat, pig and poultry meat contribution is 65.26%, 20.05%, 6.86% and 6.65% respectively (Statistics, MOAC, 2011). The demand of meat is in increasing trend. Meat and other animal products provide essential fatty acids, vitamins and minerals. The iron in meat and meat products is easily assimilated by humans and is a key to preventing iron deficiency anemia. The livestock industry therefore can be seen to have great economic and nutritional significance.

The globalization of trade has made food safety an international issue, as food contaminated in a producing country may cause food-borne outbreaks in an importing country. Food safety and quality have become increasingly important after Nepal's membership to WTO and in recent years, not only in terms of protecting the health of the consumer, but also to meet requirements for international trade. This is especially important for those areas of livestock product that have some export potential. To facilitate such trade, it is necessary to implement national standards in compliance with international standards, guidelines and recommendations for the production of safe and quality-assured foods. This includes developing the necessary analytical capacity to detect and monitor food contaminants such as pesticides and veterinary drugs during the primary production, processing and in finished food products, and also to assure the quality of the agrochemicals used. The focus on the traceability of foods could be relatively a new system for Nepal for control of food safety and quality. The ability to demonstrate the origin and the authenticity of food products is a major concern to food safety regulators such as DFTQC and DLS and to trading partners due to increasing mobility and cross-border transportation of food commodities. Failure to securely characterize food quality and safety parameters has been shown not only to have devastating economic consequences but also to create potential human and animal health problems. Food traceability and authenticity touch upon a range of diversified interests of all stakeholders in the food production chain. The consumers are concerned that the food they eat is safe and correctly labelled. Producers are concerned with that the commodity they process and trade is not adulterated or subject to fraud, and legislators concerned with the food safety and quality parameters that must be met. A major factor behind the increased interest in food authentication is the need to meet requirements for international trade. When applied in conjunction with detection and monitoring schemes, traceability mechanisms enable regulatory authorities to trace contaminated products which may be harmful to the health of the consumer to their source in order to take preventative actions to avoid reoccurrence of the contamination, and also to withdraw the contaminated products from the market if necessary. Traceability combined with traditional food safety parameters provides the ideal control system for maintaining food safety and quality and protecting the consumer. The protection of public from the food hazards associated with so called livestock sector will be the prime importance for the veterinary services in the country in coming days.

VETERINARY SERVICES, VETERINARIANS AND FOOD CHAIN

The total number of Cattle, Buffalo, Goat, Sheep, Pig, Poultry and duck is 7.2 million, 4.8 million, 8.8 million, 0.8 million, 1.1 million, 25.8 million and 0.4 million respectively. The cattle are not considered as food animal in Nepal because of socio-cultural issues (cattle slaughter is banned in Nepal). The total meat production is about 248573 metric ton derived from other animals except cattle. A total of 1495897 metric ton of milk is derived from 1.2 million buffaloes and 1 million cows. There is ample space to increase this production of milk with crossbreeding in future. Our issue here is not the total production but the quality of meat and milk that goes to stomach of hundred and millions of consumers. We can consider two main organizations i.e. DLS and DFTQC who are the responsible authority in the sector of maintaining food safety and security. The former organization has only one Veterinarian and the later has about 400 Veterinarian who are more or less busy with administrative roles. In the private sector around 226 plus veterinarian are working especially concerning with treatment of pet animals, NGOS, INGOS programs that relates to poverty reduction and allied fields. The ratio of Vet to Livestock population is very big which is probably the largest in the world. The coverage of Veterinary services from the manpower of DLS is only about 10.2%. There is a huge gap in the area of delivery of Veterinary services in the nation. In such a situation the roles of veterinarians

towards fulfilling the demand of 21st century with safe food is very difficult but this may be the starting point from where we can prepare ourselves for meeting the demand of future generation. We must not forget ''if you cannot lead the market, some other will lead and you will remain as the follower''. So let's start! Veterinarians are the leader for the food safety, public health and zoonoses control which are the major issues of the world at this stage and the days to come ahead.

National Veterinary service is not operationally effective in Nepal. For the Veterinary Services to be operationally effective, individual competencies must be structured within an institutional framework and in accordance with a general organisation including a clearly identified chain of command backed up by a suitably adapted legislative framework. This implies that the human resources must be qualified under an effective system of education and training, and that these human resources are allocated in sufficient numbers to the veterinary structure, which must itself be allocated a sufficient operational budget to fulfil its mandate. Although the institutional framework exists in Nepal, the resources allocated is often too poor, which see an ever-widening gulf separating it from developed countries when it comes to animal production and marketing of products. Animal health protection activities are organized within the framework of epidemiological surveillance and intervention systems, in which producers associations, technical organizations and the official Veterinary Services work together in a public-private partnership. These activities range from epidemiological surveillance at the national level and at borders, emergency or routine diagnosis, and sanitary interventions to control or eradicate endemic, exotic or emerging diseases. Regarding the differences in food system profiles, animal production systems and the role of Veterinary Services between developed and developing countries like Nepal, it is noticeable that the Nepal is still very active in the control of major diseases whereas the former are now more orientated towards the inspection of products and surveillance for diseases, even if there is a renewed involvement in the epidemiology of infectious diseases within the context of emerging diseases. Nevertheless, all are now concerned about how their production environment will evolve, and the degree of food self-sufficiency to be achieved and controlled in a world of renewed major uncertainties.

Most of the Veterinarians in Nepal are primarily concerned with increase production by treating the sick animals and poultry. It is the trend that can be seen in all the developing countries because of their Government priority. Poverty reduction and achieving food security is the main concern for such countries. We must not forget that every individual rich or poor have the right to live healthy irrespective of developed or poor country. The food of animal origin must be free of any hazards that can pose risk to health of Nepalese. The veterinarian should not forget that they are the key actors in ensuring quality (residue free) edible animal products such as milk, meat and eggs to the public. The implementations of WTO regulations demand that veterinarians working in food chain of animal origin should learn how to avoid hazards in food animals and disseminate this information to the farmers to safeguard the health of general public. This issue is also of paramount importance for the veterinarians employed in private sectors (pharmaceutical, slaughterhouses, food industry, hatcheries, Farms, Processing plants) and regulatory sectors responsible for assessing the fate of hazards that enter the food chain via the edible products. It is also need of the day that environmentalists, toxicologists and non-government organizations (NGO) should pay due attention towards this issue. This is necessary to conduct complete risk assessment, risk management, risk communication studies and implement certain legislative measures to safeguard the public health.

FUTURE FOOD CONTROL SYSTEM FOR NEPAL

In Nepal, food control is concentrated still on the examination of samples from the end products and the inspection of processing and catering establishments to monitor hygienic practices. Unsafe foods found during inspections are detained and removed from the market and those responsible for their production and placing on the market are prosecuted. These traditional systems of food safety are not efficient and could not respond to existing and emerging challenges to food safety, because they do not provide a preventive approach but we are helpless to do so because of shortage

of manpower and lack of stringent rules and regulation. The roles that veterinarian played in early 80s and 90s is not the same in this century as the spectrum and prevalence of hazards in foods is under continuous change. Recent epidemiological surveys around the globe have shown that bacteria in foods of animal origin are the most important causes of food-borne diseases. Most of these are transmitted by animals that do not show any clinical symptoms. The roles of Veterinarian are so diverse and vital in maintaining the wholesome food chain in the changing context. Some of the factors that is contributing to increase biological hazards in foods can be rapid population growth, rapid urbanization, Changes in farm practices, introduction of new technologies, intensive rearing of animals, increase in consumption of meat, globalization of food trade, changes in lifestyles, increase international travel, preference for fresh and undercooked foods, eating food prepared outside the home, demographic changes with increased proportion of old and immune-suppressed people and environmental pollution from improper animal manure disposal. We will discuss hazards, roles of Veterinarians and veterinary services in each step of food chain of animal origin in coming days in Nepal.

Primary Production: Farm

The role of Veterinarians at the farm level must focus on good hygienic practices, prudent use of veterinary drugs, disinfectants, insecticides, and herbicides and safe waste disposal. They should also be involved in the epidemiological surveillance programs for zoonotic diseases and food-borne pathogens, the protection of animal health and welfare, residue control programs, registration, animal identification and in the issuance of certificates for animals that will be moved from the farm. Monitoring and reporting of the animal health situation in the farm by the national Veterinary Services is an essential tool in the context of an epidemiological surveillance program. Early detection of zoonotic diseases can prevent transmission to humans at slaughter or introduction of pathogens into the food chain. It is evident that the effectiveness of monitoring and reporting is directly dependent on the cooperation of veterinarians and farmers with District Livestock services Office, Animal Health Directorate and Department of Livestock services. The veterinarian at the farm must also supervise the record keeping of all the activities at the farm, including the feed quality control and the recording of the batches of feeds received and the name and address of their suppliers. All this information must be made available to the Meat supervisor through Meat inspector in the Animal slaughterhouse where the animals are sent for slaughter or to the manager of the food establishment where the animal products are sent for processing. The role of Veterinarian in this level is very crucial in providing animal products that are safe for human consumption. The hazards at this level can be various whose control at farm level can have a beneficial or even decisive effect on the food safety of products of animal origin (including: milk and milk products, meat and meat products, eggs and egg products, honey and apiculture products).

The food safety standards implemented by DFTQC are general hygienic principles that were developed and implemented in final product and for a long period the level of hygiene was thought appropriate to control microbial foodborne disease. However, in recent days emerging (or re-emerging) pathogens have caused new and increasing problems. It has been said that "technology has overtaken hygiene." The notable increase in global trade in food has prompted a number of new international initiatives in the area of food safety, among which are those related to defining a risk analysis framework.

Secondary Production: Slaughterhouse

The role of assigned Veterinarian in the Slaughterhouse is also rightly mentioned in Animal slaughterhouse and Meat Inspection Act 2055 and its Regulation, 2057 were some of the important aspects are not clearly mentioned. Besides, the roles and responsibilities already mentioned in the Act, Meat inspector or supervisor must also check the effects of transportation on the animals, health data from the farm of origin, identification of the animals, treatment records and level of cleanliness at time of arrival

If we want to implement the Slaughterhouse and Meat Inspection Act just for the sake of

implementation to show other country than it is better to forget its implementation but if we really want food safety then we must not forget the some of the crucial issues in the inspection procedures. The Government need to recruit qualified veterinarian with full authority to judge independently in all the established slaughterhouses in the country. The ante-mortem and post mortem examination is the prime responsibility of the veterinarian working in the slaughterhouse. The Veterinarian in the slaughterhouse has to carry-out examinations for the detection of macroscopically visible pathological changes or other abnormalities. It has been seen that unnecessary incision during meat inspection further increase the risk of transferring the contamination to other carcasses that are otherwise healthy. So, wherever it is absolutely necessary, palpation and incisions for removal of any lesions, which may be present, may be carried out. The Veterinarian must supervise the implementation of Good Hygienic Practice (GHP), Good Manufacturing Practice (GMP), the HACCP and the samplings for the residues control, the microbiological testing of carcasses and the general hygiene of the slaughterhouse. He must be made responsible for record keeping and the communication of his findings to the farm veterinarian, to the Veterinary Services for any necessary actions and to meat processing establishments, cold stores and traders, as well as any information required for the traceability of the products that goes in the chain. Veterinarians and Veterinary Services are equally responsible in the primary processing of milk, fish, and eggs.

In the slaughterhouse, there are numerous potentially dangerous agents for humans which are present in the digestive tube or excreta and on the hides and skins of buffalo, sheep and goat or the plumage of birds in good health. These agents include E. coli, Salmonella and Campylobacter, which can cause food poisoning in humans. Stress caused by grouping animals together, loading them and transporting them to the abattoir can promote the passage of these pathogenic bacteria from the intestine into muscle tissue. Moreover, the greater the faecal soiling of hides, skins and feathers, the higher the risk of any pathogenic bacteria they may contain contaminating meat during the dressing or defeathering of carcasses at the slaughterhouse.

Meat Inspector should not kept under the influence of the owner of slaughterhouse while rejecting the animals or meat that is not fit for the human consumption. Meat Inspectors must be recruited and paid by Nepal Government. They should be given full authority and power to exercise in response to meat inspection. Meat Inspectors must have training and skill on inspection (why inspection, what to inspect, how to inspect and for which reason, what will happen if no inspection etc.)

Processing Plant

The responsible veterinarian must not forget that chemical contaminants in food may be naturally occurring or may be added during the processing of food. They include chemical compounds that, when consumed in sufficient quantities can inhibit absorption or destroy essential nutrients from the diet of the consumer. They can be carcinogenic, mutagenic or teratogenic, or can be toxic and can cause severe illness and possibly death. The chemical contaminants that can enter food may be Direct food additives (preservatives, flavourings, vitamins and minerals), indirect food additives (detergents, disinfectants, lubricants), heavy metals (lead, mercury, copper, cadmium, radioactive isotopes), pesticides, insecticides, fungicides, herbicides, veterinary drugs residues, persistent organic pollutants (dioxins), natural toxins and allergens. Veterinarians employed in food safety must be involved in the design and implementation of GHP, HACCP Plans and quality assurance systems in Secondary Processing Establishments for meat, milk and fish products in the country. The role of the Veterinary Services with the change of Acts (Animal health and Livestock services Act and its regulation and Animal slaughterhouse and meat inspection Act and its regulation) should be confined to assessment of the HACCP food safety programs and their auditing to verify that they are correctly implemented by the industry. It should be noted that the management of food establishments now has the primary responsibility for the production of safe food.

Retail shops

Most of the retail shops handling meat and eggs can be the major source of animal pathogen.

Regular inspection, sampling and laboratory testing for hazards from the retail outlets transfer confidence to public for getting safe food of animal origin. This role of qualified veterinarian must be made mandatory and supervise by the responsible authority so that the assigned responsibilities are timely completed and carried out effectively and efficiently.

Consumers

They can play role of vigilant and catalyst for the Veterinarian. Issues of animal welfare are not getting momentum in Nepal but it will be wrong to say it has not been started. The consumers are unaware with the consequence of unfair behaviour to animals during transportation and slaughtering. It is the duty of the Veterinarians to create awareness to consumers about the safe meat and meat products and the consequences of any ill practices in food chain of animal origin.

Reasearch

The Veterinarian involved in the field of food safety research is very less in our country but if we see from the global or even regional perspective then the role is so important which we cannot ignore. There is need to continuous improvement at the level of animal production and at all further steps of food chain. The development of simple and inexpensive detection and analytical methods for all hazardous substances and micro-organisms is the need of the hour. DLS, in order to be effective in their role and responsibilities as a key actor for increasing both the quantity and quality of food of animal origin, monitoring food industry activities at primary production and processing levels, need information and knowledge from integrated research programs. Through research, new devices can be developed, which could further support the role of DLS in the changing context. It must be the shared responsibility of DLS, NARC and the industry involved in the production of food of animal origin with concern to research in the area of food safety. However, it is an obligation of Nepal Government, which, through Veterinarians and Veterinary Services, should increase and focus research activities at primary production and processing levels that will enhance its national and international image in the area of food safety.

RECOMMENDATION

It is the fundamental rights of consumers to have safe foods, accurate and honest information, so that they can choose their diet. Veterinary activities at the farm level, aiming at protection of public health from food-borne hazards, are mainly implemented by District Livestock services office in their program as veterinary inspection by responsible veterinarians, and to a lesser degree by private veterinarians. The key actor-DFTQC has nothing to do with farms; they are mainly concern with final products perhaps due to acute shortage of manpower-Veterinarians. At the same level, programs of good hygienic practices have to implement mainly by District Government veterinarians with some assistance from veterinarians employed in industry and private veterinarians. Food safety risk management must be developed on the basis of risk analysis and risk assessment. Between risk assessment and management there should be a functional separation. Veterinarians should adapt their role and activities, in close collaboration with other sectors and professionals in a risk-based approach to cope with hazards and risks to human health from foods. Veterinary Services and veterinarians, in many countries of the world, have a leading role in the safety of foods of animal origin. The adoption of the integrated approach of food chain and the introduction of quality assurance systems, through the entire production process, provide a new challenge for the Veterinary Services and the veterinarians in general. Veterinarians should seek and accept responsibilities for developing a new quality oriented procedure. This process must cover the entire food chain from 'stable to table' and must be designed to deliver the highest levels of food safety guarantee for animal production, the food industry and the consumer. Food safety is now universally recognized as a public health priority. It requires a global approach, from production to consumption. All the steps of food chain are equally important with high consideration to primary production i.e farm. This inevitably means controlling the health status of

the animals from which food products are derived. The veterinary services must adopt following steps in order to face the need of future generation

- Risk based programs should be aimed at preventing or decreasing the transmission of zoonoses, through adequate policy frameworks, prevention and control measures, and education.
- Attention should also be paid to ecological, cultural, social and ethical aspects regarding the implementation of control programs.
- Minimize the risk of contamination (biological, chemical and physical) entering the food chain trough animal products.
- Promote the development and adoption of adequate international, regional and national regulatory frameworks.
- Enhance communication and cooperation between animal and human health sectors.
- Prioritize the national food safety related diseases and implement the control plan.
- Strengthen disease surveillance and quarantine capacities.
- Increase professional and public awareness on veterinary public health and food safety issues.
- Formulate a common plate form for academics, researchers, producers, traders, processors, regulators and consumers to discuss and disseminate vital food safety information.
- International and regional collaboration with key institutions involved in food safety and public health e.g. FAO,WHO,OIE

CONCLUSION

Ensuring safe food is paramount for the protection of human health and for enhancement of the quality of life. Safe food plays an important role, whether domestically produced and consumed, imported or exported. In addition, the production of safe food represents an opportunity for income generation and market access. The food chain approach has been well recognized by public health experts as an important step to ensure food safety from production up to consumption. Veterinarians must be well aware of the importance of drug/chemical residues in the food animals and their possible risk to the general public. They must have updated information about the proper withdrawal times of all the drugs/chemicals used in their areas of practice. They must extend this information to the livestock and poultry farmers for the production of residue free edible animal products like milk, meet and eggs. Continuous research and training is the another side of the same coin. Improved but simple and inexpensive technology is needed in the food chain of animal origin. This approach requires the commitment of all actors in the food chain, involving producers, traders, processors, distributors, regulatory bodies as well as consumers.

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VETERINARY EDUCATION IN SOUTH ASIA: A TIME OF CHANGE

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ABSTRACT

Re-structuring of veterinary educational institutions is needed for the production of quality veterinarians. Sincere dialogue among Veterinary Universities, Institutions, Colleges and Veterinary Council are essential to establish the necessary new policies and strategic directions. South Asian Veterinary Education (SAVE) network has been formed on the co-ordination of Vice- chancellor of Chittagong Veterinary and Animal Science University (CVASU) Bangladesh on 2010. The aim of the SAVE-Network is to meet the current requirements and expectations of a range of relevant stakeholders in the fields of veterinary and animal science practice and research. This network also strengthens the professional identity, and contributes to the social status of the veterinary profession. In this region, maximum number of students are enrolled in India i.e. 3200 followed by Pakistan (800), Bangladesh (400), Nepal (100) and Srilanka (75) in every year. Elective courses such as ruminant, small ruminant, poultry, lab animals, wild life and zoo, veterinary public health etc. are offered in Tamilnadu University of Veterinary and Animal Science (TANUVAS). Similarly clinical skills are developed from day-one of the course in TANUVAS. Problem based learning (PBL) is the new concept in South Asia which is initiated by CVASU, Bangladesh in collaboration with Royel Veterinary colleges, UK. Engaging with the new curriculum at CVASU, students would emerge more flexible and self-reliant, more self-directed and able to study independently. The weightage of courses on Veterinary sciences are higher in India (66.67%) followed by Bangladesh (64.60%) and Nepal (60.60%). However, animal production courses are higher in Nepal (25.80%) followed by Bangladesh (23.60%) and India (21.13%). For making learning more active teacher-student ratio should be reduced and incorporate small group work. The degree offered is also varied in different countries. The degree of veterinary medicine (DVM) is offered by Bangladesh and Pakistan where as B.V. Sc. and A.H. is offering by India and Nepal. However, University of Peradeniya Sri Lanka is offering B.V.Sc. degree. The SAVE program strongly believes that in addition to building research experience, developing skills in working across cultures, communication and flexibility are essential components. To remain relevant, academic veterinary institutions and colleges must prepare veterinarians for what may come in the future. In order to be recognized and remunerated for their knowledge, compassion, integrity, and judgment, veterinarians must first demonstrate their relevance to new societal trends.

INTRODUCTION

Restructuring the economic profile and transformation of lifestyle of the people after conflict is the challenge in the higher education development in developing countries. Defective policies in the higher education create obstacles in the improvement. At present Tribhuvan university of Nepal does not have mandate for research and extension. However the proposed Agriculture and Forestry University will have teaching, research and extension mandate. Putting aside personality differences, recognizing the strengths in others, and working together for a common goal are disciplines that we need for providing quality veterinary education.

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The world is growing more complex, and knowledge of science, animals, animal production, and animal health is expanding rapidly. Consequently, people expect more and more from the Veterinarians. The veterinary profession must become capable of routinely delivering higher and higher levels of services to all the classes of animals important to people and to achieve ever-higher levels of competence in all veterinary activities. It is not enough to provide services just to livestock and companion animals. All classes of animals are considered by the public to deserve high quality veterinary service.

Veterinary scientists are only the professional who visits the door to door of the farmers and serve them for the management and treatment of their livestock and poultry. For providing better service, quality veterinary graduates should be produced. Although there are some differences in every countries of South Asia in the farming systems, ecological variations and occurrence of the diseases, the fundamental courses of veterinary and animal sciences are same. The veterinary education at South Asia should be modified and the curriculum should be designed in consultation with the authorities of the Veterinary and Animal Science University and Colleges of Veterinary Sciences of this regions.

An attempt has been made by CVASU Bangladesh to make several changes on Veterinary teaching and learning by coordinating the Veterinary universities and colleges of South Asian region. A workshop on "Veterinary education on South Asia: new approaches to teaching and learning for evolving scenario" organized by CVASU Bangladesh was held during Sept 21-22, 2010. Some of the information discussed on the workshop are addressed in this paper. Two case studies at Tamilnadu University of Veterinary and Animal Science (TANUVAS) India and CVASU Bangladesh have been conducted and the responses on these studies were discussed on the meeting.

Networking of Veterinary education in South Asia

South Asian Veterinary Education (SEVA) network has been formed on the co-ordination of Vice-chancellor of Chittagong Veterinary and Animal Science University (CVASU) Bangladesh on Sept, 22, 2010. Tamilnadu University of Veterinary and Animal Science (TANUVAS) India, University of Veterinary and Animal Sciences (UVAS) Lahore Pakistan, Faculty of Veterinary and Animal Science Rawalpindi, Pakistan, Faculty of Veterinary Medicine and Animal Science, Peradeniya University Srilanka and Tribhuvan University Rampur Campus, Chitwan , Nepal are it's members. The aim of the SAVE-Network will be to meet the current requirements and expectations of a range of relevant stakeholders in the fields of veterinary and animal science practice and research. The present situation of Veterinary institutions in South Asia has been explored in the Table 1.

Table 1: Status of veterinary education in different countries of South Asia

SN	Particulars	Nepal	India	Srilanka	Pakistan	Bangladesh
1	Vety. University	-	10	-	1	1
2	Vety. Colleges	3 (2+1)	52	1	11	6
3	No. of students admitted /yr	40(Govt) 70(Private)	3200	75	800	400
4	No. of UG students passing out /yr	60	3000	65-70	700	350
5	No. of registered veterinarians	600	52000	2000	7703	3500

There are 10 Veterinary and Animal Science Universities in India..One each Veterinary and Animal Science University is present at Pakistan and Bangladesh. There is no Veterinary University in Sri Lanka and Nepal till date. Maximum number of students are enrolled in India in every yr. i.e. 3200 followed by Pakistan, Bangladesh, Srilanka and Nepal.

Table 2: Information on Faculties of Veterinary Science in South Asian countries

SN	Particulars	Tribhuvan Univesity IAAS,Nepal	TANUVAS India	Peradeniya University Srilanka	Rawalpindi Pakistan	CVASU Bangladesh
1	Total credit hrs.	221	213	185	223	183
2	Total semesters	10	10	10	10	10
3	Internship duration (Months)	6	6	6	6	12
4	Internship credit hrs.	Non credit hrs. (Farm/ Lab / hospital)	Non credit hrs. (3 months farm and 3 months clinical training)	-	15	22
5	Elective courses	Absent	Absent	Absent	Absent	Absent
6	Problem based learning	Absent	Absent	Absent	Absent	Present
7	Degree offered	B.V.Sc. and A.H.	B.V.Sc. and A.H.	B.V.Sc.	DVM	DVM

Total credit hour varies from 182 to 223 in different countries (Table 2). Elective courses such as ruminant, small ruminant, poultry, lab animals, wild life and zoo, veterinary public health etc. are given in TANUVAS. Course duration of undergraduate program is 10 semester to all the veterinary colleges. Regarding internship program, majority of veterinary colleges are having one semester duration for internship works whereas CVASU, Bangladesh is having 2 semester internship program. Problem based learning (PBL) is the new concept in South Asia which is initiated by CVASU, Bangladesh. The degree offered is also varied in different countries. The DVM degree is given by Bangladesh and Pakistan where as B.V. Sc. and A.H. is offering by India and Nepal. University of Peradeniya Sri Lanka is offering B.V.Sc. degree.

Table 3: Percentage of loads of different disciplines in different countries

SN	Areas of courses	IAAS, TU Nepal	TNUVAS India	CVASU Bangladesh
1	Basic and social sciences	30 (13.60)	26(12.21)	19(11.80)
2	Animal production	57(25.80)	45(21.13)	38(23.60)
3	Veterinary sciences	134(60.60)	142(66.67)	104(64.60)
4	Total	221	213	161

Figures in parenthesis indicate percentages.

Comparison of credit hours of undergraduate veterinary courses in different countries is given in Table 3. The weightage of courses on Veterinary sciences are higher in India (66.67%) followed by Bangladesh, Pakistan, Srilanka and Nepal. However animal production courses were higher in Nepal followed by Bangladesh and India.

Case-Study at TANUVAS, India

The TANUVAS, located in Chennai, is a well-established and highly prestigious veterinary institution, with an outstanding record in teaching, research and clinical provision. A number of important aspects of practice were shared, including:

- Structuring the curriculum on a model of core and elective, to ensure students gain both grounding in essential knowledge along with the opportunity to explore specialist areas of their own choosing.
- Widening the learning experience, by offering a range of extra-curricular clubs and activities for students to engage in.
- Incorporating learning experiences to develop essential first-day skills, by enhancing

students' capability in the use of English and IT, in entrepreneurship and communication, and so on.

- Addressing learning needs, e.g. through formal induction of new entrants, the use of peer teaching, etc.
- Ensuring clinical skills are developed from day-one of the course

TANUVAS Curriculum

Presentation from TANUVAS had identified features of their undergraduate curriculum. The groups highlighted particular aspects of provision that they had found to be valuable.

- The introduction of a core curriculum offered in conjunction with electives to give students specialist knowledge and expertise.
- The inclusion of particular elements such as meat science and clinical biochemistry
- The incorporation of topics of extended study such as English, basic electronics and entrepreneurship.
- The early clinical exposure given to Year 1 undergraduates. The TANUVAS was also commended for developing a system of peer learning in which senior students instructed the junior years.

The veterinary curriculum in India is comprised of six components of study:

(i) Core Courses (ii) Tracking Programs (iii) Study Circles (iv) Entrepreneurial Training (v) Internship (vi) Competence in skills

Core Courses

(a) A judicious balance has been ensured in distribution of course credits in theory and practical and sequence among basic, production, pre-clinical and clinical subjects including public health and livestock products technology.

(b) Clinical practice shall be organized in small groups of 5-10 students so that each teacher can give personal attention to each student with a .view to improve his/her skill and competence in handling of the patients.

(c) Efforts be made to encourage students to participate in group discussions and seminars to enable them to develop personality, character expression and other faculties which are necessary for a veterinary graduate to function either in solo practice or as a team member when he/she begins his/her independent professional career. An appropriate time slot for this activity be provided in the student study time table.

(d) Practical training be imparted to produce a well-balanced and all-rounder graduate.

In addition to the Core Courses above, a student has to successfully complete the Tracking Programs, Study Circles, Entrepreneurial Training, Internship and Core Competence in Veterinary skills.

Tracking Programs

These programs have been developed to allow students to exercise more control over the specific direction of their profession and motivate them for self-teaming through virtual classroom, distant learning, internet etc. A student has to compulsorily take any two programs of two credits each (2x2=4 credits) any time (one semester duration each) during second year to fifth year of B.V.Sc. & A.H. Degree Course under the supervision of one faculty member as designated by the Dean/ Principal of the College for that program. Evaluation of the students for this program shall be done internally on Grade basis (A-Excellent. B-Good, C-Average). In case of unsuccessful candidates, the program can be carried over to the next semester/year.

List of the Tracking Programs is given below:

i) Feline Medicine ii) Cryobiology of Gametes iii) Neurosciences iv) Clinical/ Interventional Nutrition v) Dermatology/integument Science vi) Alternate Veterinary Medicine vii) Ophthalmology viii) Anesthesiology ix) Small Animal Critical Care x) Non-Mammalian Medicine xi) Sports Animal Medicine xii) Drug designing Xiii- xv)- To be decided by the college/university.

These will be Non-Credit courses but shaft be mentioned in the Degree Transcript along with the grades obtained.

Study Circles

Each student of B.V.Sc. & A.H. degree course shall have to enroll himself/herself for at least two Study Circle activities during the B.V.Sc. & A.H. degree course out of the proposed Study Circles- as listed below:

i) Livestock and Livelihood Study Circle
ii) Production Systems Study Circle
iii) Ecosystems and Livestock Study Circle
iv) Equine Study Circle
v) Canine Study Circle
vi) Diagnostic Study Circle
vii) Alternate Animal Use Study Circle
viii) Fun/Sport Animal Study Circle
ix) Law and Veterinary Science Study Circle

The College shall designate an Advisor for each of the above Study Circle activities who shall supervise, guide, monitor and evaluate the activities of the Study Circles. Each enrolled student shall have to present a Seminar on the topics of his/her Study Circle any time during the Semester. The date and time of the Seminar shall be notified inviting participation of all students. The Study Circle shall also put up news, wall papers, drawings, exhibits of their subject in the college. The Dean of the college shall coordinate the activities with the Advisors for each of the above Study Circles. The evaluation of the student for each of the registered Study Circles shall be done by the Advisor who will grade them as A-Excellent, B-Good, C-Average as per their performance. The same shall be recorded in the Degree Transcript along with the grades obtained. No student shall be allowed to change the Circles during the professional year.

Entrepreneurial Training

Each student of B.V.Sc. & A.H. degree course shall be required to compulsorily undertake one of the activities of Entrepreneurial Training as listed below. This training is aimed at developing entrepreneurial skill for self-employment The university/college shall provide interest free loans out of a revolving fund (not less than Rs. 3.00 lakhs in a college) to students groups (team of up to five students), technical support and infrastructure for these activities. Inputs, day-to-day work and financial accounting shall be undertaken by the students. The profits/loss, if any, shall be kept/borne by the students. However, in case of loss, the Dean of the college through the Entrepreneurship Committee consisting of four faculty members (at least one subject matter specialist) may evaluate the reasons of such loss and provide compensation in case it is found that the loss has been inadvertent.

Proposed List of 16 Entrepreneurial activities is as follows: (i) Goat Production (ii) Sheep Production (iii) Pig Production (iv) Broiler and Egg Production (v) Pet Production (vi) Dairy Production (vii) Meat Production and Processing (viii) Fish Production (ix) Feed Production-Mineral Mixture (x) Milk Products (xi) Food safety-residue Analysis (xii) Clinical Investigatory laboratory (xiii) Quality

Control-Evaluation (Microbial) (xiv) Shoeing and Shoe Manufacture (xv) Production of Diagnostic (xvi) Pharmaceutical Formulations

The Case-Study at CVASU, Bangladesh

The new curriculum of CVASU includes problem based learning from the first year and having the long internship period i.e. one year. The objectives of the revised curriculum are as follows:

- Introduce students centred education
- Make students independent & interdependent learner
- Eliminate unnecessary duplications
- Decrease theory & increase need based learning
- Create the scope of self-directed learning for the students
- Rectify the mistakes by the students
- Make the faculties interdependent & more challenging
- Provide the students more time for hands on practice
- Increase the social bondage between faculties & students

CVASU New Curriculum

- While the identity of specific subject disciplines had been removed, the necessary knowledge remained incorporated into the learning experience, but the overall load had been reduced.
- The groups also saw benefits for the learners. They considered that, by engaging with the new curriculum, students would emerge more flexible and self-reliant, more self-directed and able to study independently.
- There was a general consensus that the work undertaken had been a success, and that the curriculum change now had potential to enhance student capability, while maintaining core knowledge, and sustaining scientific rigor. The Internship Program was also recognized as playing a crucial role in developing such student capability. The groups acknowledged that CVASU had been visionary, and that the university had taken considerable risk in making fundamental curriculum change in terms of scope and integration of content.
- Inevitably there were some concerns. The majority of the groups identified problem-based learning (PBL) as a major issue. These concerns focused primarily on the capacity of the institution to deliver PBL. Would the staff have the necessary expertise? Relating to curriculum ownership, were particular staff being disenfranchised by the process? In addition there were resource implications. How would the students be able to access the necessary information?
- A number of groups felt that important content was missing. This related to aspects which extended learning beyond the formal course to include: English, computer/IT skills, business, math and entrepreneurship. Some groups also sought minor adjustments in relation to weighting and sequencing of particular elements of the content, and with regard to credit allocation in the course.

Some Strategies for modifying the undergraduate curriculum

1. Reduction of content: reduce theoretical component.
2. Avoid unnecessary duplication of subject material.
3. Integration of discipline content: promote integration of subjects.
4. Develop a core content combined with electives to meet specialists / research needs of

students and their career aspirations.

5. Modify course content to meet regional needs.
6. Increase emphasis on hands-on learning: content less theory-based.

and increase emphasis on developing Animal Husbandry/Clinical skills.

7. Stronger links with a range of industrial stakeholders.

Possible Outcomes of Networking

1. Enhancing students learning: Student exchanges, curricular integration, open access E learning and internship program.
2. Training: Faculty workshops and faculty exchanges (inter-institutional/inter-departmental)
3. Mechanisms for changes:
   a) Formation of organization committee:
      - Membership: representatives from each institution, stakeholders and sponsors
      - Local and regional authority
      - Infrastructure for Committee support

   b) Centre of excellence

   c) Identify lead institutions

   d) Identify faculty expertise across institutions: Specialist database

   e) Collaboration with Medical School networks

   f) Form an education society or association
4. Resources: E-library, teaching materials and day-one skills ideas
5. Sharing / communications: Web site, online discussion forum, E-mail forum, Newsletter, video-conference and discussion groups:
6. Scholarship/communal activity
   - Research projects: Publications
   - Financial support (grant application)
   - Community of experts

CONCLUSION

Delegates of the Chittagong workshop, Bangladesh agreed to create the South Asia Veterinary Education Network (SAVE-Network), to enhance the quality of the student learning experience on veterinary courses across the South Asia region. In carrying out their work, all institutions associated with the SAVE-Network will be regarded as equal partners; with developments promoted through active sharing of issues, ideas and practices.

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STRATEGY OF LIVESTOCK SERVICES TO INCREASE THE PRODUCTION AND PRODUCTIVITY OF LIVESTOCK IN NEPAL

B. K. Nirmal[1], N. B. Rajwar[2], U. C. Thakur[2]

ABSTRACT

Agriculture Perspective Plan (APP), Livestock Master Plan (LMP), Tenth five year Plan, Three years interim plan of livestock services have been reviewed to analyse and present the current status of production and productivity of livestock. Livestock contributes 36-47% of household cash, 18% and 27% in Gross Domestic Production (GDP) and Agriculture Domestic Production (AGDP) respectively (MOAC, 2010). Commercialization of livestock farming, security of food and nutrition, poverty reduction by income and employment generation and replacement of imports and promotion of exports of livestock products are the major thrusts of Department of Livestock Services (Three year interim Program, DLS, 2011). Integrated approach of components of animal health, genetic improvement, feed and forage development, capacity enhancement and market management at potential areas are the demands of farmers for rapid increase of production and productivity of livestock. The model of public private partnership (PPP) through cooperatives is the best strategy for sharing the resources and equating the benefits among all economic levels of the society. The coordination among extension, research and education should be strengthened in a common platform of farmer's school. Value addition of livestock products, least cost production, organic farming, mitigation of effects of climatic change, livestock insurance, control of highly pathogenic emerging and economically important diseases are some of the issues to be addressed in the present strategy of livestock development. The budget allocation should be increased up to 10% of national budget in agriculture sector and 5% in livestock sector . There is need of budget allocation in the department of livestock services as follows: Animal Health and sanitation-20%, Animal breeding-20%, Feeding and Nutrition-40%, capacity enhancement-10% and market management-10% , The detail of present situation and future strategy of each components of livestock development has been discussed in this paper.

INTRODUCTION

Livestock is the backbone of rural economy in Nepal. Livestock contributes about 27% to Agriculture Gross Domestic Production (excluding processing industry such as hides/ skin, carpets etc.) .The growth rate of agriculture sector lies between 2.5 to 3.0% per year which is far below than the targeted growth rate (MOAC, 2011).

The estimated number of cattle and buffalo are 7.19 million and 4.83 million respectively out of which only 0.95 million of cattle and 1.25 million of buffaloes are milking and 12% of cattle and 30% buffaloes are said to be of improved breeds which produce 3000 lit and 1500 lit/anum of milk respectively. Large number of indigenous breeds of cattle and buffaloes are either unproductive or less productive producing 450 lit or 900 lit of milk/year respectively. The total milk production is 1.5 million MT annually in which buffaloes contribute 70% and the cattle contribute around 30%

1 National Livestock Breeding center
2 Department of Livestock services

(MOAC, 2011). 0.8 Million of sheep and 8.84 million of goats are reared for wool and meat purpose whereas 1.06 million pigs and 25.76 millions of poultry are also reared for meat and egg purpose. Around 5% sheep, 6% of goat, 34% of pig and 55% of poultry are of improved varieties. The number of layers poultry and duck are 7.12 million and 0.17 million which produce 62.9 million of eggs annually (MOAC, 2011).

In Nepal, 25% of population is still under poverty. The contribution of dairy, meat and eggs are 62.6, 32.4 and 5% respectively. Per capita requirement of animal products in Nepal is14 kg of meat, 57 litre of milk and 48 eggs per year (DLS, 2011). There is a potentiality to increase livestock sector contribution up to 45% to AGDP (APP, 1995). Three year interim plan (2010-2013) has the target to achieve the increment of livestock production with the growth rate of 5.85%, 4.99% and 13.27% for milk, meat and eggs respectively. Livestock can utilize the vast natural resources like natural pastures (12%), forest (40%) and agriculture lands (28%) and convert these to high value nutritive food like milk, meat, eggs, draught power and manure to support agriculture production system (MOAC, 2004).

There is quite alarming situation of deficit of milk, meat and egg in the country in the present context. The dairy industry is in short supply of half million litre of milk everyday where as large amount of goats (cost of 400 million NRs.) are being imported from neighbouring countries. Nepal is self-efficient in egg production however imported low quality of eggs are observed in almost all urban, peri urban and rural areas of border districts. 65.5% meat is produced by buffaloes, as they are slaughtered when they stopped to produce milk or become old or sick. The number of sheep is reducing day by day due to limitation in rangelands therefore most of the carpet wools are imported from other countries.

MATERIALS AND METHODS

Agriculture perspective plan (APP, 1995-2015) and livestock master plan have been reviewed in this study. Dairy master plan, policies of Agriculture sector and Livestock sector have been consulted and reviewed. The information from the tenth five year plan, three year interim plan (2010-2013) , current situation of livestock development , policy and strategies have been collected from several sources and analysed. The data was tabulated and presented into graphs to give the clear picture of present situation of livestock development. This paper is also based on several articles and personal communication. International training and workshop proceedings have been cited for the best suitable recommendations. Long-term plan of animal genetic resource conservation (2012-2022) was studied and also the discussion with farmers, stakeholders and entrepreneurs were done to collect the information for this paper.

RESULTS AND DISCUSSION

The history of Department of Livestock Services (DLS) initiated with establishment of a dispensary in Singhdurbar with the use of homeopathy dispensary by foreigner to protect the exotic breeds of animals., The modern veterinary and livestock development started with the establishment of first veterinary dispensary in 1939 (BS 1996). Livestock services were strengthened from 1957 (BS 2014) to cope with Rinderpset outbreak (DLS, 2008).

The present long term plan (APP, 1996), National Agriculture Policy (2004) and interim plan of livestock development (2010-2013) have guided the core objectives of Livestock Services. At present, there is a need to ensure the food and nutrition security by increasing the livestock production and productivity and also to establish the commercial livestock farming for generation of income to the rural people and to protect the environment and support the regional economic balance. Since around 70% of human diseases are originated from animal origin, the objective of DLS is also to ensure the safety of animal health, human health and environment by controlling devastating emerging zoonotic diseases. Department of Livestock Services should act to promote the export of livestock products and replacement of import through supplying the raw materials to the livestock

industry and accordingly there is a necessity to promote commercial livestock farming, resource centre development, conservation of genetic resources and management of market of livestock products. Promotion of self-employment and creating the opportunities of employment in livestock based industries will help in poverty reduction. Regulatory function of DLS for quality monitoring of livestock products and related industries to provide the quality products to the people is also one of the most important objectives of the department (DLS, 2008).

Present Situation of Livestock Development

The present situation of Livestock development has been presented in the tabular and graphical form which clearly indicates that there is large number of domestic animal in the country; however the number of productive animals is very less. Livestock population and its average annual growth rate during last 14 years have been presented into table no.1. It has also shown the percentage of improved animals and it clearly indicates that it is very less in case of milk and meat producing animals. Annual Growth rate of livestock products has been shown in figure no.1 and it can be observed that the growth rate is very less even negative rate in the case of wool production.

Table 1: Livestock Population and its growth rate in Nepal:

Livestock Species	Number (million)	Average annual Growth rate	Percentage of improved
Cattle	7.19	0.49	12
Buffalo	4.83	2.29	26
Sheep	0.80	-0.93	5
Goat	8.84	2.36	6
Pig	1.06	4.02	34
Poultry	25.76	4.94	55

Source: Average growth Rate is of 14 years (MOAC, 2007/08)

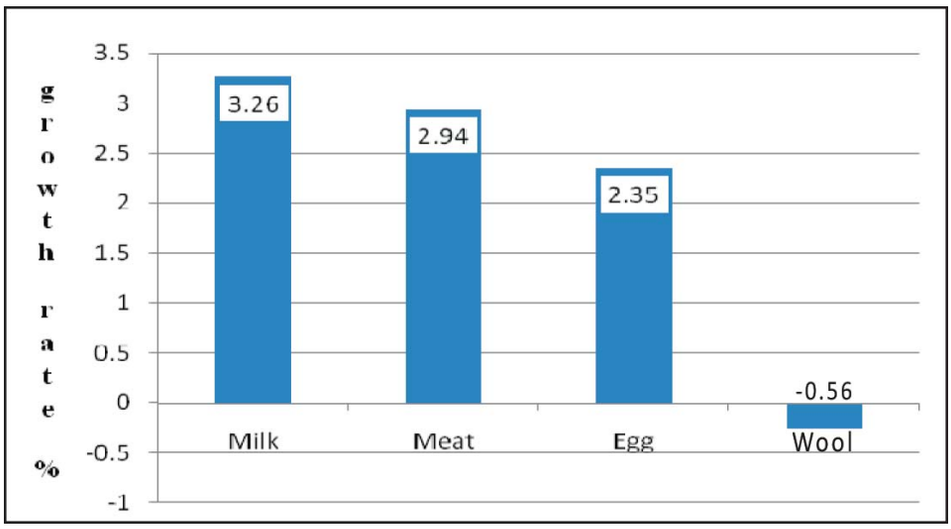

Figure 1: Annual Growth Rate of Livestock Products (MOAC, 2010/11)

Large number of livestock and their products are still being imported and large sum of money is being spent on import. The cost of meat animals imported has been presented in table no.2 and figure no.2. The cost of meat animal imported was as large as 781.5 million NRs. during 2006/07; however it is in decreasing trend afterwards.

Table 2: Trend of import of animals and animal products:

S.N.	Commodity	2004/05	2005/06	2006/07	2007/08	2008/09	2009/10	Percentage in change compare to last year
1.	Buffalo(no.)	135124	179111	203604	173160	35061	46208	31.79(+)
2.	Goat(no.)	270151	279101	275259	279098	333305	476623	42.99(+)
3.	Day old chicks (no.)	27916	97493	334764	440077	411286	787661	91.51(+)
4.	Fish (MT)	2547.38	2058.11	2261.23	2034.77	7033.37	4334.86	38.36(-)

Source: Annual Technical Report, Directorate of Animal Health, 2010.v

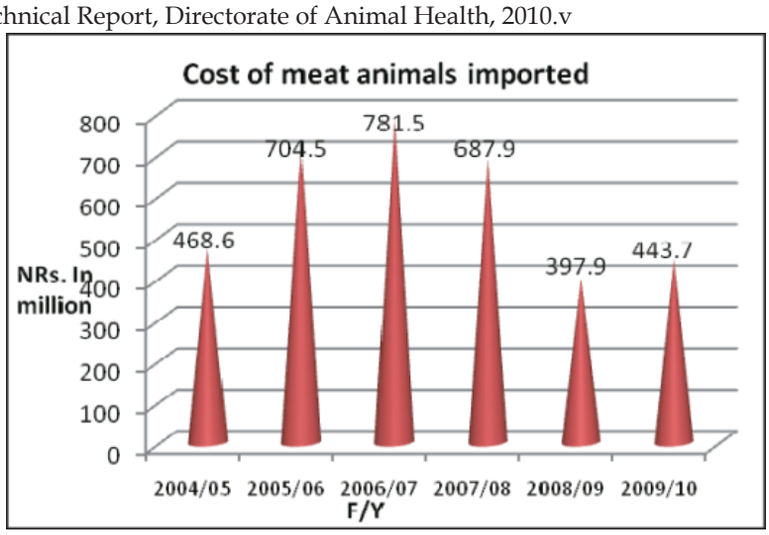

Figure 2: Estimated cost of meat animals imported annually
(Source: Annual Technical Report, Directorate of Animal Health, 2010)

Department of Livestock services have given more emphasis on the functioning of animal quarantine to prevent the entry of livestock diseases which in turn increase the production and productivity of livestock. Table no.3 shows the trend of quarantine activities. The increasing trend of vaccination is the positive signs of preventing the diseases.

Table.3: Trend of quarantine activities:

Fiscal year	Quarantine inspection(no.)	Quarantine checks(no.)	Sample collection(no.)	Vaccination (no.)
2005/06	35485	937	1150	279101
2006/07	37385	610	269	275259
2007/08	27433	813	716	279098
2008/09	29501	895	866	333305
2009/10	30920	467	866	476623

Source: Annual Technical Book, Directorate of Animal Health, 2010

The Situation of Animal Nutrition is poor. Availability of Livestock feed (DM basis) is 8.3 million MT which is 30% deficit than total requirement. Deficit in Forage Seed is 28.4%. About 60% of the livestock are reared only on maintenance ration. Budget allocation in feed is usually < 10% of total livestock allocated budget and Coverage of forage in total irrigated land is 0.20 %.(National Pasture and Animal Feed Centre, 2012)

Emerging Challenges and constraints for hindering the increment of livestock production and productivity

It has been estimated that the deficit of the milk to the dairy industry is around 500,000 litre per

day. The number of unproductive and low productive animals is enormous and their productivity is very less. The number of cattle and buffalo are 120 million among which 2.2 million are milking. Native cattle and buffalo produces 450 lit, and 900 lit of milk per day respectively. Buffalo is the most economically important livestock in Nepal but their number is declining as dairy buffaloes are slaughtered in many places. Genetic improvement of livestock is very limited and research is often not sufficient. Coverage of AI is just 9% whereas 91% of livestock is bred through natural services by bulls having unknown origin and merit. Un-systemic breeding of livestock is very common . Similarly, inbreeding is critical problem among indigenous breeds of animals. Resource centres for improved breeds of animals are very limited. Per capita annual requirement and supply of meat, milk and eggs are 14 kg meat, 57 lit. of milk 48 eggs and 9 kg. meat, 51 kg. milk and 23 eggs respectively (Nirmal, 2010).

The Government services are just limited around 20% of total livestock and very limited private services are accessible to farmers. There is always a threatening of infectious diseases to the livestock. Concrete policy of livestock insurance does not exist. Foot and mouth disease is quite prevalent throughout the country which is the major threatening to the productivity of livestock.

Around 33% of feed is deficit which in turn makes the buffaloes as seasonal breeders and milkers. Silage feeding and teat dipping are not practiced practically. Integrated program of animal health, breeding and nutrition do not reach to the farmers. Metabolic profiles of productive animals are not monitored. Nepal is quite vulnerable country to the effects of climate change. The implication / impact of climate change on livestock sector, accounts the overall effects on breeding, feeding, livestock diseases and health and other miscellaneous inputs.

There is ban on export of cattle and import of exotic and improved breeds are not possible due to policy of the neighbouring countries. Being the member of World Trade Organization, Nepal has to fulfil all the requirements of sanitary and phyto-sanitary agreement. Proper quality infrastructure systems have not yet been established. Capacity building opportunities of institutions and human resources are limited. Marketing of the live animals and livestock products are not yet fully systematic.

Processing and manufacturing of livestock products is not fully quality control/assurance based. Foot and Mouth disease (FMD) , Peste des petits ruminants (PPR), classical swine fever, Hemorrhagic Septicemia (HS) and Black Quarter (BQ) were found to be most frequently occurring five infectious diseases among top ten reported diseases (Khatiwada *et al.*, 2010). Emerging epidemics like HPAI has serious consequences to food security, food safety, public health and trade owing to huge socioeconomic importance.

Recent developments and efforts

To cope with the milk deficit, genetic improvement of indigenous cattle and buffalo is must. The implementation of Dairy Cattle and Buffalo Improvement Program (DCBIP) is based on Pedigree and Performance Recording System (PPRS) and intended with the objectives to select the sire and dam with top breeding values for breeding purpose. Around 850 farms, 6000 cattle and around 2500 buffaloes have been selected in twenty two districts of Nepal. DLS is attempting to establish resource centres of cattle and buffaloes at farmer level through implementation of 'extensive cattle and buffaloes farming" but there is no operational system. The program needs strong technical backstopping and record keeping, breed selection and artificial insemination.

Embryo transfer (ET) program has already been implemented once and the bulls from ET were procured to produce quality semen. National Livestock Breeding Centre (NLBC), Pokhara has well equipped laboratory for semen production. The semen production is increasing day by day. During 2010/11, the total number of semen produced is 250,496 which is almost 30% more than that of previous year. On the demand of farmers, semen has been collected from the elite bulls of Holstein Friesian (HF). This was largely required by the farmers of HF breeds, under intensive system of cattle rearing with good feeding and husbandry practices. Jersey appears to be more

appropriate in smallholder farmers in mid hills and high hill for upgrading native breeds of cattle (Paudel, 2010).

With the Program of Livestock Breed Improvement (PLBI), A.I. mission has been implemented into 25 districts from this year (2011/2012) to increase the coverage of A.I. through public private partnership by establishing community livestock breeding centre. Additional 250 Artificial insemination centre have been established to provide easy and accessible services to all farms.

In the country, some nucleus herds and their resource centres have been established and developed especially for goat, pig and poultry. Poultry is very much privatized in Nepal. More than 150 hatcheries and 300 feed industries are functioning all over the country.

A ten year (2010-2020) long term policy of Animal genetic Resource Conservation (AnGR) was made to implement strategically for the sustainable development and conservation of indigenous livestock of all species (Nirmal, 2010). The places have been identified and implemented to utilize the genetic resources. There is some effort for value addition in livestock products especially in cheese like high value products in tourist area.

Being the member country of OIE, promotion of quality veterinary services is done through government organizations; however privatization is adopted to provide good veterinary services to the farmers. The establishment of quarantine and their functioning is done to reduce the entry of the diseases at boarders and within country. Central veterinary laboratory, regional veterinary laboratories, avian laboratory, FMD and transboundary diseases control laboratories perform the vital role in diagnosing and controlling the diseases. The epidemiological and surveillance study is also done to prepare the contingency plan and control strategy for several zoonotic and infectious diseases of livestock.

In the recent years, more than 3000 meat stalls/ milk stalls have been established and improved hygienically through the public private partnership throughout the country. This has established a system and process to start new stall in the country for hygienic and healthy production of livestock products.

Certain pocket areas have been established to introduce the potential forage and fodder especially in some of the milk shed districts like Makwanpur, Chitwan, Nawalparasi, Ilam, and Rupendehi, Kaski , Tanhun, Palpa and other similar districts. Some of the measures have been taken to reduce the cost of production of livestock products through forage and fodder feeding. The best of agri-byproducts and residues were used in many districts of Nepal.

Table no.4 shows the target for major livestock products for three year interim plan (2010-2013). The target could be met if the stability of government, resource allocation and commitment of the concerned stakeholders remain intact.

Table 4: Three Year Plan Target for Major Livestock Products, (2010-2013, MoAC)

S.N.	Products	Unit	Base year	Target	Annual Growth %
1.	Milk	000 MT	1496	1774	5.85
	Cattle	000 MT	449	532	5.81
	Buffalo	000 MT	1047	1242	5.86
2.	Meat	000 MT	248	287	4.99
	Buffalo	000 MT	156	169	2.7
	Goat	000 MT	52	57	3.11
	Pig	000 MT	17	26	15.21
	Chicken	000 MT	23	35	15.02
3.	Eggs	Million	640	930	13.27

Opportunities and Potentialities

There is huge potentiality of livestock development in Nepal. It is a member country of WTO and has two big markets of livestock products as China and India. Due to geographical structure, large areas of mid hills, around 30% of forest and 10% of shrubs, there is large resources for livestock farming. Cost of production of livestock products can easily be reduced by low cost production technology. In recent years, large group of professionals and educated people have been attracted to livestock enterprise. The commercialization of livestock can give surplus products which lead to the promotion of export and replacement of imports supporting income generation and employment.

Nepal experiences a good system of Public Private Partnership. There is an ample opportunity of organic farming. Studies have shown that genetic productivity of indigenous livestock is quite encouraging. The country has suitable climate and environment for livestock farming.

The economy of the country is dependent on remittance especially in rural areas. Remittance can be utilized on livestock farming to raise the livelihood of rural people .There is good scope of utilization of micro-financing and sustainable return on cost benefit basis. Low productive lands, which is another potential resource, can easily be utilized for livestock farming.

RECOMMENDATIONS

All the policies of breeding, feeding, animal health, marketing and human resource development should be developed and strategies should be made accordingly. Planning should be done on the basis of such policies and strategies only. There should be concrete policy of livestock farming. Current act and regulations should be amended as per requirement. Long term policy for Animal Genetic Resources (AnGR) should be established for sustainable development, utilization and conservation.

Integrated animal health, breeding, feeding services and market management should be implemented and resources should be allocated on the basis of requirement, animal health-20%, Breeding-20%, Feeding and Nutrition-40%, capacity enhancement-10% and Market management-10%(remodelled from Chase, 1981).

To increase the milk production and productivity of indigenous animals, a campaign of widespread Artificial Insemination (A.I.) mission should be continued at least for 10 years for genetic improvement. The coverage of A.I. should be reached at least 40% and almost 1 million improved dairy cattle and 1 million buffaloes should be maintained in the country for sufficient supply of milk. The private sector should be involved for the management of A.I. program. Government should give subsidy to the milk cooperatives to establish Liquid Nitrogen plants. LN2 storage tanks and depots should be stalled in potential locations to facilitate timely and efficient supply of LN2. Production of frozen semen and AI in swine, Goat and Sheep should be implemented in potential areas and districts. AI Mission must be followed by Forage Mission.

A stock of frozen semen of proven genetic merits must be available to the farmers. Semen from at least four pedigree tested bulls must be available to the inseminators to prevent inbreeding. National Livestock Breeding Centre (NLBC) should increase number of bulls required for semen collection. As NLBC is collecting semen from 28 cattle and 12 buffaloes at present, it must purchase more bulls to make this numbers four times of the present animals (160 bulls) (Paudel et.al., 2010). Inbreeding should be prevented through regular replacement and exchange of old bulls in all districts. A widespread awareness program should be initiated for the control of inbreeding with proper strategy.

Research results recommend that an exotic blood level of 50-75% should be maintained for dairy cattle. Semen from HF bull of 75% exotic blood level will be appropriate to inseminate a HF cross

bred cows of 50% exotic level to achieve 62.5% progeny. Genetic characterization, DNA analysis and phenotypic analysis of all Nepalese breeds should be done and and their economic traits should properly be defined. A well-established gene bank is required. Embryo transfer technology and sexed semen production technology should immediately be introduced. Elite herds of cattle and buffaloes should be established from private sector which might need additional technical support from the government.

For breed security of different livestock, region wise resource centres of dairy breeds of livestock should be maintained on the basis of potentiality. Nucleus herd should be maintained. All the government farms should run outreach program for establishment of resource centres (DOLP, 2011). At least 1000 female cross bred animals and 5000 meat animals should be maintained as cross bred resource centre through public private partnership.

Since there is trend of slaughtering buffaloes, culling the calves, it should immediately be monitored and calf rearing scheme should be implemented. Cattle and buffalo rearing centre and male buffalo calf fattening centre should be established in potential areas. There is an immediate need of amendment of the law to export / import of live cattle helping to cull less productive animals of both sexes including inferior male calves.

Forage and fodder, pasture and range land development is the only means to produce least cost animal products to enter into competitive world market. Forage mission and pasture and range land management is the immediate demand of livestock development in Nepal. Seed processing, grading, quality testing, packaging and labeling should be done with certain standard and truthful level. For quality seed of forage and grasses, contract farming should be done in government farms (DOLP, 2011). There should be animal feed balance sheet for all districts.

The productivity of dairy cattle/buffaloes should be maintained at the production level of 15-20 kg/day and 5000-6000 kg/ lactation. Possibility of generating Nepalese Jersey and Nepalese HF which can be maintained on fodder and forage should be materialized. Agriculture byproducts should efficiently be utilized as livestock feed. It can be enriched through fortification. Pure Jersey bull semen is appropriate for upgrading native cattle so that 50% blood level is achieved in F1 generation (Paudel, 2010). Feeding of total mixed ration should be practiced in potential areas of milk production. Teat dipping, silage preparation, milk replacer, urea molasses and mineral block, total mixed ration (TMR) etc. are necessary practices for enhancement of production and productivity. Cooperatives should be developed as centre for service delivery (extension and micro financing). Fodder can massively be produced on contract by 'land surplus' farmers. Enriched Straw Pellets and Urea treatment of straws can reduce the cost of milk and meat production. Biological treatment of straw and rumen by-pass protein are the source sof supplementation of protein in feed to high producing animals.

High value products should be produced by value addition from indigenous livestock products. These products should be developed as mitigation tools of of climate change effects and promoter agro-tourism. There is an urgent need to expand and strengthen market infrastructure and market information system on public private partnership model.

A coordinated approach should be applied for extension services. Department of Livestock Services (DLS), Nepal Agriculture Research Council (NARC) and academic institutions should work together on the basis of farmers' demand. A joint field visit or campaign and farmer's field school could be a good means to work together. The functional linkages can be strengthened among research, extension and education.

Periodic health monitoring and metabolic profile estimation should be done for all breeding dams and sires. This can be maintained through livestock insurance system. Teat dipping must be done in all commercial farms.

Developing and implementing Village cattle Upgrading Breed Model (VCUB) should be performed

for two generations. All cattle in the village will be upgraded by supplying the adequate number of either jersey or HF bulls to produce cross bred cattle by castrating all of the young males of the village in inaccessible areas with AI helping in supplying cross heifers to supply milk to the dairy industries or to a school milk program in remote areas (Shrestha, 2010).With the strategies to increase Livestock Gross Domestic Production (LGDP), livestock commercialization and diversification can be done by one village one product (OVOP).

"Livestock- business parks" should be developed on corridor basis for market, technology and information and communication. Food safety and quality management system can be applied from farm to industry to retails. Improvement of meat and milk stalls for hygienic and healthy production is very important to supply the quality animal products with the concept of hygiene from farm to fork. Cooperatives should be developed as centre for service delivery.

National veterinary services should be functional as per OIE guidelines. The structural limitation of the Department of Livestock Services is one of the hurdles for livestock development. So, restructuring of Department of Livestock Services and the national veterinary services as per OIE guidelines is necessary to obtain positive results. Strengthening service delivery such as extension, regulatory and quality control, animal quarantine and diagnostic and analytical services is must. Risk analysis should be done to know the possible threats due to particular diseases. Detailed economic analysis of the livestock diseases should also be done by multi-disciplinary team.

CONCLUSION

Adoption of technologies like improved breeds, shading, sprinkling, increasing air circulation, improved management practices (stocking rates, rotational grazing, improved pasture species) and efficient use of improved resource (land, water and feed) are expected to help to minimize harmful impacts of livestock keeping. Other risk management measures could be weather based insurance schemes, climate information system (early warning), risk fund schemes and feed storage. At the same time, changes of production systems e.g. breed selection for greater tolerance and increased productivity, intensification of livestock production, substitution of livestock species (draught resistant, monogastric for ruminants).

Livestock productivity can be enhanced by feed management, breed improvement, shed improvement, commercial farm establishment, strengthening of private / AI services, expansion of animal health coverage, management of market through Public Private Partnership.

Coordination among Research, Extension and Education is necessary for over all development in the livestock sector. Privatization of service providers is required on mutual benefit sharing. Coopeatives can be the best choice for collective services in more efficient and economic manner. Facilities for information and communication and capacity enhancement are must for rapid livestock development.

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RUMINANT HEALTH AND PRODUCTION

CONCEPTION RATE IN REPEAT BREEDING BUFFALOES USING HORMONE GnRH AND MINERAL-VITAMIN MIXTURES UNDER FARMERS MANAGED CONDITION IN CHITWAN, NEPAL

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ABSTRACT

A study was conducted in chitwan, nepal from 15 august, 2010 to 15 august, 2011 to evaluate the conception rate in repeat breeding buffaloes using hormone gnrh (gonadotropin releasing hormone) and mineral-vitamin mixtures supplementation. Fifty-two repeat breeding buffaloes selected at random from different individual farmers were divided into four treatment groups t1, t2, t3 and t4 each having 15, 15, 12 and 10 animals respectively. Group t1 was administered with hormone gnrh only, t2 with mineral-vitamin mixtures supplementation, t3 in combination with gnrh and mineral-vitamin mixtures and t4 as control with existing normal feeding. Per rectum pregnancy diagnosis was performed every sixty days later after each insemination. Significant conception rate of 53.34%, 66.67% 75.00% and 10.00% in t1, t2, t3 and t4 respectively was observed. Number of conception achieved within two months and four months differed significantly ($p<0.05$) between the treatment groups respectively. The highest conception rate of 58.34% was observed in t3 within two months indicating better and quick response to conception whereas the highest conception rate of 46.67% was observed in t2 by four months indicating better conception result but with late response. Hence, results concluded that the use of hormone gnrh in combination with mineral-vitamin mixtures had better response to conception rate in repeat breeding buffaloes.

Keywords: repeat breeding, conception rate, GnRH, mineral-vitamin

INTRODUCTION

Buffaloes play a prominent role in overall social developments by maintaining sustainable food producing system and power for agricultural operations in developing countries having 60-80% of the agrarian population (Nanda & Nakao, 2003). Although, buffalo contributes about 70% of the total milk and 64% of total meat production, Nepal imports 11,674 live buffaloes at the cost of NRs. 30,644,813 from India for various purposes to meet the consumers' demand (MoAC, 2008/09). To be self-reliant in this commodity it is necessary to increase the number of buffaloes together with their improvement in production level which could be aided by the approach of infertility management. Improving the productivity of buffalo requires an understanding of their potential and limitations under each farming system, development of simple intervention strategies to ameliorate deficiencies in management, nutrition and healthcare (Perera, 2008). Among various reproductive disorders like repeat breeding, anoestrus, genital prolapse, dystocia, abortion, uterine torsion, one of the major causes to economic losses is repeat breeding and seems to be important reproductive problem (Rabbani, Ahmad, Lodhi, Ahmad, & Muhammad, 2010). The repeat breeding cow is one that has

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clinically normal reproductive tract with normal or nearly normal oestrous cycles and oestrus periods and has been bred two or more times to a fertile bull but failed to conceive (Roberts, 1971). Hence, so far the problem of repeat breeding persists in considerable number; annual birth rate cannot be improved and overall production will remain below the optimum. In Nepal, very few researches have been conducted in the past in repeat breeding buffaloes, so in the lack of enough scientific information no specific guidelines have been developed to combat the problem of the repeat breeding buffaloes. Hence this study tries to develop the valuable guidelines regarding the management for the repeat breeding buffaloes by increasing its conception rate through hormone GnRH (Gonadotropin Releasing Hormone) and mineral-vitamin mixtures supplementation.

MATERIALS AND METHODS

Study was carried out in Chitwan District, Nepal under farmers managed condition, from August 15, 2010 to August 15, 2011. Three infertility camps were conducted in different VDCs (Bhandara, Chainpur and Shivanagar) of Chitwan. Besides these camps, individual farmer contacts and farmers seeking services for infertility management in buffaloes from their contact dairy cooperatives around the area of three more VDCs (Mangalpur, Shardanagar and Gunjanagar) were also included in the study.

With breeding history of buffaloes taken from the farmer and per rectum palpation fifty-two repeat breeding buffaloes of non-infectious cause were segregated from individual farmers to conduct treatment trial. Selected animals were divided at random into four treatment group T1, T2, T3 and T4. Treatment T1 was provided with hormone GnRH, T2 with mineral-vitamin mixtures, T3 with both hormone GnRH and mineral-vitamin mixtures and T4 as control, simply kept under observation with its existing normal feeding. GnRH (Receptal® Intervet India Pvt Ltd. Pune, India) @ 2.5 ml was administered intramuscularly once to the treatments T1 and T3 irrespective of the presence of corpus leutem or dominant follicle in the ovary. Mineral-vitamin mixtures (Chelated Agrimin Forte Powder®, Virbac Animal Health Pvt Ltd., Mumbai, India) one kg containing Vit A 700, 000 IU, D3 70, 000 IU, E 250 mg, nicotinamide 1000 mg, cobalt (Co) 150 mg, Cupper (Cu) 1200 mg, iodine (I) 325 mg, iron (Fe) 1500 mg, magnesium (Mg) 6000 mg, manganese (Mn) 1500 mg, potassium (K) 100 mg, sodium (Na) 5.9 mg, zinc (Zn) 6900 mg, phosphorus 12.75%, selenium (Se) 10 mg, sulphur (S) 0.72%, and calcium (Ca) 25.0% was given orally @ 50 gm per day per head to the treatments T2 and T3. Control group T4 were kept under supervision till two more matings simply under existing farmers' feeding management. Before trial the entire animal under study was dewormed with albendazole 1500 mg (Albomar® Virbac Animal Health Pvt Ltd., Mumbai, India) @ 10 mg/kg body weight so as to rule out the repeat breeding animal of parasitic issue. Upon heat detection after treatment application animals were naturally and artificially inseminated to those which were previously inseminated naturally and artificially respectively with consideration of the same quality of semen. After two months following the every insemination pregnancy diagnosis was done per rectum. Number of animals that got conceived was recorded for every treatment.

For statistical analysis SPSS 16.0 software (SPSS Inc. Chicago, USA) was used for cross tabulation among the percentage of conception and various treatment group. Fisher's Exact test (Snedecor & Cochran, 1989) was applied to see any association between conception rate and treatment effect with respect to type of insemination and time to achieve conception. For all the analysis level of significance was set at probability $P< 0.05$.

RESULTS AND DISCUSSION

Overall conception rate

Overall conception rate in repeat breeding buffaloes using hormone GnRH and mineral-mixtures under farmers managed condition in Chitwan 2010/2011 is presented in Figure 1. Out of 52 repeat breeders, overall 28 (53.84%) were pregnant within four months with different treatment groups with varying conception rate in different treatments T1 8/15 (53.34%), T2 10/15(66.67%), T3 9/12 (75.00%) and T4 1/10 (10.00%) as shown in Figure 1. Observed percentage of conception among various treatments was statistically significant at $p<0.05$.

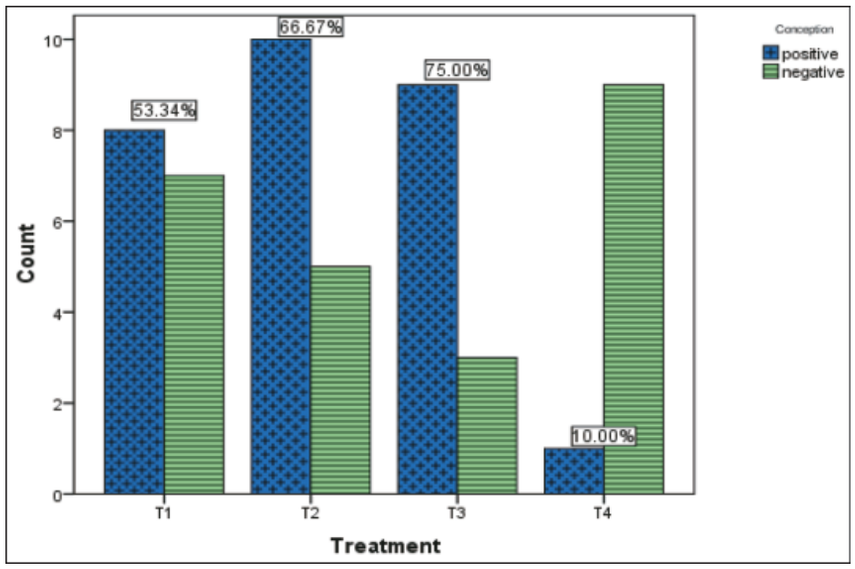

Figure 1. Overall conception rate in repeat breeding buffaloes using hormone GnRH and mineral-mixtures under farmers managed condition in Chitwan 2010/2011

Conception rate of 53.34% in T1 (hormone GnRH only) as presented in Figure 1 crossponds to 54% in repeat breeder cows (Stevenson, Frantz & Call,1988). However, slightly less 42.8% conception was found in Nilli-Ravi buffaloes (Ahamd, Saeed, & Bashir, 2002) and slightly high 68.75% conception was observed at P<0.05 (Anjum, Usmani, Tunio, & Abro, 2009) and 61.54% by (Ahmed, El-khadrawy, Emtenan, Amal, & Shalaby, 2010). However, in contrast overall conception was 100% within 2 months in the treatment with GnRH and PGF2α in buffalo cows and heifer buffalo (Sah & Nakao, 2006). This high conception rate in contrast to this study may be due to the selective administration GnRH to the repeat breeders having only dominant follicle but not CL where as in this study there was random use of GnRH hormone irrespective of dominant follicle and CL in ovary. A smaller population of antral follicles or an abnormal endocrine status during folliculogenesis is major cause of repeat breeding (Maurer & Echternkamp, 1985) and GnRH influences FSH and LH from anterior pituitary and causes pre-ovulatory surge to cause ovulation from the dominant follicle (Janakiraman, 1988). Hence the hormone GnRH can alleviate the conception in repeat breeding by maintaining and synchronizing the hormonal status in repeat breeders.

In mineral mixture supplemented group (T2), conception rate was found to be 10/15 (66.67%) as in the Figure 1, was in accordance with the result of 63.64% (Ahmed, El-khadrawy, Emtenan, Amal, & Shalaby, 2010) but Sah and Nakao (2006) showed conception of 58.4% in buffaloes cows and 50.0% in heifers within one month where as 100% conception after 6 months in heifers as well as buffalo cows. For optimum reproductive performances minerals play important role in several biological process and some of them are components of hormones which directly regulate endocrine activities (Kumar, Pandey, Razzaque, & Dwivedi, 2011). Das, Dutta, Deka, Biswas, Sarmah & Dhali (2009) has proved that Ca, P, and Zn play crucial role for the normal ovulatory process and Mg and Mn below optimum serum level causes anovulation in crossbred cows. Besides, vitamin A deficiency also has been reported as cause of repeat breeding and feeding β-carotene to repeat breeders found improved conception rate (Celik, Avci, Aydin, Bulbul, & Bulbul, 2009). Similarly, Vitamin A and Selenium has proved improved conception rate in repeat breeding animals (Qureshi, Siddiq, Lodhi, Muhammad & Jamil, 2010). Therefore, it can be concluded that mineral-vitamin mixtures supplementation improves the conception rate in repeat breeding buffaloes.

The highest conception rate 9/12 (75%) was found with T3 treated with hormone GnRH and mineral-vitamin mixtures (Figure 1). This high conception rate with respect to the other treatment groups may be due to repeat breeding being multi-factorial cause involving number of extrinsic factors as well as intrinsic factors coupled to the individual animal (Gustafsson & Emanuelson,

2002) and also Singh, Dadarwal, Honparkhe, & Kumar (2009) concluded that hormonal aberrations and its combination with others factors constitute major causes of repeat breeding. Hence hormonal therapy together with mineral-vitamin mixtures in this group might have played the significant role to improve the conception in repeat breeding buffaloes with respect to other treatment groups.

Lowest conception rate 1/10 (10.00%) was observed under control groups T4. This result may happen due to the fact that an animal was repeated simply due to the faulty or poor timing of mating (Nakao, 1988). Due to the lack of knowledge farmers in Nepal sometimes mate the buffalo forcibly thinking animals to be in estrus even when buffalo did not accept the bull (Sah & Nakao, 2006) which gives false inference regarding repeat breeding.

Month-wise conception rate

Month-wise conception rate in repeat breeding buffaloes using hormone GnRH and mineral-vitamin mixtures under farmers managed condition in Chitwan 2010/11 is presented in Figure 2. Conception achieved within two months and four months varied significantly ($P<0.05$) between the treatment groups respectively. But, within the treatments in T1 frequency of conception achieved within two months and four months was equal 4/15 (26.67%) each. However in T2 more number of conception 7/15 (46.67%) was achieved in four months which was lower than T1 4/15 (26.67%) as shown in Figure 2. This indicated that although better response was given by T2 as compared to T1 but success in achieving conception was time taking. This is in accordance with Ahmed, El-khadrawy, Emtenan, Amal, & Shalaby (2010) who reported high pregnancy rate within two months with mineral mixtures treated groups as compared to GnRH. Later response may be due to the more number of services required for the conception in repeat breeders (Yusuf, Nakao, Ranasinghe, Gautam, & Long, 2010). Again with T3 the highest conception rate 7/12 (58.34%) was achieved only in two months and only 2/12 (16.67%) in four months. Hence all these data reflects that better result of conception rate with mineral-vitamin mixtures in combination with hormone GnRH together with quick response than other groups.

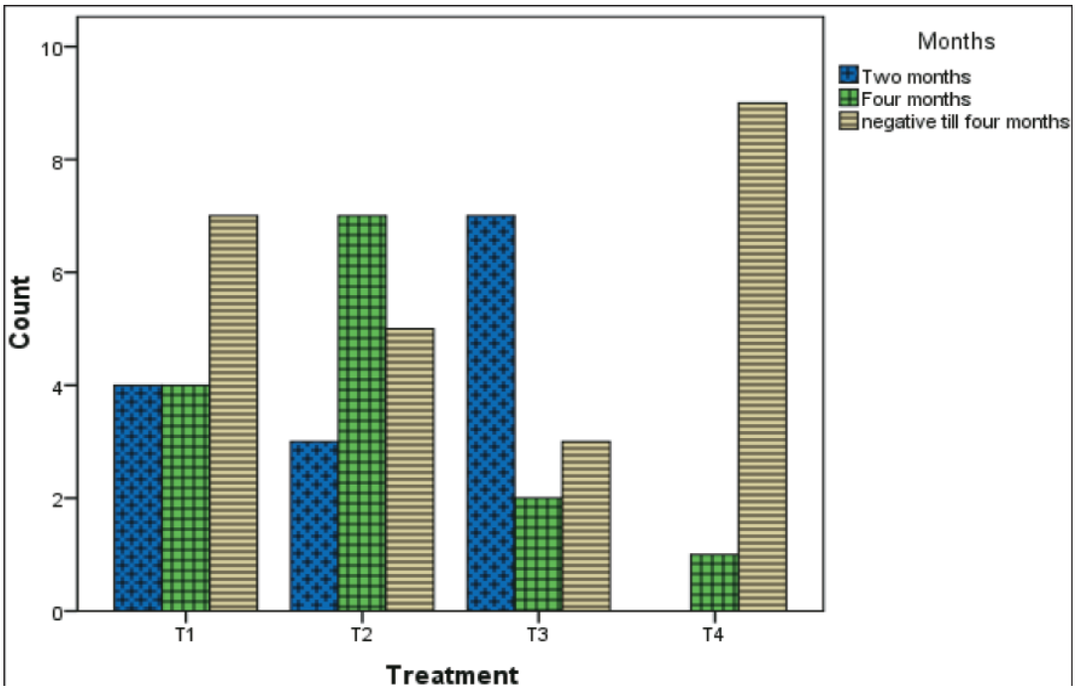

Figure 2. Monthwise conception of repeat breeding buffaloes using hormone GnRH and mineral-vitamin mixtures under farmers managed condition in Chitwan 2010/11

Better and quick response of conception rate within two months with GnRH and mineral-vitamin mixtures treated groups may be because it is suggested that GnRH together with minerals such as Cu and Zn are required to maintain the level of progesterone and hence pregnancy (Suzuki *et al.*, 1999).

Type of insemination and conception rate

Conception rate in naturally inseminated (NI) and artificially inseminated (AI) repeat breeding buffaloes using hormone GnRH and mineral-vitamin mixtures under farmers managed condition in Chitwan during 2010/11 is presented in Table 1. The result of artificially inseminated animals differed significantly (P<0.05) among treatments while remained insignificant in naturally inseminated. The result 65.00% overall conception rate as shown in Table 1 in artificially inseminated repeat breeders is more or less similar with the findings 60.00% (Singh, Saravia, Bage & Rodriguez-Martinez, 2005). In addition he suggested that conception rate may be improved in repeat breeding by frequent AI in relation to spontaneous ovulation. Hence results also conclude that improvement in overall conception rate in naturally inseminated is poor 15/32 (46.87%) in comparison to artificially inseminated (65.00%). Also control group T4 was found with conception of 1/7 (14%) in naturally inseminated (Table 1) which might have happen due to the faulty inseminations or poor timing of mating before (Nakao, 1988).

Table 1: Conception rate in naturally inseminated (NI) and artificially inseminated (AI) repeat breeding buffaloes using vitamin-mineral mixtures and hormone GnRH under farmers managed condition in Chitwan during 2010/11

Treatment groups	Conception	
T1 (GnRH)	5/9 (55.56%)	3/6 (50.00%)
T2 (Mineral-vitamin mixtures)	4/8 (50.00%)	6/7 (85.71%)
T3 (GnRH + mineral-vitamin mixtures)	5/8 (62.50%)	4/4 (100%)
T4 (Control)	1/7 (14.28%)	0/3 (0.00%)
Total	15/32 (46.87%)	13/20 (65.00%)

Values in parentheses show conception percentage. P<0.05 differ significantly between treatments. NI=Natural insemination, AI=Artificial insemination

CONCLUSION

On the basis of this study it could be concluded that repeat breeding buffaloes responded quickly and excellently to hormone GnRH when mineral-vitamin mixtures also supplemented to it with better conception rate in artificially inseminated than naturally inseminated. Also, mineral-vitamin mixtures supplemented alone responded well, but had late response than with hormone GnRH in repeat breeding buffaloes.

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ENTERIC METHANOGENESIS IN RUMINANTS AS A MAJOR CONCERN IN GLOBAL CLIMATE CHANGE ISSUE AND MITIGATION STRATEGIES TO REDUCE THE EMISSIONS

Y.S.Bajagai[1]

ABSTRACT

Ruminants produce methane as a by-product of fermentative reaction which is one of the major constituents in the pool of anthropogenic green house gases in the atmosphere contributing to global warming. Enteric methane emission from domestic ruminants is the important anthropogenic sources of methane which contribute approximately 23% (81 Tg of CH_4) of the total global anthropogenic annual methane production and this is the second largest (fossil fuel is the first) source of anthropogenic methane emission. In Nepal, total annual emission of enteric methane from domestic ruminants is roughly 522 Gg in which share of buffalo, cattle, goat and sheep are roughly 52%, 39%, 8% and 1% respectively. Enteric methanogenesis is increasingly drawing attention due to 25 times higher global warming potential (GWP) of methane as compared to CO_2. In addition, production of methane by ruminants causes a significant amount of feed energy loss which could be used for animal growth and production if methane production is prevented. In addition, reduction in GHGs emission from ruminant animals can be done fairly quicker than from other sectors requiring less effort and financial investment making the strategy more feasible. Therefore, reduction of methane production in the rumen has been a major goal of animal nutritionists and microbiologists since long. There are several options studied to inhibit CH_4 production in ruminants. Direct inhibition of archaeal methanogens, manipulation of ruminal fermentation to reduce H_2 (principle metabolite involved in CH_4 production) production, use of alternative H_2 sinks and oxidation of methane by using methane oxidizing bacteria (MOB) or methanotrophs are some of the major strategies studied so far to reduce the emission of CH_4 from ruminants.

INRODUCTION

Global climate change

Any alternation in the climate over time caused either by human induced activities or natural phenomenon has been defined as climate change (IPCC, 2007b). Global warming of the atmospheric temperature due to anthropogenic emission and accumulation of greenhouse gases (CO_2, CH_4, N_2O etc.) is one of the most debated and prominent results of the climate change (IPCC, 2007b; World Bank, 2010) resulting widespread and long lasting effects in ecosystem, agriculture, health, soil, water resources etc. (IPCC, 2007a, 2007b). Rise in global atmospheric temperature, rise in sea level and melting of glaciers in snow zone of the earth are major mega fingerprints of global climate change (IPCC, 2007a) (figure 1).

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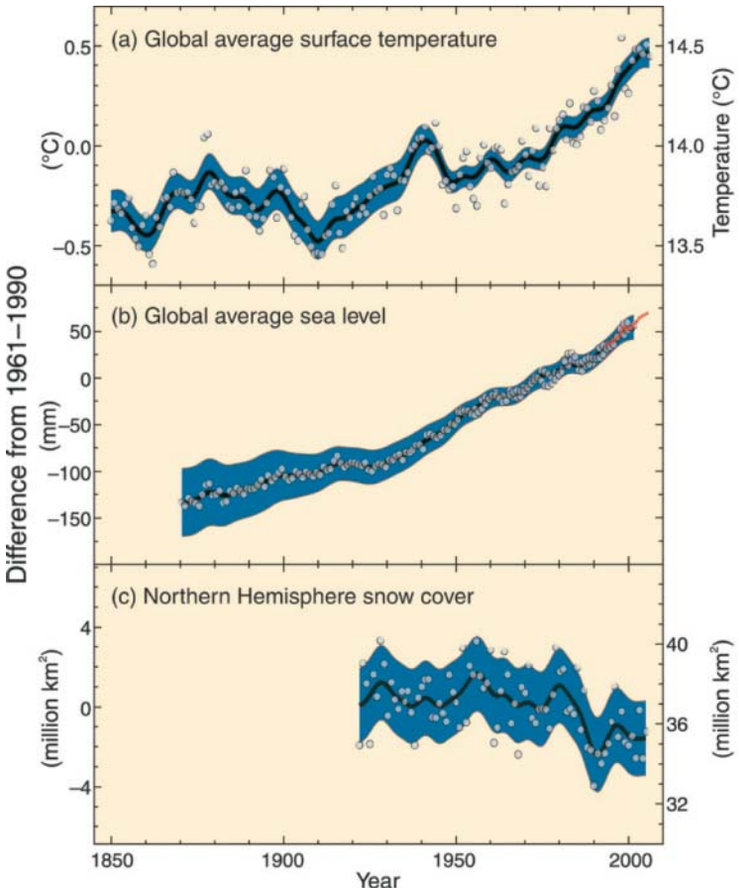

Figure 1: Major impacts of global climate change. Reproduced from IPPC (2007a).

Continuation of present rate of rise in global temperature may cause extended and widespread societal and environmental disruptions which could be beyond the bearable limit of present-day societies (Richardson, Steffen, Schellnhuber, Alcamo, & Barker, 2009) and therefore has attracted the attentions of scientists, policy makers and governmental authorities towards this prominent issue (Grubb, Vrolijk, & Brack, 1999).

Role of methane (CH_4) in climate change

Methane is one of the major greenhouse gases contributing to global warming. Although proportion of methane in atmosphere is very low as compared to CO_2, relative contribution of CH_4 for global warming is high due to high radiative forcing contributed by this gas (Solomon *et al.*, 2007). Agriculture is the predominant source of methane and ruminants emit 91 Tg of CH_4 per year to the atmosphere which is 26% of total methane production by anthropogenic sources (Denman, Brasseur, Chidthaisong, Ciais, Cox, Dickinson, Hauglustaine, Heinze, Holland, Jacob, Lohmann, Ramachandran, da Silva Dias, Wofsy, *et al.*, 2007).

The global atmospheric concentration of methane reached 1774 ppb in 2005 compared to 1732 ppb in the early 1990s and only 715 ppb in the pre-industrial era (figure 2) (IPCC, 2007b). This increase in atmospheric concentration of CH4 is responsible for radiative forcing of +0.48 ± 0.05 W m–2 which is second only to that contributed by CO_2 due to CH_4 having a 25 times higher global warming potential (GWP) compared to CO_2 (Solomon, *et al.*, 2007). Therefore, reduction in CH_4 emission is more effective and probably an easier strategy than reducing CO_2 production (Hogan, Hoffman, & Thompson, 1991).

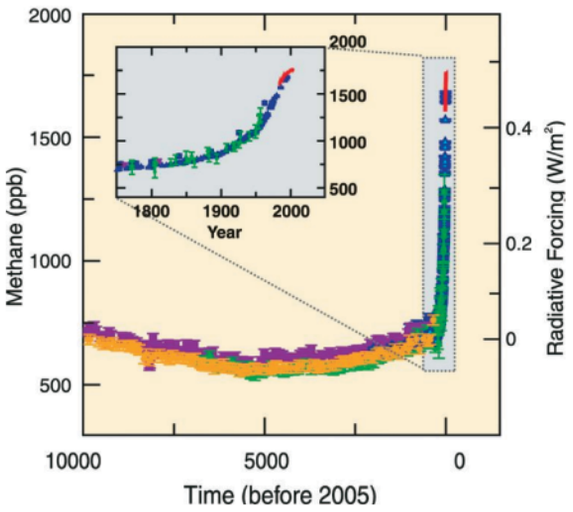

Figure 2: Trend in the increase in atmospheric concentration of CH_4 over the last 10,000 years and since 1750 (inset) with corresponding radiative forcing. Reproduced from IPCC (2007b).

In addition, production of methane by ruminants causes significant amount of energy loss (K. A. Johnson & D. E. Johnson, 1995) which can be used for animal growth and production if methane production is prevented. Thus, reduction in methane emission from ruminants has twofold benefits. Firstly, it will help to reduce the global warming due to greenhouse gases and secondly, it reduces energy loss and growth and productivity of ruminant animals will be increases with the same amount of energy supplied.

METHANOGENESIS IN RUMINANTS AND ITS CONTRIBUTION IN GLOBAL WARMING

Enteric Methanogenesis

Even though none of the mammalian animals has enzymes to digest complex polysaccharides in plant cell wall (cellulose, hemicelluloses and pectin), ruminant animals can utilize these fibrous materials due to degradation of these substances by microorganisms in the forestomachs. Digestion of plant polysaccharides in animal diet is possible due to anaerobic biodegradation of these compounds by Ruminococcos, Fibrobacter, Butyrovibrio and other microorganisms (bacteria and fungi) in the gastrointestinal tract leading to the formation of respective monomers from complex polysaccharides. These monomers (especially hexose) are fermented by microorganisms through glycolysis into pyruvate which ultimately converted into volatile fatty acids mainly propionate, acetate and butyrate (figure 4). Structural and cellular carbohydrates in plant cell are major substrates for microbial fermentation leading to methane production (Prins, 1979).

Large number of bacteria, protozoa, and fungi live in close association in rumen giving final product of rumen digestion as volatile fatty acids (VFAs), CO_2 and H_2. Carbohydrate fermenting bacteria do not produce methane but they do produce H_2, CO_2, and formate as substrate to produce CH_4 by methanogens (Wolin, Miller, & Stewart, 1997). Hydrogen is formed when pyruvate is converted into acetyl-CoA as an intermediate to produce VFAs (Prins, 1979).

Large proportion of rumen methanogens are in symbiosis relation with ciliated protozoa both inside the cell and attached on the surface of the protozoa as endosymbiont and ectosymbiont respectively (B. J. Finlay *et al.*, 1994; Tokura, Ushida, Miyazaki, & Kojima, 1997; Vogels, Hoppe, & Stumm, 1980; Williams & Coleman, 1997). More than one third of ruminal methane is produced by the methanogens associated with protozoa (B. J. Finlay, *et al.*, 1994) before converting into methane (Wolin, *et al.*, 1997).

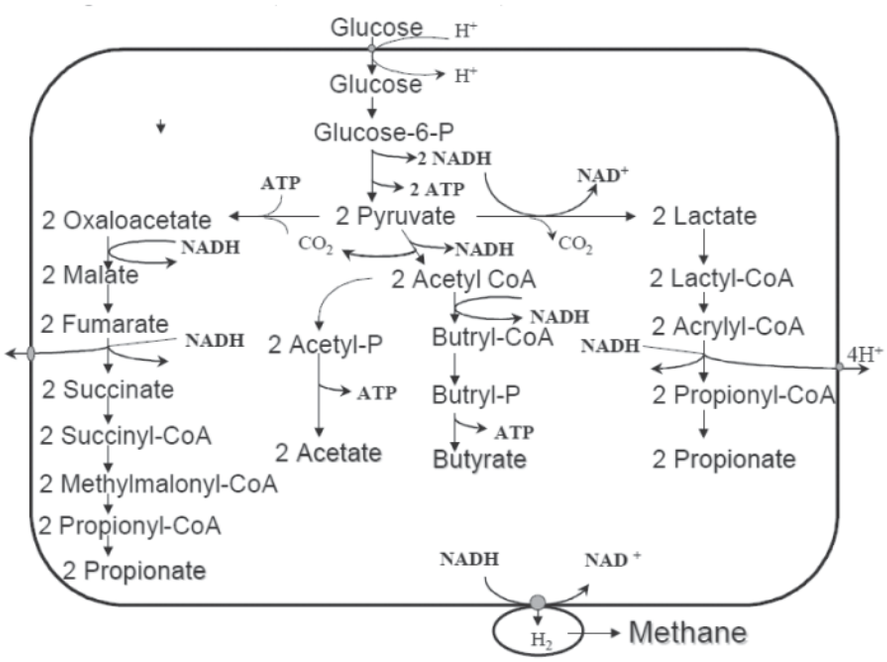

Figure 3: Microbial fermentation reaction in rumen generating and consuming hydrogen. Reproduced from Nolan (1999)

Most of the rumen microbes oxidize sugars with reduction of NAD to NADH through Embden-Meyerhof-Parnas pathway (Miller, 1994; Moss, Jouany, & Newbold, 2000; Wolin, *et al.*, 1997). NADH produced during oxidation of sugars must be re-oxidized to NAD for regular fermentative digestion in rumen (Miller, 1994). NADH is re-oxidized to NAD and H_2 only if the partial pressure of H_2 in rumen is remained below 1kpa by continuously removing H_2 gas from the rumen lumen (Miller, 1994). Methanogens carry out this function of H_2 removal by producing methane from H_2 and CO_2 to ensure continuation of fermentation reaction normally (Miller, 1994). Acetogenic bacteria (acetogens) in the rumen also utilizes H2 but cannot outcompete methanogens and these acetogenic bacteria are studied as an possibility to be exploited as an alternative hydrogen sink in the rumen (Chaucheyras, Fonty, Bertin, & Gouet, 1995; Joblin, 1999; Lopez, McIntosh, Wallace, & Newbold, 1999). Fast turnover of the rumen do not allow other substrate than H2, CO_2, and formate to be used by archaea to produce methane (Wolin, *et al.*, 1997). Formate is also broken down into H_2 and CO_2 .

In the absence of H_2 consumer (e.g. methanogens) the fermentative reactions leads to unusual end products (Miller, 1994). For example, in the absence of methanogens (H_2 consumer) in the rumen *Ruminococcus albus* would produce ethanol instead of acetate, *R. flavefaciens* would produce succinate instead of acetate and *Selenomonas ruminantium* would produce propionate instead of acetate (Miller, 1994). Proportion of different VFAs influence the amount of H2 produced in the rumen (Moss, *et al.*, 2000). Methane production is directly proportional to acetate and inversely related with propionate (Moss, *et al.*, 2000).

Contribution of ruminants to total anthropogenic green house gases in atmosphere

Emissions of methane have increased by 40% since 1970 with the largest contribution from the agriculture sector (Barker *et al.*, 2007). Domestic ruminant animals are one of the important anthropogenic sources of methane which contribute approximately 23% (81 Tg of CH4) of the total anthropogenic annual methane production (Figure 4) (Wuebbles & Hayhoe, 2002), and this is the second largest (fossil fuel is the first) source of anthropogenic methane production (Hogan, *et al.*, 1991; Wuebbles & Hayhoe, 2002). This value is found to be different in different papers (Denman, Brasseur, Chidthaisong, Ciais, Cox, Dickinson, Hauglustaine, Heinze, Holland, Jacob, Lohmann,

Ramachandran, da Silva Dias, S.C., *et al*., 2007; Reay, Smith, & Hewitt, 2007; Wuebbles & Hayhoe, 2002). The emission rate may vary year by year or the variation in results may be attributable to different methods used to calculate the emissions (Thornton & Gerber, 2010). Approximately two thirds of total methane production by domestic ruminants is contributed by cattle and the rest is shared by other domestic ruminants like buffalo, sheep, goats etc. (Crutzen, Aselmann, & Seiler, 1986; K. Johnson & D. Johnson, 1995).

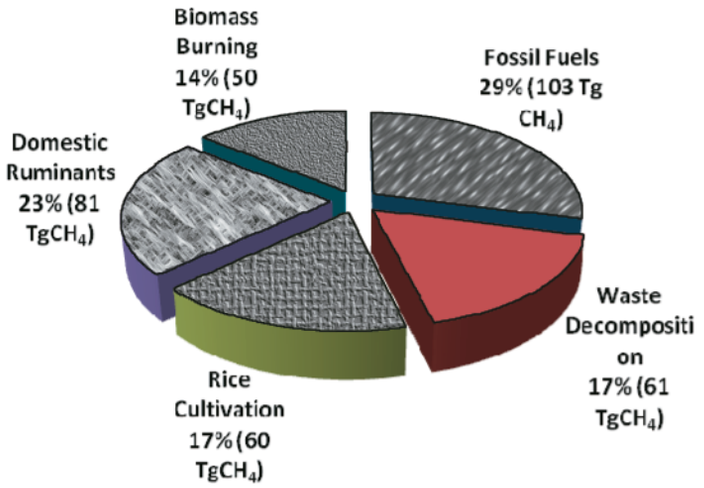

Figure 4: Contribution of individual sources to total anthropogenic annual methane production. Source: Wuebbles & Hayhoe (2002)

Several factors influence the enteric methane emissions from ruminants. Daily dry matter intake, digestibility of the feed, amount of fibres and soluble carbohydrate in diet, type of volatile fatty acids (VFAs) produced during fermentation (acetate: propionate ratio) etc, effect the amount of enteric methane production (Hart, Martin, Foley, Kenny, & Boland, 2009; Hindrichsen, Wettstein, Machmüller, & Kreuzer, 2006; Moss, *et al*., 2000). Similarly, animal species, breed and composition of the microbial population in the rumen and rumen pH also affect methane production (R. S. Hegarty, Goopy, Herd, & McCorkell, 2007; K. Johnson & D. Johnson, 1995; Lana, Russell, & Van Amburgh, 1998; Sejian, Lal, Lakritz, & Ezeji, 2010).

ENTERIC METHANE PRODUCTION FROM DOMESTIC RUMINANTS OF NEPAL

Number of domestic ruminants (cattle, buffalo, sheep, and goat) is considerably large in Nepal as compared to its land area and population. Per capita domestic animal is one of the highest in the world. Therefore, domestic ruminants are significant source of anthropogenic green house gas emissions. Although our animals emit less CH4 as compared to those in developed countries due to small animal body size, productivity and feeding and management practices, total emission is significant due to large number of animals in the country.

Calculation of Greenhouse Gas emissions from domestic livestock of the country by using the Tier 1 emission estimation method of IPPC (IPPC, 20006) reveals that buffaloes are the largest source of CH4 followed by cattle. Although population of cattle is larger than that of buffalo, emission factor of buffalo is larger in our region than that for cattle (IPPC, 1996) which gives larger value of emission from buffalo. Total annual emission of enteric CH4 from domestic ruminants is roughly 522 Gg in which share of buffalo, cattle, goat and sheep are roughly 52%, 38%, 9% and 1% respectively. These emissions are calculated by using default emission factors of IPPC for these categories of animals (IPPC, 1996). According to the rule of thumb of IPPC (IPPC, 2006) buffalo and cattle are significant livestock species responsible for enteric CH4 emission and thus warrant higher level methods (Tier 2 or 3) to estimate total emissions form these animals.

Table 1: Emission of enteric Methane from domestic ruminants

Categories of Animal	Enteric CH4 (Gg per year)	Proportion of total emission (%)
Cattle	201	38.5
Buffalo	271	52.0
Goat	46	8.8
Sheep	4	0.7
Total	522	100

APPROACHES TO REDUCE METHANOGENESIS IN RUMINANTS

There are several strategies studied to inhibit methane production in ruminants (Boadi, Benchaar, Chiquette, & Masse, 2004; Buddle *et al.*, 2011; Martin, Morgavi, & Doreau, 2010; Moss, *et al.*, 2000; Sirohi, Michaelowa, & Sirohi, 2007; Wall, Simm, & Moran, 2010). As H2 is the principal metabolite for methanogenesis, approaches to inhibit methanogenesis are chiefly directed either towards reducing H2 production in the rumen or finding alternative sinks for H2 (figure 5). Direct inhibition of methanogens by biological control agents, immunization and in vivo oxidation of CH4 are some other strategies which are being considered as possible mitigation tools for ruminant methanogenesis (Klieve & Hegarty, 1999; A.-D. G. Wright & Klieve, 2011). The recent completion of genome sequencing of the widely distributed archaeal methanogen *Methanobrevibacter ruminantium* will probably open new horizons to develop biotechnological tools to fight against ruminal methanogenesis (Buddle, *et al.*, 2011; Leahy *et al.*, 2009).

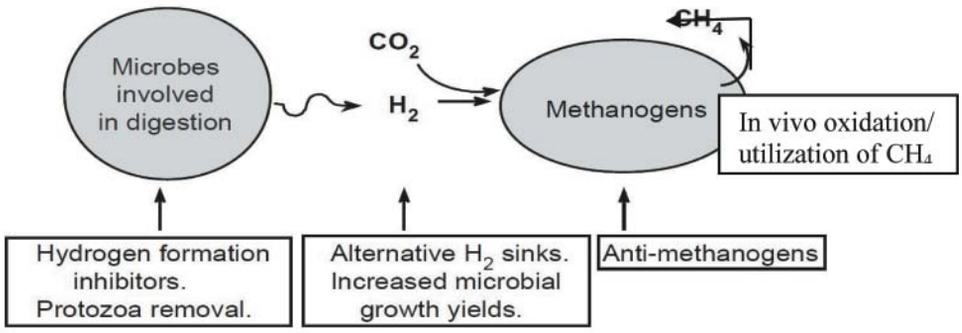

Figure 5: Approaches to reduce methanogenesis in ruminants. Adapted from Joblin (1999)

Direct inhibition of methanogens

Use of biological control agents like archaeal virus (bacteriophages) and bacteriocins to inhibit methanogens have been studied. Archaeal viruses which selectively infect methanogens are suggested as novel control agents for ruminant methanogenesis but further study and exploration is needed to materialise this option (Klieve & Hegarty, 1999). Similarly, bacteriocins (bacterial toxin), which selectively inhibit other microbes, were reported to reduce methanogenesis both *in vivo* (Santoso *et al.*, 2004) and *in vitro* (Callaway, Carneiro De Melo, & Russell, 1997; Lee, Hsu, Mantovani, & Russell, 2002).

In addition, use of vaccines against methanogens is another area which has been studied as a possible method to reduce ruminal methane emission(A. Wright *et al.*, 2004). Active components present in essential oils were also reported to be effective both in vivo and in vitro to inhibit methanogenesis by direct inhibition of the methanogen population (Busquet, Calsamiglia, Ferret, Cardozo, & Kamel, 2005; Busquet, Calsamiglia, Ferret, Carro, & Kamel, 2005; Evans & Martin, 2000; Kamra, Agarwal, & Chaudhary, 2006; Mohammed *et al.*, 2004; A. Patra, Kamra, & Agarwal, 2006; A. K. Patra, Kamra, & Agarwal, 2010; A. K. Patra & Saxena, 2010; Wang, Marx, Lora, Phillip, &

McAllister, 2009). Due to being lipophilic (Sikkema, De Bont, & Poolman, 1995), active compounds in essential oils attach to the lipid bilayer of the cell membrane causing loss of membrane integrity and disruption of ion gradients across the cell, resulting in the death of the microorganisms (Griffin, Wyllie, Markham, & Leach, 1999; Sikkema, *et al.*, 1995; Ultee, Kets, & Smid, 1999).

Decreasing H_2 production by manipulating ruminal fermentation

Any of the nutritional or management strategies which inhibit the production of H_2 gas in the rumen, reduces the emission of methane by limiting the supply of H_2 to the methanogen population. Production of acetate and butyrate during fermentation reactions results in H_2production but production of propionate takes place without forming H_2 (Martin, *et al.*, 2010). Therefore, selecting feedstuffs which decrease the acetate: propionate ratio will result in reduced methane emission (Moss, *et al.*, 2000). Generally, diets high in soluble sugars (like concentrates based diet) produces less methane per unit of fermentable organic matter due to the reduced acetate: propionate ratio as compared to diets high in fibre (like roughage based diet) (K. Johnson & D. Johnson, 1995; McAllister, Okine, Mathison, & Cheng, 1996).

Similarly, particle size of the feed also affects the final product of fermentation which in turn affects methane emission. The production of acetate increases when animals are fed with coarse feedstuffs than fine or ground feedstuffs (Le Liboux & Peyraud, 1999). Manipulation of ruminal fermentation by using ionophores as feed additives has been widely studied. Ionophores include monensin, lasalocid, tetronasin, narasin, salinomycin and others. When used in ruminant diets, ionophores were found to reduce methane emission (Mathison *et al.*, 1998; Moss, *et al.*, 2000; Sauer *et al.*, 1998) by selectively inhibiting acetate producing bacteria and stimulating propionate producing bacteria (C.J. Newbold, Wallace, Watt, & Richardson, 1988; Van Nevel & Demeyer, 1996). However, it has been reported that the effect of ionophores is short lived (Rumpler, Johnson, & Bates, 1986).

A proportion of rumen methanogens are in symbiotic relationships with ciliated protozoa both inside the cell and attached to the surface of the protozoa as endosymbionts and ectosymbionts respectively(B. Finlay *et al.*, 1994; Tokura, *et al.*, 1997; Vogels, *et al.*, 1980; Williams & Coleman, 1997). More than one third of ruminal methane is produced by the methanogens associated with protozoa through interspecies hydrogen transfer produced from protozoa to methanogenic archaea (B. Finlay, *et al.*, 1994). Therefore, defaunating agents can reduce methanogenesis due to decreased H2 production and disrupting symbiosis between protozoa and methanogens (B. Finlay, *et al.*, 1994; Tokura, *et al.*, 1997). Saponins, phytochemicals present in many plant species, have been reported to act as a defaunating agent by disrupting the membrane permeability of protozoa (Bangham & Horne, 1962; Klita, Mathison, Fenton, & Hardin, 1996; Lu & Jorgensen, 1987). Supplementation of ruminant diets with saponin can result reduced methane emission (Klita, *et al.*, 1996; Lila, Mohammed, Kanda, Kamada, & Itabashi, 2003; Lu & Jorgensen, 1987; Mao, Wang, Zhou, & Liu, 2010; Wina, Muetzel, Hoffmann, Makkar, & Becker, 2005). However, ruminal adaptation to saponin limits the in vivo use of this plant extract (Ivan *et al.*, 2004; C. J. Newbold, ElHassan, Wang, Ortega, & Wallace, 1997; Teferedegne *et al.*, 1999).

Tannins from different plants were also found to be effective in reducing methanogenesis in ruminants (Animut *et al.*, 2008a, 2008b; Carulla, Kreuzer, Machmuller, & Hess, 2005; Grainger *et al.*, 2009; Huang *et al.*, 2010; Jayanegara, Togtokhbayar, Makkar, & Becker, 2009; Tavendale *et al.*, 2005; Zeleke, Clément, Hess, Kreuzer, & Soliva, 2006). It has been suggested that tannins inhibit cellulolytic bacteria by interacting with their cell wall, cell membrane, extracellular proteins and microbial enzymes resulting in less fibre digestion which ultimately reduces the availability of H2 for methanogens to produce CH4 (Carulla, *et al.*, 2005; Makkar, Blummel, & Becker, 1995; McSweeney, Palmer, Bunch, & Krause, 2001; Smith, Zoetendal, & Mackie, 2005). Digestion of protein was reported to be impaired while using condensed tannin or tannin source in ruminant feed due to binding of the proteins by tannins (Animut, *et al.*, 2008a; Beauchemin, McGinn, Martinez, & McAllister, 2007). Ruminal adaptation has also been reported (Brooker *et al.*, 1994; Nelson, Thonney, Woolston, Zinder, & Pell, 1998).

Using alternative H2 sinks

Removal of hydrogen gas produced in the rumen by using alternative hydrogen sinks limits the availability of H2 to the methanogens thereby reducing methanogenesis. Reductive acetogenesis is one of the potential strategies which could be exploited as an alternative H2 sink (Joblin, 1999). Reductively acetogenic bacteria (reductive acetogens) in the rumen also utilize H2 to produce acetate, but cannot outcompete methanogens. However, they have been studied as possibility to be exploited as alternative hydrogen sink in the rumen (Chaucheyras, *et al.*, 1995; Joblin, 1999; Lopez, *et al.*, 1999). Reductive acetogenesis has twofold benefits; firstly, it helps to reduce methane emission by limiting H2 supply to methanogens and secondly, the resultant acetate could be used as an energy source by the host animal (Martin, *et al.*, 2010). Similarly, use of fats and oils (unsaturated fatty acids) in ruminant diets causes a reduction in methane emission (Dohme, Machmüller, Wasserfallen, & Kreuzer, 2000; Dong, Bae, McAllister, Mathison, & Cheng, 1997; Machmüller, 2006; Machmüller & Kreuzer, 1999) due to the incorporation of hydrogen by fatty acids during hydrogenation of unsaturated fatty acids(R. Hegarty, 1999; McAllister, *et al.*, 1996).

Methane oxidation

Methane oxidizing bacteria (methanotrophs) are found in a wide variety of environments (Hanson & Hanson, 1996). Stocks and McCleskey (1964) reported the presence of methane oxidizing bacteria in the rumen of cattle. These methane oxidizing bacteria could be a promising strategy, if they could be established in rumen successfully.

Promoting efficiency of animal production

Strategies which increase efficiency of livestock production result in a net reduction in methane production due to the relative decrease in methane emission per unit of product (e.g. meat, milk) (Moss, *et al.*, 2000). Increased efficiency of livestock production may result in a decrease in the population of livestock, which in turn reduces GHGs emission per unit area (Moss, *et al.*, 2000).

Others

Increasing levels of feed intake promote intestinal digestion with reduction in ruminal fermentative digestion which results in reduced methane emission when expressed relative to digestible energy (K. Johnson & D. Johnson, 1995; Mathison, *et al.*, 1998). Some other less feasible approaches like a wholesale reduction in livestock numbers has also been envisioned as an alternative approach to reduce methane emission (Sejian, *et al.*, 2010; Thorpe, 2009). Similarly, genetic selection or breeding strategies to evolve ruminant animals with less methane emission is another approach for emission reduction (Wall, *et al.*, 2010).

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LIVESTOCK FEED SITUATION IN NEPAL AND POTENTIAL INTERVENTION STRATEGIES TO ALLEVIATE FEED DEFICIT

B. Sharma[1]

ABSTRACT

Livestock is an integral part of agricultural production system in Nepal. National Planning Commission (NPC) estimates 25% of the population are living in absolute poverty. Poverty is one of the factors of accelerating environmental degradation. Livestock rearing is the income generation activities in rural areas of Nepal. Conservation of the degraded dry forest land is managed by the poor community in Nepal. The sustainable livestock programme, which focuses on indigenous knowledge and technology along with modern ways of livestock farming, is the way for ecological balance. Nepal has a great biological diversity.Department of livestock Services (DLS) facilitates for livestock farming based on animal feed which focus on forage based livestock keeping and enhancing environment friendly production system to minimize environmental degradation. Nepalese livestock are one third underfed. Animal production and productivity is directly related to livestock feed situation. Therefore, scientific management of pasture land and cultivation of leguminous forage crops in the field for winter forages are important steps to alleviate deficit in livestock feed.

INTRODUCTION

Agriculture sector is basis of livelihood of Nepalese farmers, contributing to 31% of the country's GDP and employing 66% of its labor force (MoAC, 2007). Government of Nepal (GoN) has given top priority to agriculture and livestock sector in poverty reduction and food and nutritional security programme. Livestock contributes about 31% to the agricultural GDP of the country among which the largest amount is derived from the hills (53%) followed by terai (38%) and the least from the mountains (9%) (APP, 1995). In 2012, livestock sector contributes 26% of AGDP. Livestock and livestock products are an important source of household cash income (about 20% of total income) especially in the hills and mountains.

The total Dry Matter (DM) availability in the year 1995/96 is 15.2 Million ton, but the requirement was 20.9 Million ton which is 24.5 % deficit and it can be forecasted that the deficit will certainly increase to 29.7% in the year 2014/15 according to the high DM requirement which is 25.6 Million ton (APP, 1995). Some data indicates that about 45% yellow maize, 98% soybean meal, 95% till cake are being imported to fulfill the national demand of animal feeds (Bhattarai, 2007).

From the perspective of food scarcity, the most challenging enterprise is livestock. The population of man and animal is being increasing which might result to the competition for food between them. Furthermore, it is reported that about 65-70% of the cost of production is being invested in animal feeds and fodders (Upreti, 2008). So, the most considerable inclination of our programmes should be towards forage based livestock farming by utilizing the waste, unused, barren lands and pasture lands.

Crop residues, crop weeds, and forest supplies make up the diet of the livestock in Nepal. Agricultural lands contribute about 60 percent of the total feed requirement, mainly in the form of low quality

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crop residues, and forest and grazing lands contribute the remaining 40 percent.

The 2001 population census shows that over 70 per cent of the total households have livestock. Livestock form an integral part of the social fabric for many people while they serve as a capital reserve available for hard times.

Nepal is a mountainous country with a great biological diversity. There are varied floras and fauna in different Eco-zones. Livestock rearing is more in mountain and high hills (Sharma, 2002). The contribution together with production of food (meat, milk, and eggs), fibre, hides/skins and transportation amounts to about 15% of GDP which is 28% of Agricultural GDP (Annonaa, 1995).

Livestock population, in relation to arable land and animals per person, are large by Asian standards, with approximately 7.19 million cattle, 4.83 million buffalo, 8.84 million goats, 0.80 million sheep, 1.06 million pigs, 25.7 million fowl and 0.37 million ducks (Agricultural Statistics Division, 2009/2010).

In Nepal, 12% of cattle and 35% of buffaloes are improved and they are primarily kept in stall feeding system. The country experienced about 15 per cent increase in the number of livestock during 1984 to 1998. Over half of the total livestock are reared in the Hills (5.8 million LU), followed by the Terai (4.1 million LU, and the Mountain (1.3 million LU). In case of stocking rate, the Terai has highest LU/ha (58 LU/ha), followed by the Hills (20 LU/ha) in comparison to the national average of 6.5 LU/ha(Sharma B., 2005).

In Nepal, most of the pasturelands are located in the mountains while the livestock pressure has been noticed in the temperate zone-the hills. This has degraded the pastureland and increased pressure on forests. Forests alone contribute 30 per cent of the animal feed, 47 per cent of their feed is met from crop residues and remaining from grazing in the shrub lands and grazing lands. Rangelands supply a low amount of forage. Although forage yield in the alpine meadows is about 3.08 MT-higher than other areas, only 37 per cent of the rangelands are accessible for forage collection. (Dinesh Pariyar, 2005). Leasehold forestry gives some degraded forest land to rural poor and it becomes vital to increase their livelihood by agriculture and livestock practices along with conservation of forest.

In Nepal, women are actively involved in livestock Production. Fodder collection, grazing and milking are generally performed by both women and men, whereas activities like feed preparation, feeding, cleaning sheds and preparing milk products are women's domain (Annonc, 1995). Rural production Livestock, particularly dairy production, is a major source of income for women (APP, 1995) and sale of livestock and livestock products accounts for nearly 55% of total farm family income. Trading in livestock, especially sheep and goats, and wool and woollen products, is the major livelihood strategy of hill and mountain people in Nepal as food grain production in these regions is inadequate. From the early days, weaving has been a part of daily life in the hills and mountains. The per capita consumption of milk, meat, and fish in Nepal is 44.1, 8.1 and 0.8 kg respectively. This indicates a much lower consumption of animal protein by the Nepalese population than in developed countries (Mishra & Shrestha 1996).

At present per capita consumption of milk, meat and eggs is 60 lit, 13 Kg and 29 number respectively (MoAD, 2012).

The livestock keeping system in Nepal is as follows:

(a) Transhumance systems

This system is practiced in the temperate, sub-alpine and alpine regions where cattle, buffaloes, sheep and goats migrate from one place to another throughout the year. This system utilizes forage resources available from temperate, sub-alpine and alpine pastures during the monsoon season and from crop stubble during the winter season.

(b) Sedentary systems

This system prevails at the lower altitudes of the mid-hills (900 - 1000 m) and utilizes all the available

forage in and around the village. The main grazing areas during summer are the scrub-lands and community grazing lands around the village. The livestock population is sedentary and consists of working oxen, dry buffaloes, a small number of cattle, and goats. The animals spend half their time grazing. Other forage resources are crop by-products and tree fodders during winter, and grasses and weeds from crop-lands during the summer (Sharma, B. 2005).

(c) Stall-fed systems

This system prevails mainly in the low to mid-hills (400 - 900 m) and peri-urban areas, and comprises milking buffalo, exotic or cross-bred cattle and buffaloes. The system predominates in areas of intensive cultivation and marketing, where the availability of crop by-products is adequate to maintain the animals in winter. In addition to crop by-products, tree fodder, grasses and weeds collected from farm-lands are also an important forage source. In recent year's cultivation of exotic grass in farm land and other lease land boost the production of forage and ultimately help for improved livestock feeding system in mid hill and Terai region of Nepal (Sharma B. and Karki Y., 2012).

Potential Land Resources for Fodder and Pasture Development

The major land resources for increased forage supply are grasslands, degraded forests, shrub lands, uncultivated agricultural and the terrace risers in the inner Terai and Hills. It has been estimated that about 1.04 m ha of rangelands and 0.93 m ha agricultural and related farmlands could be utilized for improved pasture management and fodder production respectively (TLDP, 2002). So far only about 1.27 percent of the total potential grasslands and degraded forests have been brought under improvement. Besides, community forest area under the high-tension line and the roadsides of total 15,458 km roads (4577 km black-topped; 3696 km link roads and 7185 km unpaved roads; 1914 km feeder roads and the roads constructed by VDCs etc.) could be utilized for forage production and pasture management.

Livestock is the main source of maintaining the soil fertility. It provides nine million tons manure annually to three million hectares cultivated land through the use of feed from grazing land (19%), crop by-products(37%), forest biomass(35%) and secondary plant residues (9%) in Nepal (Sherchand,2001).

Livestock Feed Balance situation in Nepal

Table 1: Livestock Feed Balance

Sources	Area ('000 ha)	Bari Risers/ Bonds	Khet Risers/ Bonds	Fallow land	Tree Fodder	Kitchen Residue	Total TDN ('000 MT)	% Supply
Agricultural Land	819	353.8	223.3	79.2	179.8	209.7	1067	17.6
Pakho	1551							
Khet								
Forest Land							1672	27.5
Coniferous	2247						112	
	1332						227	
Hardwood	1932						186	
Mixed Wood	1283							
							1146	
Shrubs								
Grazing Lands	1757						764	12.6
Crop Residues							2569	42.3
Total							6072	100
Requirement							9300	
Deficit							3228	34.7

Source: (Forage seed production area mapping, TLDP, 2002); (MOAC, 1999/2000); (Singh, 2002)

The data on the above table clearly indicated that the overall annual feed deficit in the country is estimated at 34.7 percent on TDN basis.

MATERIALS AND METHODS

The data has been collected from GoN, Ministry of Agriculture Development (MoAD) and Department of livestock Services (DLS).

The feed deficit is severe in the hills region (-56%) followed by the Terai (-42%). The mountain region is at surplus (26%) (DLS, 2002). This clearly indicates that feed, particularly in the form of green fodder is the major factor limiting livestock production. Because, when animals are underfed they would questionably get more susceptible to diseases and parasites. In the shortage of fodder, farmers try to compensate the nutrient deficit by supplementing the diet with expensive concentrate feed. The increasing cost of treatment against various disease and nutritional disorder and concentrate feeds has led to higher cost of animal production in Nepal. This would have serious implication on the competitiveness of domestic product with imported products as the sustainability of the agricultural system in the long run.

Problems and Prospects

The agro-ecological and cultural diversity of Nepal provides the potential to cultivate and develop tropical to temperate forage and pasture species. However, there is still deficit of forage biomass production as per the requirement. This huge deficit of green fodders is attributable to low production, lack of propagation of existing species, lack of conservation of native forage and pasture species and lack of quality seeds.

The availability of feed and fodder for livestock, especially during winter and early summer, is the major constraint on livestock productivity in all the existing systems. The feed requirement and supply situation for the livestock population is presented in Table 1 below. These data suggest that there is a serious shortage of nutrients for animals. Some of problems are listed

- Lack of animal feed act
- Lack of programs that supply the national demand of feed ingredients
- Lack of coordinated approach for composite animal feed production combining green fodder, roughages and concentrates.
- Lack of round the year feed to livestock to meet increasing demand of animal protein to growing human population.

Table 2: Estimated TDN (total digestible nutrients) requirement and supply

	TDN equirement (MT 000)			TDN supply (MT 000)			Deficit
	Large ruminants	Small ruminants	Sub total	Cultivated land	Forest & uncultivated land	Sub total	
Mountain	782	289	1071	310	755	1065	6
Hills	3626	654	4280	1518	989	2507	1773
Total	4408	943	5351	1828	1744	3572	1779

Source: LMP (1993)

This shortage of nutrients supply to the animals can reflect that there is shortage of forages and fodders, the cheapest source of animal feed. To reduce 34% feed deficit, certainly we should plan different programmes related to forage and fodder development and this is only sustainable and prophet when we first implement the programmes that assures the supply of forage grass as a reliable source of animal feed.

In addition, the commercialization of livestock enterprise has huge demand of animal feed resources. So, low cost feed formulation and inclusion of cheapest animal feed resource i.e. grass in the feed making composite feed formulation are further prerequisites for forage and pasture development program.

RESULTS AND DISCUSSIONS

Livestock is the main source of organic manure required for maintaining the soil fertility. It provides nine million tons manure annually to three million hectares cultivated land through the use of feed from grazing land (19%), crop by product (37%), forest biomass (35%), and secondary plant residues (9%) in Nepal (Sherchand , 2001).

Leasehold forestry program basically focuses on mid hill areas of Nepal stretching from east to west. It has been good example of income generation program for poor farmers in remote areas. The overall goal of the programme is a sustained reduction in the poverty of the 44,300 poor households who are allocated leasehold forestry plots through increased production of forest products and livestock products.

Mixed farming is probably the most appropriate agricultural production system from an environmental perspective because it is, at least partially, a closed system. The waste products of one enterprise (crop residues), which would otherwise be loaded on to the natural resource base, are used by the other enterprise, which returns its own waste products (manure) back to the first enterprise. Because it provides many opportunities for recycling and organic farming and for a varied, more attractive landscape, mixed farming is the favourites system of many agriculturalists and environmentalists. The key role of maintenance should be given to private sector for making sustainable development.

On the whole, the Nepalese livestock are underfed at least by about one-third (TLDP, 2002) and its production has not increased. Majority of farmers in Nepal are subsistent. Their livelihood depends on agriculture. To cope with exigent need of farmers there should be innovation of new technology, which must be environment friendly. To preserve environment is also one of the basic duty of the society. Improved livestock farming becomes vital for conserving natural resources. When farmers practice stall-feeding, there will be less land degradation. Large number of livestock grazes in small areas, which causes soil erosion, loss of young shoot and shrubs. These activities certainly leads to more steep water flow to the downhill area, streams and finally to the river. It may cause flood and loss of top soils. Whenever, there is stall feeding practice certainly there is less deforestation. It eventually leads for promotion of natural resource and positive impact on environment too.

The sustainable livestock program, which focus on indigenous knowledge and technology along with modern way of livestock farming is best suited to different ecological areas. Cattle, buffaloes, sheep, goat, pigs and poultry are the livestock species reared across different agro-ecological zones.

However, NPAFC, CLDP, LFLP and other organizations are enforcing the similar activities to establish and develop forage resource centres, the success is very poor. In some districts, the activities are successful. Therefore, there is need of expansion of successful stories to other districts too. The project can implement the activities according to forage seed and agro ecological zones and apply more appropriate technology, so that the deficit forage seeds can be produced and ultimately, feed deficit will be mitigated.

Policies for livestock and wildlife production and conservation need to be formulated as a matter of high priority in all agro-ecological zones. Integrated research - development - extension projects should be initiated with support from local bodies and farmers' group.

There must be some sort of training on marketing component, which should be explored. The bio-security measures should be worked out and published for general public and stakeholders.

Forage based livestock feeding system can be enhanced in mid hills and mountains region of Nepal. Terai region agricultural by products, forage and some feed can be incorporated to make viable livestock business.

The feeding of livestock with grains makes cost of milk production high. It will eventually compete with human food.

The TDN available in the district can be assessed and number of livestock which can be rear in that district shall be calculated.

The policy of exporting unused cattle to Bangladesh and India shall be liberal, quarantine and administration process shall be eased. It will have positive impact on feed balance situation in Nepal.

Improved livestock farming becomes vital for conserving natural resources. When farmers practice stall feeding, there will be less deforestation and less land degradation. It eventually leads to promote natural resources with positive impact on environment.

CONCLUSIONS

Pasture vegetation improvement shall be done by:

Identification and elimination of the unwanted weeds and shrubs from the grazing land and surrounding area shall be done properly. Conservation of the native vegetation by adopting proper grazing management system by the herders and minimize the grazing pressure on the rangelands. Introduction of new potential forage species without replacing the key plant species of the pastureland would be done. Increase dry matter percentage of the forage biomass in terms of grasses and legumes, more preference to the legumes so that total DM digestibility of the animals will be increased.

However NPAFC, CLDP, LFLP and other organizations are enforcing the similar activities to establish and develop forage resource centres, to extend forage development programmes and to manage pasture lands, the success is very poor. In some districts, the activities are successful. So, there is need of expansion of successful stories to other districts too. The project can implement the activities according to forage seed and agro-ecological zones and apply more appropriate technology, so that the deficit forage seeds can be produced and ultimately, feed deficit will be mitigated.

Similarly, forages when combined with other feed ingredients, concentrate rations or when value added by treating with urea or making urea molasses mineral block (UMMB), will be more nutritive and give better results. So, along with commercialization of livestock industry, there is need of commercialization in the field of fodder and pasture development also.

The most important and crucial part of commercialization of animal feed is recent technology which can be easily adapted by small scale farmers too. There is need of technology and concept development by which farmers would motivate towards commercialization for preservation of animal feeds/fodders for winter period. Also, they can motivate only in production of fodders and feeds and selling to livestock entrepreneurs.

Malnutrition in animals and human being are interdependent. There are limitation of fodder and forage production in winter season. Revolving fund for purchasing of forage seeds on time is important for sustainable programme.

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STUDY OF CLINICAL MASTITIS AND TRENDS OF ANTIBIOTIC SENSITIVITY TO MASTITIS PATHOGENS

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ABSTRACT

Milk samples were obtained from the farmers and the technicians working in the field. Out of 780, 630 (80%) samples were found positive in California Mastitis Test (CMT). All 630 CMT positive samples were included in this study. Five hundred and twenty seven samples showed growth. The highest isolate observed was Staphylococcus sps followed by Escherichia Coli, Streptococcus sps and Pseudomonas sps and so on. Tetracycline, Streptomycin, Enrofloxacin, Kanamycin, cephalexin, Gentamycin and cloxacillin were the antibiotics used to study the trend of antibacterial sensitivity to the above isolated organisms from the mastitis milk. Tetracycline, Streptomycin, Enrofloxacin, Kanamycin and Gentamycin seem still highly sensitive to the most of the isolates. However, cloxacillin showed lesser sensitive to the all of the isolates. When the trend of this sensitivity was compared in two study periods except in Staphylococcus there was variable in sensitivity of the antibiotics in other isolated organisms.

INTRODUCTION

Mastitis is the inflammation of mammary gland causing suppression of milk production. Bovine mastitis is an important disease of the dairy animals. Though it is caused by a number of microorganisms, Staphylococcus Streptococcus is the predominant causative organism for the mastitis (Blood and Henderson, 1979). Though, Streptococcus was the predominant cause of the mastitis now it is replaced by Staphylococcus (Blood and Henderson, 1979). Mastitis is also of three types, Clinical, Sub clinical and chronic forms. In clinical mastitis the symptoms are visible like swelling of mammary gland, change of color of the milk, consistency of the milk where as it is not clear in sub clinical ones (Blood and Radotitis, 1989).

The technicians, village level worker in the field level treat the mastitis. Due to improper and haphazard use of antibiotics in treating mastitis leads to the resistance to it. So, it is important to know the sensitivity of the antibiotics to the different organism causing mastitis before switching to the treatment. Therefore, this paper aimed to study the pattern of microorganisms causing clinical mastitis over a 2 years period and antibiotic sensitivity pattern to each of the organism isolated from the mastitis milk submitted to the CVL. Study tried to determine the whether susceptible pathogens had changed its sensitivity to the antibiotics during this time period.

MATERIAL AND METHODS

In this study, those milk samples were included that were brought from the field or the farmers themselves suspected for the mastitis. A total of 780 milk samples were included that were submitted to CVL in 2 years of period (2003 to 2004). Received samples were from different places of Kathmandu valley.

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All received samples were subjected to the California Mastitis Test (CMT). The positive samples to CMT (630) were subjected to primary culture in Nutrient Agar. A loopful of CMT positive milk was inoculated on the Nutrient agar plate, and then incubated at 370C for 18 to 24hrs. The plate was observed for the colony character or the pattern. Mixed colonies were subjected to subcultures on the Nutrient Agar again. Then the organism was identified based on colony characteristics; Gram staining characteristics; motility growth on differential (McConkey Agar) and selective media (EMB Agar); and biochemical characteristics such as Oxidase, Catalase, Coagulase, Urease and Indol. Once pathogens were isolated and identified in pure culture, organism was subjected to antibiotic sensitivity test using disk diffusion assay according to HIMEDIA.

Disk diffusion assay was conducted in accordance with the method given by Cowan and Steel in 1981. Briefly, a loopful colony of each isolate was added to a sterile normal saline and incubated for 2 hours. The isolate was streaked smoothly onto the nutrient agar using sterile swab dipping into the saline. The plates were incubated for 24 hours at 370C. The zone of inhibition was measured and the isolates were recorded as sensitive to a particular antibiotic zone of inhibition was of 3 mm radius according to Antimicrobial Susceptibility Test Disk, HIMEDIA.

RESULTS

Out of 780 received milk samples, 630 samples were found positive for mastitis (80%) by CMT. All the CMT positive samples were subjected to culture. The growth of organisms was observed in 527 (83.7%) samples only, where no growth is was observed in 103(16.3%) samples. The major isolates were *Staphylococcus sps* (55.8%); *E.coli* 22.4%; *Streptococcu sps* (14.4%); and *Pseudomonas sps* (2.9%) as shown in Table 2. The number of different isolates recovered in 2 different years of study was also compared. According to that *Staphylococcus sps* was found 60.12% in 2003 whereas 50.72% in 2004; *E. coli* 15.6% in 2003 and 30.4% in 2004; *Streptococcus sps* 14.4% in 2003 and 14.4% in 2004 and *Pseudomonas sps* 3.9% in 2003 and 1.4% respectively. The trend of isolated organisms was almost similar in two years period. However, number of *Staphylococcus sps* and *Pseudomonas sps* was more in 2003 than 2004. In contrast, rate of isolation of *E. coli* was higher in 2004 (Table 2).

The most sensitive antibiotics to *Staphylococcus sps* was found Streptomycin, followed by Enrofloxacin, Kanamycin, Gentamycin, Tetracycline, Cephalaxine and Cloxacillin respectively (Table 3).

Similarly, Tetracycline was the most sensitive antibiotics for *E. coli* that was followed by Streptomycin, Kanamycin, Enrofloxacin, Gentamycin, Cephalaxine and Cloxacillin respectively (Table 4).

In the same way, sensitive antibiotics for *Streptococcus sps* were Enrofloxacin, Tetracycline, Streptomycin, Kanamycin, Gentamycin, Cephalaxin and Cloxacillin respectively as shown in Table 5.

However, the sensitivity pattern for *Pseudomonas sps* is different and i.e. Enrofloxacin, Gentamycin, Kanamycin, Cephalaxine, Cloxacillin and Tetracycline respectively as shown in Table 6.

Comparison of the trend of antibiotic sensitive among *Staphylococcus sps*, *E.coli* and *Pseudomonas sps.* for 2 years, showed similar pattern in both the years except in Cloxacillin. However, in Streptococcus sps except Gentamycin the trend is almost similar in 2 years.

Table1: CMT and the growth of the organism

S.N.	Year	Total Sample	CMT positive	No. of samples positive for microorganism	No. of samples Negative for microorganism
1.	2003	400	356	307	49
2.	2004	380	274	220	54
		780	630	527	103

Table 2: Isolates from CMT positive samples in 2003 and 2004

S.N.	Isolate Organism	2003		2004		Total	
		no.	%	no.	%	no.	%
1	6.6.1.1.1 Staphylococcus	196	60.1	140	50.7	336	55.8
2	E.coli	51	15.6	84	30.4	135	22.4
3	Streptococcus	47	14.4	40	14.4	87	14.4
4	Pseudomonas	13	3.9	5	1.4	18	2.9
5	Bacillus	7	2.1	0	0	7	1.2
6	Proteus	4	1.2	3	1.0	7	1.2
7	Klebsiella	5	1.5	0	0	5	0.8
8	Corynebacterium	2	0.6	0	0	2	0.3
9	Micrococcus	0	0	2	0.7	2	0.3
10	Aerococcus	0	0	2	0.7	2	0.3
11	Haemophillus	1	0.3	0	0	1	0.1
	Total	326		276		602	

Table 3: Trend of Antibacterial susceptibility of Staphylococcus species isolates to different antibiotics used in 2003 and 2004.

S.N.	Antibiotic	2003	2004	Total
1	Streptomycin	138/138 (100.0%)	140/140 (100.0%)	278/278 (100.0%)
2	Enrofloxacin	188/188 (100.0%)	93/94 (99.0 %)	281/282 (99.6 %)
3	Kanamycin	196/196 (100.0%)	104/107 (97.2%)	300/303 (99.0%)
4	Gentamycin	178/182 (97.8%)	133/140 (95%)	311/322 (96.6%)
5	Tetracycline	135/145 (93.1%)	117/119 (98.3 %)	252/264 (95.5%)
6	Cephalexin	110/121 (90.9%)	81/90 (90.0%)	191/211 (90.5 %)
7	Cloxacillin	75/118 (63.5%)	61/76 (80.3%)	136/194 (70.1%)

Table 4: Trend of Antibacterial susceptibility of E. coli species isolates to the different antibiotics used in 2003 and 2004.

S.N.	Antibiotic	2003	2004	Total
1	Tetracycline	34/34 (100.0%)	75/76 (98.7%)	109/110 (99.1%)
2	Streptomycin	45/46 (97.8%)	83/84 (98.8%)	128/130 (98.5%)
3	Kanamycin	49/51 (96.1%)	69/69 (100.0%)	118/120 (98.3%)
4	Enrofloxacin	39/42 (92.8%)	38/38 (100.0%)	77/80 (96.3%)
5	Gentamycin	43/43 (100.0%)	78/84 (92.8%)	121/127 (95.3%)
6	Cephalexin	31/31 (100.0%)	22/25 (88.0%)	53/58 (94.5%)
7	Cloxacillin	32/44 (72.7%)	55/59 (93.2%)	87/103 (84.5%)

Table 5: Trend of Antibacterial susceptibility of Streptococcus species isolates to the different antibiotics used in 2003 and 2004.

S.N.	Antibiotic	2003	2004	Total
1	Enrofloxacin	39/39 (100.0%)	32/32 (100.0%)	71/71 (100.0%)
2	Tetracycline	38/39 (97.4%)	36/37 (97.3%)	74/76 (97.4%)
3	Streptomycin	24/24 (100.0%)	38/40 (95.0%)	62/64 (96.9%)
4	Kanamycin	44/47 (93.6%)	32/32 (100.0%)	76/79 (96.2%)
5	Gentamycin	39/39 (100.0%)	35/40 (87.5%)	74/79 (93.7%)
6	Cephalexin	22/24 (91.7%)	15/17 (88.2%)	37/41 (90.2%)
7	Cloxacillin	18/31 (58.1%)	18/28 (64.3%)	36/59 (61.0%)

Table 6: Trend of Antibacterial susceptibility of Pseudomonas species isolates to the different antibiotics used in 2003 and 2004.

S.N.	Antibiotic	2003	2004	Total
1	Enrofloxacin	12/12 (100.0%)	5/5 (100.0%)	17/17 (100.0%)
2	Gentamycin	12/13 (92.3%)	5/5 (100.0%)	17/18 (94.4%)
3	Kanamycin	11/12 (91.7%)	4/4 (100.0%)	15/16 (93.8%)
4	Streptomycin	10/10 (100.0%)	4/5 (80.0%)	14/15 (93.3%)
5	Cephalexin	7/9 (77.8%)	4/5 (80.0%)	11/14 (78.6%)
6	Cloxacillin	9/13 (69.2%)	2/2 (100.0%)	11/15 (73.3%)
7	Tetracycline	6/9 (66.7%)	3/5 (60.0%)	9/14 (64.3%)

DISCUSSION

Milk samples submitted to this laboratory is generally from cattle dairy farms suspected for mastitis by technicians or farmers. The positive for CMT was found 80% which is higher than the previous studies by Manandhar, 2001 (22.21%) and Khakurel, 1996 (17.20%) and Dhakal, and Tiwari, 1992 (30%). and Pradhan and Ghimire, 1988 (71.64%). This higher percentage may be due to the samples submitted to the lab is already suspected for mastitis.

Among 527 isolates, *Staphylococcus sps* (55.8%) was found to be the highest followed by *E. coli* (22.4%), *Streptococcus sps* (14.4%) and *Pseudomonas sps* (2.9%) comparison to other organisms. So, this retrospective study shows that the main cause of the mastitis in the dairy animals is *Staphylococcus sps* (55.8%). This finding is similar to the study carried out by Manandhar, 2001, and Pradhan and Ghimire, 1988 in which the causative organism for mastitis was predominantly *staphylococcus sps*. However, this finding does not agree with the findings of the study carried out by Dhakal, 1996 in which the predominant isolate was coli form (30.23%) instead of *Staphylococcus sps*. (26.16%). Similarly, this differs in findings of Chakrabarti, 19-- in which, *Streptococcus sps* was higher i.e. 73%, and 29.1% in Roberson et.al. 19-- where as *Staphylococcus sps* was 5%, 2.9% respectively. However, this finding is similar in the veterinary medicine (Blood and Handerson, 1979). This suggests that the most of the mastitis-causing organism is *staphylococcus sps*.

However, the trend of antibiotic sensitivity to all organisms taken in consideration, Streptomycin, Enrofloxacin, Kanamycin, were the most sensitive to *Staphylococcus sps* followed by, Gentamycin, Tetracycline, Cephalexin and Cloxacillin. This finding totally differs from other studies in which Streptomycin was the most resistant to this organism (Pradhan and Ghimire, 1988). In case of *Streptococcus sps*, the most sensitive antibiotic was Enrofloxacin followed by Tetracycline, Streptomycin (97%), Kanamycin, Gentamycin, Cephalaxin and Cloxacillin. This is similar to the study of Babu et.al., 1979 in which Streptomycin was sensitive (92%). However, Pradhan and Ghimire found the streptomycin was the most resistant to *Streptococcus sps*. In case of E. coli, Tetracycline (99%) was the most sensitive one followed by Streptomycin, Kanamycin, Enrofloxacin, Gentamycin, Cephalexin and Cloxacillin respectively. This is different in other studies like in Pradhan and Ghimire (1988) and Erskine et al (2002), where Tetracycline was moderately sensitive. In case of *Pseudomonas sps*, the most sensitive one was Enrofloxacin that is similar to *Streptococcus sps*. In contrast, Gentamycin was the most sensitive to *Pseudomanas sps* cited by Erskine et al (2002). But the least sensitive one was Tetracycline that is similar to the study of Erskine et al (2002).

In all the cases of the antibiotic sensitivity trend the Cloxacillin was the least sensitive except in *pseudomonas sps*.that is not beyond 60% where as this cloxacillin was found resistant to almost all the organisms in the studies carried out by Dhakal (1996) and Jha et al (1994).

This is to be taken in consideration that Streptomycin, which used to be resistant seems to gain sensitive again. This might be due to the least use of this chemical in those years. Similarly, Gentamycin was pushed back to third and fourth position. It is because of massive use of this chemical in the field by the technicians and farmers themselves without testing in lab. Enrofloxacin and Tetracycline still can be used as before as they show good sensitivity trend to these organisms.

Comparison of the trend of antibiotic sensitive to organism *Staphylococcus sps*, *E.coli* and *Pseudomonas sps.* for 2 years, showed similar pattern in both the years except in Cloxacillin. However, in *Streptococcus sps* except Gentamycin the trend is almost similar in 2 years. This study is similar to Erskine *et al.* 2002 in which also proportion of bacterial isolates determined to be susceptible did not change during the 7 years period for the majority of bacterial antibacterial interaction tested.

CONCLUSION

Higher percentage of mastitis was found in the submitted milk samples than the field studies. This study reports the most commonly caused mastitis organism was *staphylococcus sps*. Similarly, this study suggests that Enrofloxacin was the most sensitive to the *Pseudomonas* and *Streptococcus sps* where as Streptomycin and Tetracycline were the most sensitive to the *Staphylococcus sps* and *E.coli*. This change in an antibiotic sensitivity pattern is due to the antibiotic used in the field. However, the trend of antibacterial sensitivity to those organisms was not different in two years period. Overall there was no indication of increased resistance of mastitis isolates to antibacterial that are commonly used in the field.

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A REVIEW ON STATUS AND ECONOMIC IMPACTS OF TRANS-BOUNDARY ANIMAL DISEASES IN NEPAL

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ABSTRACT

Trans-boundary animal diseases (TADs) are highly contagious diseases of livestock in the world and of economic importance and a major constraint in the international trade of livestock and livestock products. The attempt has been made in this paper to include the present status of the TADs and also the information available on the economic impacts of some of the TADs in Nepal. Although there are various infectious diseases of livestock and poultry prevalent in the country, among them the foot and mouth disease (FMD), Peste des petits ruminants (PPR), haemorrhagic septicemia (HS), classical swine fever (CSF), Newcastle disease (ND), highly pathogenic avian influenza (HPAI) and sheep and goat pox are priority TADs. Approximately 22 million livestock are susceptible to FMD apart from wild ungulates. Out of the possible seven, only four serotypes of FMD virus, e.g. O, A, C and Asia-1 were recorded in Nepal. Serotype 'C' has not been found in the country since 1996. The adverse impact of PPR is increasingly serious causing high morbidity and mortality in small ruminants. From 2001-2009 there were 2547 outbreaks of PPR in the country. Nepal is at high risk of HPAI because both migratory birds and illegal importation of poultry seems to be responsible for the outbreaks. The H5N1 viruses identified in Nepal was of clade 2.2 in 2009 outbreak. In 2010 outbreaks H5N1 clades 2.3.2 and 2.2 were identified. So far no human cases due to H5N1 have been reported in the country. Economic impacts of TADs in terms of losses in the production and productivity, price and market effects and adverse effects on trade, nutrition and food security etc. have been reviewed.

INTRODUCTION

Livestock is an integral part of agricultural production system, which plays a vital role in national economy of Nepal. This sector contributes about 13% to the national GDP and 31% to agricultural GDP. There are approximately 7.17 million cattle, 4.68 million buffaloes, 0.80 million sheep, 8.47 million goats, 1.04 million pigs and 24.40 million poultry (MOAC, 2009). The annual production of milk, meat and eggs in the year 2009 in Nepal was 1496 thousand MT, 248 thousand MT and 643 million respectively. Within livestock, dairy accounts for 63 percent of the total value added, followed by meat (32 percent) and eggs (5 percent). Commercial livestock production is expanding fairly rapidly in areas close to large population centers. The poultry industry has been a leading sub-sector in commercialization. The commercial pig industry is small but expanding. The milk sub-sector has been doing well overall, with marked seasonal differences in production and supply.

Trans-boundary animal diseases are highly contagious diseases of livestock in the world and of economic importance and a major constraint in the international trade of livestock and livestock products. Many developed countries are now free from most of these diseases & prevent introduction of the diseases to their countries by banning imports from infected developing countries. TADs have also implications on human health because some affect humans as well as animals such as mad cow disease, Rift valley fever, West Nile fever and highly pathogenic avian influenza (HPAI).

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The attempt has been made in this paper to include the present status of the TADs and also the information available on the economic impacts of some of the TADs in Nepal.

CURRENT STATUS OF MAJOR TADs IN NEPAL

In Nepal mass vaccination for Rinderpest (RP) was stopped in 1992 and from 1995 onwards RP eradication process was started. The country obtained certificate for freedom from rinderpest infection in 2002. Following are the major TADs prevalent in the country.

Foot and Mouth Disease (FMD)

Foot and Mouth Disease is a highly infectious viral disease commonly affecting all cloven footed animals including cattle, buffalo, sheep, goat and pigs. Approximately 22 million livestock are susceptible to FMD apart from wild ungulates in Nepal. Irrespective of seasons, the disease is being observed throughout the country in all the three agro-ecological zones including terai, hills and the mountains. From 2000-2009 the eco-zone wise distribution of FMD outbreaks was highest in the hills (45%) followed by terai (35%) and in the mountains (20%). During year 2000-2009 the month-wise pattern of FMD outbreaks revealed that this disease is prevalent throughout the year. However, occurrence of the disease was found to be slightly high in the month of May and June and again during November and December (VEC, 2010). On an average from 2001 to 2010, 870 outbreaks per year have been reported in the country (table 1). Uncontrolled animal movement within the country and unrestricted importation of animals (goat, sheep and buffaloes) from neighboring countries pose additional threat to spread of FMD.

Table 1: FMD Outbreaks from 2001 to 2010

Year	No. of Outbreak	No. of Cases	No. of Death	Case Fatality %
2001	1904	51003	861	1.68
2002	546	7261	118	1.62
2003	2048	57076	1265	2.21
2004	879	19525	202	1.03
2005	1042	19949	461	2.31
2006	710	17389	105	0.60
2007	481	13590	145	1.06
2008	151	5278	109	2.06
2009	646	80357	1644	2.04
2010	294	16478	246	1.49

Source: Annual Epidemiological Bulletins (2001-2010) of Veterinary Epidemiology Center, Kathmandu, Nepal

Out of the possible seven, only four serotypes of FMD virus, e.g. O, A, C and Asia- 1 have been recorded in Nepal however since 1996, serotype 'C' has not been found in the country. Prevalence of serotypes of FMD virus from 2001 to 2010 revealed that serotype O is in increasing trend (82%) followed by serotype Asia-1 (15%) and serotype A (3%). Moreover, the results of genetic and antigenic typing of FMD virus isolates from Nepal submitted to WRLFMD, Pirbright, UK revealed that serotype O isolates from Nepal belong to the Middle East-South Asia (ME-SA) topotype. In 2003 both PanAsia and Ind2001 strains of serotype O were prevalent in Nepal. Likewise in 2007 PanAsia-2, in 2008 PanAsia-2 and Ind2001 strains of serotype O were found. In 2009 and 2010 Ind2001 strain of serotype O was found. According to the Pirbright laboratory based on Virus Neutralization Test, FMD virus strain O BFS or O IND R2/75 included in the vaccine is likely to confer protection against O type in Nepal.

Vaccination coverage against FMD is very low in the country. FMD vaccine is not produced in the country. The formal import record of FMD vaccine is only 0.15 million doses in the year 2010 (CAQO, 2010). This clearly shows that the practice of FMD vaccination is very low.

Peste des Petits Ruminants (PPR)

PPR is a highly contagious and infectious viral disease of domestic and wild small ruminants. The PPR entered in Nepal in the year 1995. The adverse impact is increasingly serious causing high morbidity and mortality in small ruminants. From 2001-2009, there were 2547 outbreaks of PPR in the country (VEC, 2010).

Though PPR vaccine is produced in the country since the year 2000, coverage of vaccination is too small to minimize the number of outbreaks. Compared to the 9.27 million goat and sheep in the country the vaccination coverage/year presented in table-2 is not even one third of the total population except in the year 2001/02 (Khatiwada, 2011).

Table 2: Goat and sheep vaccinated against PPR under national PPR control programme

S. N.	Fiscal Year	Vaccination (million doses)
1	2001/02	3.2
2	2002/03	2.4
3	2003/04	2.7
4	2004/05	2.5
5	2005/06	1.0
6	2006/07	1.0
7	2007/08	1.0
8	2008/09	1.2
9	2009/10	1.5

Avian Influenza

The first outbreak of highly pathogenic avian influenza (HPAI) was reported in backyard chicken in Jhapa district of eastern Nepal on 16th Jan, 2009. Second outbreak was reported in a different village of the same district in backyard chicken on 20th Feb., 2009. The HPAI outbreaks in 2010 were confirmed in Kaski district and then in Rupendehi, Chitwan, Dang, Nawalparasi, Banke and Kailali districts.

Table 3: HPAI outbreaks in Nepal in 2009 and 2010

S.N.	District	Date	Cases	Destroyed	Total	Type of birds
1	Jhapa	16-Jan-09	14	24689	24703	Backyard birds
2	Jhapa	20-Feb-09	150	2871	3021	Backyard birds
3	Kaski	26-Jan-10	153	11128	11281	Backyard ducks, chicken & commercial birds
4	Banke	4-Feb-10	351	286	637	Backyard birds
5	Chitwan	16-Feb-10	30	194	224	Backyard birds
6	Rupendehi	16-Feb-10	256	358	614	Backyard birds
7	Dang	25-Feb-10	2	0	2	Backyard birds
8	Kailai	2-Mar-10	40	83	123	Backyard ducks & chicken
9	Nawalparasi	8-Mar-10	216	4551	4767	Backyard birds
10	Chitwan	28-Oct-10	66	11437	11503	Commercial Layers
	Total		1278	55597	56875	

Source: Pathak, 2011

The H5N1 viruses identified in Nepal was of clade 2.2 in 2009 outbreak. In 2010 outbreaks clades 2.3.2 and 2.2 were identified. So far no human cases due to H5N1 have been reported in the country. Nepal is at high risk of HPAI because both migratory bird and illegal importation of poultry seems to be responsible for the outbreaks.

Haemorrhagic Septicemia (HS)

HS has been reported from all parts of the country throughout the year. HS is regarded as one of the important diseases of farm livestock and is a serious cause of concern for farmers as the infected animals succumb to death. Pasturellamultocida of serotype B:2 was isolated from investigated outbreaks in western hills of Nepal (Joshi and Joshi, 2003). Some information on HS outbreaks from 2001-2010 is presented in table 4.

Table 4: HS outbreaks from 2001-2010

Year	No. of Outbreak	No. of Cases	No. of Death	Case Fatality %
2001	1041	9949	1297	13.03
2002	878	7223	857	11.86
2003	768	4321	557	12.89
2004	665	11692	487	4.16
2005	532	5337	457	8.53
2006	668	2492	310	12.43
2007	442	2784	577	20.72
2008	335	2121	205	9.66
2009	163	1170	137	11.7
2010	272	3002	139	4.63

Source: Annual Epidemiological Bulletins (2001-2010) of Veterinary Epidemiology Center, Kathmandu, Nepal

Month-wise pattern of HS shows that this disease is prevalent throughout the year. However, there are more instances of the disease in the months of June to August which coincides with the rainy months of Nepal (VEC, 2011).

The HS vaccine is produced in the country and also imported from foreign countries. Although routine HS vaccination is done in the dairy pockets in the crossbred animals however the coverage of vaccination in the local animals are at low level. The protective immunity of locally produced alum precipitatedvaccine was found to be for three months only (Joshi and Joshi, 2003). The strategic period for vaccination would be at the beginning of the summer and autumn seasons and oil adjuvant vaccine would be better than alum precipitated vaccine for preventive vaccination due to its longer immunity.

Classical Swine Fever (CSF)

In Nepal pig rearing represents an integral part of livestock industry and is one of the fast growing livestock commodities. There are tremendous scopes of pig farming in Nepal, many factors are however, responsible to hinder the sustainability of pig industry. Among them infectious diseases in pigs particularly CSF is a major constraint to pig production system in Nepal.

CSF has been in existence in the country for a long time and has reached endemic status. Every year outbreaks of CSF have been reported in one or other parts of the country. CSF is identified as causing substantial deaths in pigs. During 2000-2009; based on clinical sign and symptoms and occasionally on postmortem examination, a total of 184 outbreaks of CSF were reported in the country (VEC, 2010). However many outbreaks in the field go unreported making it difficult to know the actual status of the disease. Since 2010, the National FMD and TADs Laboratory, Kathmandu has started laboratory diagnosis of CSF using CSF virus antigen detection ELISA. So far CSF outbreaks in the pigs of Makwanpur, Bhaktapur and Kathmandu districts have been confirmed by the laboratory.

Systematic routine vaccination is an ideal approach to protect the pig population against this disease. Although CSF vaccine is produced in the country, the coverage of vaccination is at a small

scale. Major reason for the frequent outbreaks of CSF in pigs is due to irregular or not vaccinating the pigs against this disease. This might be because of lack of awareness about the preventive measures for CSF among the pig rearing farmers.

Newcastle Disease (ND)

The major constraint for production of backyard chicken of many developing countries including Nepal is the ND because this disease is capable of causing high mortality in unprotected flocks. Even the small scale broiler farmers ignore or are not aware of the importance of ND and do not vaccinate broilers against ND therefore outbreaks of ND have been frequently recorded in commercial flocks as well.

Table 5: Outbreaks of ND from 2001-2010

Year	No. of Outbreak	No. of Cases	No. of Death	Case Fatality %
2001	211	39319	7713	19.7
2002	190	61296	6123	10.0
2003	135	44447	6573	14.8
2004	48	57321	1239	21.7
2005	147	27479	3532	12.9
2006	87	36645	4940	13.5
2007	46	5500	1197	21.8
2008	31	16726	2585	15.54
2009	128	23968	5900	24.61
2010	310	112836	4356	3.86

Source: Annual Epidemiological Bulletins (2001-2010) of Veterinary Epidemiology Center, Kathmandu, Nepal

Sheep and Goat Pox

Sheep and goat pox is prevalent in the in the country causing high mortality in kids and posing severe economic losses due to retarded growth and lowered quality of leather and wool. The outbreak of sheep and goat pox recorded during 2001 to 2010 is presented in table 6.

Table 6: Outbreaks of sheep and goat pox from 2001-2010

Year	No. of Outbreak	No. of Cases	No. of Death	Case Fatality %
2001	34	1245	81	6.5
2002	12	104	0	0
2003	14	102	1	0.98
2004	12	290	1	0.34
2005	29	604	30	4.96
2006	46	278	0	0
2007	4	100	0	0
2008	4	43	0	0
2009	10	34	0	0
2010	10	807	15	1.86

Source: Annual Epidemiological Bulletins (2001-2010) of Veterinary Epidemiology Center, Kathmandu, Nepal

Bluetongue in Sheep

Jha and Tamang (2009) reported that surveillance of bluetongue in sheep conducted in 11 districts

of Nepal; in 10 districts the sheep were positive for bluetongue virus (BTV) antibody with various prevalence rates. Further testing at the Pirbright Laboratory, UK revealed that from blood samples of sheep from Nepal, BTV was also detected by RT-PCR and the isolates were confirmed to be BTV serotype 23.

ECONOMIC IMPACTS OF TADs IN NEPAL

Production and productivity

The economic impacts of TADs can be complex and go beyond the immediate impact on the directly affected agriculture producers. All TADs have the potential to kill the affected animals, but the severity of the disease will vary depending upon the factors such as animal species, breed, age, nutrition, disease agent etc. Many TADs have 50-90% mortality rates in susceptible animals (Otteet al, 2004).

Infectious diseases of livestock form one of the main causes of reduced livestock production, mainly through mortalities and productivity losses. Moreover, cost involved in treatment is huge financial burden. These losses pose threat to managing rural farmers' livelihood means (Lohani and Rasali, 1993). A conservative estimate shows that HS causes an annual loss of NRs. 40.8 million to Nepalese livestock sector (Lohani&Rasali, 1993). Economically, HS is regarded as one of the important diseases of farm livestock and is a serious cause of concern for farmers as the infected animals succumb to death.

The economic importance of FMD due to the loss of productivity following infection is enormous. Infection in draft cattle causes a serious problem in tillage on which the preparation of agriculture land is fully dependent. In dairy cows sharp drop in milk production, abortion and chronic mastitis is very common. In suckling calves the mortality rate is even high. Various study reports clearly indicated that FMD is the major livestock diseases that cause enormous economic losses in the livestock production system. Though economic losses due to FMD has not been systemically quantified in Nepal, an economic loss in terms of 20 % reduction in milk and 10 % reduction in meat production is estimated to be 66 million US $ per year (Gongal, 2002). But the actual economic loss would be much higher if the reduction in breeding efficiency and draught power of animals are added. A study of the economic impact of livestock diseases in rural areas of Nepal estimated that FMD could account for 26% of the overall economic losses in livestock production (Lohani and Rasali, 1993).

Mandal (2010) conducted a study on evaluation of economic losses due to FMD in 2009 in Rajbiraj municipality of Saptari district and Duhabi and Mahendranagar VDC of Sunsari district. The economic loss was calculated taking into consideration of milk loss for a period of 1 month after the onset of the disease outbreak. The loss in milk production was found to be 31.13%.

Since the entry of PPR in Nepal there have been great losses to the goat and sheep rearing farmers. Most of the goats are reared by rural poor farmers. The high morbidity and mortality rate of this disease has caused enormous losses however there is no any literature available on the actual losses so far due to PPR in the country.

Price and market effects

In addition to impacts on production, there can be variations in prices, which are determined by the supply and demand effects induced by the TADs. Market effects can similarly induce variations in wages for farm and processing employment and can otherwise spread through to upstream and downstream activities. Depending on the market for the affected agricultural products, an affect of outbreak can lead suddenly to higher prices if most production is domestically consumed, or to lower prices if most production is exported and quarantine prevents such export but not domestic consumption (Otte*et al.*, 2004).

Price and market effects of H5N1 outbreaks in 2009 in Nepal:

When the first outbreak of H5N1 was confirmed in Nepal in 2009, there was decrease in consumption of poultry products; price of chicken was down from NRs 230 to NRs 170 per kg immediately after government confirmed the first case of avian influenza in Nepal. In some places even the price of the poultry meat went down to NRs 120-130 per kg. Poultry farmers also refrained from rearing the birds. Later on the chicken prices had touched an all time high of NRs 250 per kg in June 2009 due to sharp decline in poultry farming after the outbreak of H5N1 bird-flu in the country in January (MDTL & PACL, 2011).

When the government banned importing poultry products from India after the detection of bird flu there, hatcheries started buying parent chickens from third countries. There was the availability of about 35 to 40 percent parent chickens only in the country. Low production prevailed for about next three months. Many farmers did not produce chicks for weeks till the situation returned to normalcy (Republica, 2009).

Trade (national and international)

India and China are Nepal's biggest trading partners. Cattle, buffaloes and goats are imported for milk or meat production, draft power and breeding. Eggs, fish, hide and skins are major livestock products that are traded with India. Mass movement of live animals across the Indo-Nepalese border and uncontrolled animal movement within the country have been responsible for outbreaks of FMD, PPR and CSF and other TADs. The pattern of disease outbreak is mostly related to the frequency of animal movement across the border and within the country.

As Nepal is a member of World Trade Organization, TADs are a major barrier in international trade of livestock and its products. Due to presence of FMD in Nepal, China did not allow to enter Nepalese dairy products into Tibet during 2002 (Thakuri, 2006).

The livestock sector is facing a major challenge in cross-border collaboration due to unregulated and unchecked animal movement both within the country and across international borders. The predominance of informal trading practices for livestock and livestock products and lack of standardization and absence of harmonized practices in matters related to information, laboratory, quality control and quarantine systems are also discernible challenges.

Food Security and Nutrition

Trans-boundary animal diseases can often have significant negative impacts on food security and nutrition in developing countries. The growth of international trade in agricultural produce buffers the potential impacts on food availability, but there can still be major impacts on poorer communities that do not have access to substitute supplies (FAO, 2001).

Agriculture is the main source of livelihood for over three quarters of the population in Nepal. The shortage of fodder and lack of easy access to veterinary services, combined with animal disease outbreaks, continually threaten the food security of families reliant on livestock, a vital livelihood asset for rural communities, especially the landless (FAO, 2010).

Every year various infectious disease outbreaks such as FMD, PPR, CSF and HS hit the livestock and cause high case fatality. There is no doubt that these outbreaks affect on various aspects including food security and nutrition situation especially in poor and rural areas. However the actual socioeconomic impacts due to TADs in Nepal still needs to be evaluated.

Health and Environment

The main threat to human health arises from zoonotic diseases. Such diseases appear to have increased in recent years, the human deaths associated with HPAI in South-east Asia have shown that HPAI can also be a great threat to public health and could become a major human pandemic.

In Nepal rabies, anthrax and tuberculosis are major zoonotic problems. Fortunately no human case due to HPAI was recorded in the country but threat has not become over because of the antigenic changing pattern of the avian influenza virus.

Increasing concern is arising over threats to the environment, either from TADs themselves or from the control measures used against them. Control measures have become a matter of serious concern since attention has focused on pesticide dangers and stockpiles of unused pesticides (FAO, 2001).

Livelihood and Employment

Livestock are important in supporting the livelihoods of poor farmers, consumers, traders and labourers throughout the developing world. The greatest impact of livestock in sustainable development designed to help the poor is enhancement of livestock-production systems. Animal diseases are crucial constraints in this: the animals of poor people are particularly vulnerable to disease because of the expense, absence or unsuitability of animal-health and production inputs. Poor farmers have few animals and few reserves on which to survive during lean times and use for recovery, so the loss of individual animals has a proportionally greater impact (FAO, 2002).

Epidemic diseases threaten national livestock industries by direct effects. These include high levels of morbidity and mortality, control or eradication programme costs and restrictions to trade in livestock and livestock products. Livestock producers, workers in livestock industries and consumers are all affected. Animal diseases have multiple direct and indirect effects on human welfare. Village chickens are owned throughout the developing world by poor farmers, for whom ND is a major constraint (FAO, 2002).

In Nepal, 66% of the populations are directly engaged in agriculture. The agriculture is mainly subsistence-oriented, and crop-livestock or crop-livestock-forest integrated farming systems. Majority of the poor farmers in the rural areas have a few small ruminants, poultry and/or pigs. Mostly the prosperous farmers have large ruminants but the poor farmers may have a few as well. Livestock are crucial source of financial capital for the rural poor. For many, livestock ownership is the only form of shaving available. Livestock can be a critical reserve against emergencies and decrease vulnerability to financial shocks from ill health, crop failures and other risks. Therefore looking at the importance of livestock for the rural farmers it is quite obvious that the occurrence of TADs in livestock can lead to heavy economic losses and also create negative social impact on livelihood and employment of the people.

Financial Costs

The financial costs can include disease surveillance cost, vaccine production and vaccination cost, bio-security and management cost and compensation cost etc. The major activity for the control and prevention of TADs require surveillance program which helps for early detection and early response. In Nepal although there are various TADs prevalent in the animal population and disease outbreak occurs time and again but the epidemiological surveillance is limited to only few diseases e.g. HPAI.

Better prevention and control of trans-boundary animal diseases can be achieved when public services and producers work together to include practical and cost-effective bio-security measures in all good animal production management practices (FAO, 2010).

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PREVALENCE OF SALMONELLA IN GOAT MEAT IN KATHMANDU VALLEY

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ABSTRACT

A qualitative bacteriological study was conducted in Kathmandu valley with the objective of finding out the prevalence of Salmonella in goat meat from different meat shops of Kathmandu valley.Out of 101 samples,13.86% were positive for Salmonella spp.The prevalence of salmonella indicates the unhygienic handling of meat and risk to the consumers.The result of this study did not meet the acceptance level of goat meat given by Sharma (1999) and standards set by ISO in which Salmonella spp. should not be present in 25 gram of meat sample. All these findings show unsatisfactory sanitary condition in the local meat markets of Kathmandu valley.

INTRODUCTION

Meat and meat products originating from all domestic farm animals except cattle are consumed in Nepal. Among the farm livestock reared by the farmers in Nepal, goats, pigs and boilers poultry are reared specifically as meat animals (Joshi & Shah, 2003). The per capita meat consumption is 8.5kg. Meat consumption in urban areas, particularly in the major cities is much higher than the national average, although meat consumption pattern is not similar throughout the country (Shankhi, 2006).

Meat and other meat products contribute significantly to high incidence of food borne disease and zoonotic diseases. Microbes in meat and meat products are the main causes of food borne diseases in human. The surfaces of raw meats are contaminated with variety of microorganisms. The magnitude of this microbial contamination reflects the microbial population of the environment from where the meat was taken, the method of handling, the time and condition on raw meat. Poor slaughtering facilities and meat handling practices contribute greatly to the spread of disease. Further, poor sanitary condition at herd or flock level, improper screening of disease animals, lairage of abattoirs with deteriorating conditions are very much suitable for the growth of microorganisms and cross contamination of carcass. The presence of extremely large numbers of microorganism suggests that some undesirable events have occurred and that the meat and meat products are indeed susceptible to further deterioration (Prasai, 2001).

Meat serves as an excellent medium for the growth of microorganisms. Bryan, (1973) listed approximately 200 diseases that transmitted to man by foods. The lists of pathogens which can be transmitted from animals to humans by food contain about 16 kinds of bacteria, 3 groups of viruses, 22 parasites and 5 protozoa (Singh *et al.*, 1995). The microorganisms responsible for lowering the sanitary quality of meat are mainly derived from external environments. The pathogenic bacteria like Escherichia coli, Salmonella spp., Clostridium spp., Staphylococcus spp., Campylobacter spp., etc. not only spoil meat but also cause food poisoning and other illnesses to consumers. Measures

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should be adopted in such a way that these organisms should not be present in meat and meat products (Adams *et al.*, 2003).

Access to safe and wholesome meat is a pre-requisite for sound health and well-being of people but in current situation of Nepal the quality of meat is very poor. Fresh meat butcher shops are the major retail outlets for meat in Nepal. Main contamination is through unclean water used while transporting and slaughtering. Due to the insufficient supply of clean water in Kathmandu valley, butchers frequently use untreated ground water for meat processing (Prasai, 2001). Nepal has already become a member of the World Trade Organization (WTO). So, safe and wholesome meat is a must in order to be able to compete in the market. Therefore the meat should meet the standards established internationally as per the sanitary and phyto-sanitary (SPS) agreement. The quality management systems like ISO and Hazard Analysis and Critical Control Point (HACCP) are still not followed effectively in Nepal (TLDP, 2003).

As Nepal has not established any standards for goat meat, it is very important to conduct several researches in this area which ultimately may help establish standards acceptable in Nepalese market. Microbiological aspect is the important factor to study the quality and safety of meat. Only few studies have been conducted on the microbial load in goat meat so far. The present study has mainly focused on the assessment of microbial load as well as isolation of some organisms. The study also compares the microbial load in the samples with international standards.

METHODOLOGY

Altogether 101 dressed goat meat samplesfrom different meat shopsof Kathmandu, Bhaktapur and Lalitpur districts of Kathmandu Valleywere collected randomly.All the laboratory analysiswereundertaken at the microbiology laboratory of Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan. A quantity of about 200 gms of cut meat samples were collected and labeled accordingly from the different regions of thecarcass, such as brisket, neck and thigh in a sterile plastic bag kept in ice box in the morning time and finally carried to the laboratory for the further processing.Bacterialflora was isolated from samples according to the methods described in theBacteriological Analytical Manual of the Food and Drug Administration (updatedDecember, 2007).

Isolation of Salmonella

Twenty-five gms of meat samples were minced in mincing machine aseptically into small pieces. Five gms of the homogenized sample with 25ml buffer peptone water was incubated at 370 C for 24 hours for pre-enrichment media.One milliliter of pre-enriched sample was poured into 9ml of Selenite F-broth and incubated at 370 C for 24 hours for enrichment.One loopful of the enriched sample from Selenite- F broth was inoculated in selective media i.e. BGA (Brilliant Green Agar) and one Petri dish was kept as control. Then Petri dishes were incubated at 370C for 24 hours. After overnight incubation, the red colonies from BGA were picked up andre-culture was done on Nutrient Agar for the isolation of pure culture and incubated at 370C for 24 hours.Gram's staining of the pure isolate from the Nutrient Agar plate was also done to study on morphology of the cultured organisms.

Biochemical tests

The organisms were inoculated in various biochemical mediaand the results were observed on following day. The typical non-lactose fermenting colonies for the conformation of Salmonella spp. were used from the selective media. TheIndole production test, Motility test, TSI test and Citrate utilization testof more than one colony were done separately from single plate. The biochemical test guideline is given in Table no. 1.

Table1: Biochemical test guideline for the identification of Salmonella spp.(Merchant & Packer, 1983).

Bacteria	Motility	Indole	Citrate	Glucose	Lactose	Sucrose	H2S
Salmonella	+*	-	+	AG	-	-	+

+ = positive reaction or fermentation; -= negative reaction; AG = production of acid & gas.

* S. pullorum and S. gallinarum are non-motile.

RESULTS

Out of 101 samples, 68 samples were found positive for non-lactose fermenters among which 14 samples were positive for Salmonella spp. and remaining 54 were non-lactose fermenting bacteria other than Salmonella spp.

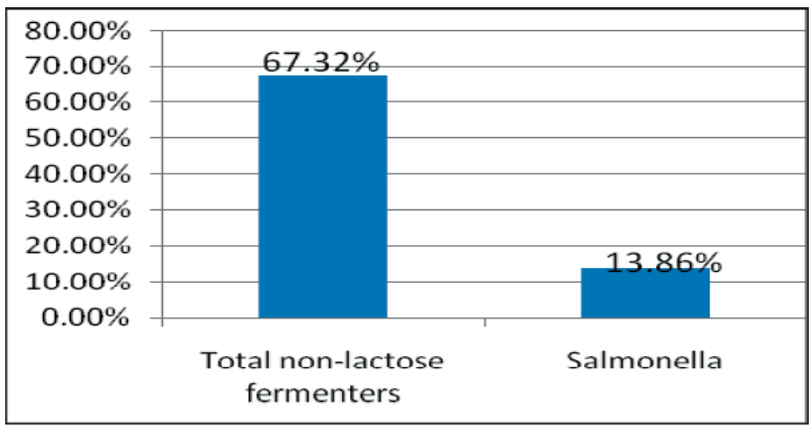

Figure 1: Prevalence of Salmonella spp. and other non-lactose fermenters

DISCUSSION

The prevalence rate of Salmonella spp. in Kathmandu valley was found to be 13.86% which was close to the results given byMaharjan (2009), i.e. 11.9%;Joshi (2003), i.e. 11.3% andHeredia (2001), i.e.11.4% but it is found to be higher than the result given by Manandhar*et al.*, (2006) i.e. 3.3% (1/31). The result of this study was lower than the results given by CFRL (2000), i.e. 22%, Shrestha (2005), i.e. 21% and NARC (1999), i.e. 22%.It might be due to the variation in the sample size, season and unhygienic practice of meat shop-keepers. Furthermore, the result of this study did not meet the acceptance level of goat meat given by Sharma (1999) and Standards set by ISO in which Salmonella spp. should not be present in 25 gram of meat sample which supports the result of Mukhopadhyay*et al.*, (2004) in which no Salmonella spp. were found in any of meat sample. It might be due to the reasons that the meat sellers are still not getting familiar to the quality of meat products and human health properly or they were ignoring this. All these findings had shown unsatisfactory conditions of sanitation in the local meat markets of Kathmandu valley.

CONCLUSION

Out of 101 goat meat samples from different meat shops of Kathmandu valley,13.86% were positive for Salmonella spp.On the basis of this study, it can be concluded that the degree of contamination is at rejection level from food safety point of view i.e. there is still a room for improvement in the practices and sanitation done in slaughtering and processing of goat meat in Kathmandu valley. The sources of contamination might be due to contaminated water used for dressing and washing of carcass, feces of the goat, environment or the unhygienic practice of the butcher itself for the materials or equipment to be used in the slaughter slabs.

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EPIDEMIOLOGY OF GASTROINTESTINAL HELMINTHS IN GOATS OF CHITWAN

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ABSTRACT

An epidemiological study was carried out during August to December 2011 to determine the prevalence and intensity of helminths infection in goats of Chitwan. Total 330 fecal samples, of which 180 in post rainy season and 150 in winter season, were examined. The fecal samples were examined qualitatively by differential floatation and sedimentation technique and Quantitative (egg per gram) examination was done by Stoll's counting method. A questionnaire survey was conducted among 30 randomly selected goat owners regarding management practices and use of anthelmintics. The study revealed 205 fecal samples out of 330 samples (62.12%) with significant EPG. The prevalence was significantly higher ($\chi 2=5.311$, $P<0.05$) in post rainy season (68.33%) than in winter season (54.6%).The prevalence was higher in adult and female animals. The Strongyloides (22.92%) was most common helminth followed by Haemonchus (20.97%), Moniezia (20%), Trichostrongylus (19.15%), Trichuris (16.58%), Fasciola (16.58%), Dictyoculus (12.19%), Nematodirus (10.24%), Paramphistomum (8.78%), Oesophagostomum (8.29%), Ostertagia (8.29%), Chabertia (8.29%), Dicrocoelium (4.39%) and Capillaria (1.95%). This finding shows that infection was high in goats with gastrointestinal helminths. The prevalence had a defined seasonal trend with higher prevalence during post rainy season. All ages of goats were affected with one or more helminths parasites. Most of the farmers are unaware of helminths parasite problems.

INTRODUCTION

Parasitic disease (gastrointestinal nematodes, liver fluke and external parasites) are regarded as the most important cause of reduced productivity among goats in Nepal. Parasitic diseases are ranked first by farmers and this view has been further supported by studies showing higher prevalence of parasitic infestation in goat. The problem of gastrointestinal helminths is easy to understand but difficult to mitigate. Various researches and studies have been conducted in the past regarding the problems of gastrointestinal parasites and their control but the problem of gastrointestinal (GI) parasitism is still very common and economically important. The major parasites of concern differ by the prevailing host animal species and climatic conditions in a particular geographic location and no farm animal species in general is free from GI parasitism. Due to lack of adequate studies on parasitic epidemiology and appropriate control strategies large number of goat population is harboring parasitic infestation. The loss due to death of animal and decrease in production is high in Nepalese context and the problem is overwhelming in small ruminants. Understanding of the biology and epidemiology of gastrointestinal parasites of goat will led to improvement in control measures and a decrease in population losses however, only a limited number of studies have been undertaken to provide information on the prevalence, distribution and epidemiology of various species of parasites in goat of Chitwan. This study is designed to determine the prevalence and intensity of gastrointestinal helminthes in goat in relation to season, host, age and physiological

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status and is an attempt to bridge the gap in knowledge of these aspects. This study on helminths will definitely aid to devise effective control strategies and it is advisable to plan effective integrated control strategies based on the epidemiological findings.

MATERIAL AND METHOD

This study was conducted from August, 2011 to December, 2011. Total of 330 fecal samples were collected from Shaktikhor and Phulbari VDCs and Bharatpur municipality of Chitwan district in two seasons: Post rainy and winter. Samples were collected from three age groups of goats: kids (< 4months), young (5-12 months) and adults (above one year) including males and females from all age groups. 180 fecal samples were collected in post rainy season (60 from each area) and 150 samples were collected from winter season (50 from each area). Samples were collected irrespective of use of anthelmintics use. The samples were collected in plastic zipper bag with cotton soaked in 3% formalin. Samples were processed in Parasitological Unit of Central Veterinary laboratory (CVL).

Fecal samples were analyzed qualitatively (floatation and sedimentation) and quantitatively (Stoll's method) as per the method given by Soulsby (1986).Helminths eggs were identified based on morphology of eggs (Foreyt WJ, 1997). 0n the basis of EPG, level of infection was categorized into 3 groups as light (100-500 EPG), moderate (100-500 EPG) and heavy (>1500 EPG) infection as given by Soulsby (1982) and samples with moderate and heavy infection were considered as significant. A questionnaire survey was conducted among 30 randomly selected goat owners regarding management practices and use of anthelmintics. The questionnaire also captured the status of the animal such as body condition or any other observed signs of ill health. The epidemiological risk factors captured were age, sex, location and season. The prevalence was calculated by dividing the number of samples showing significant EPG by the total number of samples examined. Percentage (%) was used to measure prevalence .The Chi-square test was used to evaluate statistical significance of the association between the risk factors and prevalence and/or the existence of clinical signs of parasitic infection at 5% level of significance.

RESULTS AND DISCUSSION

Overall prevalence

Of the 330 samples 205(62.12%) samples had significant EPG for one or more parasites. Among the samples having significant EPG 84 were males and 121 were females. All age groups were affected. The most common parasites encountered were *Strongyloides* (22.92%), *Haemonchus* (20.97%), *Moniezia* (20%), *Trichostrongylus* (19.15%), *Trichuris* (16.58%), *Fasciola* (16.58%), *Dictyocaulus* (12.19%), *Nematodirus* (10.24%), *Paramphistomum* (8.78%), *Oesophagostomum* (8.29%), *Ostertagia* (8.29%), *Chabertia* (8.29%), *Dicrocoelium* (4.39%), *Capillaria* (1.95%) and *Gigaria* (1.95%). Altogether 15 genera of helminthes parasite were found in present study among them one cestode (*Moniezia*), three trematodes (*Fasciola, Paramphistomum* and *Dicrocoelium*) and eleven were nematodes. *Capillaria* and *Gigaria* were only found in wet season.

The prevalence rate of Chitwan (62.12%) is almost similar to those reported by Ijaz *et al.* (2008) (63.33%) and Shirale and Maske (2007) (65.47%) but lower than that reported by Bashir (2009) (70.53%) and Parajuli 2007) (81.53 %) and was higher than the result given by Leonard (2009) (58%). The higher prevalence might be due to inappropriate and inadequate drenching practices, sharing of common pasture (forest), resistance due to indiscriminate use anthelmintics.

Prevalence by season

Table 1: Seasonal Prevalence of helminths infection.

Season	Total no samples	Positive	Negative	Prevalence (%)
Post rainy season	180	123	57	68.33
Winter season	150	82	68	54.6
Totals	330	205	125	62.12

There was definite seasonal variation in the occurrence of helminths infection as reflected by the fecal egg counts. The prevalence of gastrointestinal helminths was 68.33% and 54.6% respectively during post rainy and winter seasons. The proportion of samples that had a significant EPG during rainy season was significantly higher than during the winter season ($X2=5.311$; $P<0.05$) hence it can be concluded that the prevalence of helminths infection was higher during the rainy season as compared to the winter season. This is in consistent with previous findings (Basir, 2009; Madu and Richards, 2007). *Capillaria* and *Gigaria* were found only in post rainy season while all other 13 parasites were found on both seasons. *Strongyloides* was more frequent in post rainy season and *Nematodirus* in winter. This is in agreement with findings of Di Gebro *et al.*, (2006).

The prevalence was higher during the post rainy season as compared to winter season. The relative humidity and warm temperatures seemed to provide condition favorable for the development of pre-parasitic stages of nematodes and intermediate host (snails) of flukes. This is in agreement with findings; total gastrointestinal helminthes burden (Basir, 2009) and the fecal egg counts (Nwosu *et al.*, 2007) were positively related to climatic conditions, especially rainfall and relative humidity. Shirale and Maske (2007) also found high helminths infection during monsoon season. The presence of infection in the goats even during the winter season when environment conditions preclude the development and survival of their pre-parasitic stages could be an indication of persistence of the adult stage within the host

Prevalence by age

Table 2: showing age wise prevalence of helminths infection.

Age	Total sample	Total positive	Total negative	Prevalence (%)
0-4 months	76	45	31	59.21
5-12 months	121	71	50	58.68
>12 months	133	89	44	66.92
Total	330	205	125	62.12

The prevalence of helminths infection in the table in absolute figures reflects a higher occurrence in the adults followed by the kids with young goats having the least. While the absolute figures might seem to indicate otherwise, the prevalence of helminths infection in this study did not show any statistical significant($X2 =1.27$; $P>0.05$) trend related to the age of the goats. This is in contrast with the findings of Boomker *et al.* (1994) who found that the prevalence was inversely related to age, however, agrees with those of Basir (2009) and Magona & Musisi (2002), who also found that age does not play a major part. *Moniezia*, *Trichuris* & *Strongyloides* were more prevalent in kids and young goats while *Fasciola*, *Haemonchus* & *Trichostrongylus* were more prevalent in adult goats.

Prevalence by sex

Of the 330 goats samples collected, 140 were males and 190 were females. 84 males and 121 female were positive for one or more parasites respectively.

Table 3: Showing sex wise prevalence

Sex	Total sample	No. positive	No. negative	Prevalence (%)
Males	140	84	56	60.71
Females	190	121	69	63.68
totals	330	205	125	62.12

While the absolute figures indicates a higher prevalence in female goats, there was no statistically significant difference in the prevalence of helminths infection between males and females (X2=0.176; P>0.05). This contradict the finding of Urquhart *et al.*(1988), who reported the existence of some evidence that entire male animals were more susceptible than females to some helminths infections.

Prevalence by location

Table 4: Location wise prevalence

Location	Total sample	No. positive	No. negative	Prevalence (%)
Shaktikhor	110	71	39	64.54
Bharatpur	110	62	48	56.36
Phulbari	110	72	38	65.45
Totals	330	205	125	62.12

The prevalence was highest in Phulbari followed by Shaktikhor and least in Bharatpur. *Fasciola* & *Haemonchus* were more common in *Phulbari* area whereas *Moniezia* & *Trichuris*- were more common in Bharatpur area and *Dictyocaulus*, *Haemonchus*, *Nematodirus* and Trichostrongylus were more common in Shaktikhor area. No trematode parasite was found in Shaktikhor area & *Strongyloides* was found in all areas. The lower prevalence of helminths infection in Bharatpur area may be due to better availability of veterinary care and facilities.

CONCLUSION AND RECOMMENDATIONS

Infection with gastrointestinal helminths is inarguably the most important constraint to goat production in Chitwan. Goats are reared in semi-intensive farming system. The hot and humid climate, lowlands and forest based grazing practices in Chitwan significantly contributes to helminths infection in goats. Similarly lack of deworming in some places and inadequate and inappropriate deworming practices in other places always makes goats vulnerable to helminths infection due to higher level of contamination from pastures and forages. The present study showed that major helminths parasites belonging to genera *Strongyloides*, *Haemonchus*, *Trichuris*, *Trichostrongylus*, *Nematodiurs*, *Dictyoculus*, *Fasciola*, *Paramphistomum*, *Moniezia*, *Oesophagostomum* and *Chabertia*. The prevalence had a defined seasonal trend with higher prevalence during the wet season. There was however, no evidence to suggest that age and sex had any influence on the prevalence of infection. Management practices and different locations influence the prevalence of gastrointestinal helminths infection in goats.

The result of this study implies that more emphasis should be given to regular deworming in goats. The farmers should be made aware of harmful effect of parasites and benefits of deworming. As goats have been infected with various helminths parasites, the anthelmintics should be selected accordingly. Various trainings and awareness programs relating to management and feeding aspects of goat farming should be provided to farmers. The concerned authorities like DLSO should take initiatives for providing better veterinary facilities and drugs. The efficacy of marketed anthelmintics drugs should be regularly tested to ensure their effectiveness. Similarly the susceptible host like kids, pregnant and lactating animals should be housed separately. The goats shouldn't be grazed in low and marshy lands and forages from such area should be properly dried before feeding to animals. In light of this study there is need of formulating and following

appropriate deworming schedule and there should be continuous monitoring of worm burden in goats. In addition, such kind of studies should be carried out in other areas including larger sample size to determine the actual picture of helminth infection in animals.

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ASSESSMENT OF SUB-CLINICAL MASTITIS AND MANAGEMENT ASPECTS OF DAIRY ANIMALS: A CASE STUDY OF LAMJUNG DISTRICT

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ABSTRACT

A community cross sectional study was conducted in two VDCs viz: Chandreshwor and Archalbot of Lamjung district to assess sub-clinical mastitis and management practices of dairy animals from August to December, 2011. A total of 63 dairy livestock were selected randomly and questionnaire survey was conducted to owner of each animal. About 10 ml of milk sample from each quarter was collected in a sterilized syringe, labeled and dispatched for further investigation. California Mastitis Test (CMT) was performed in the farmer's shed and cultural examination was conducted on Nutrient agar and Mac Conkey agar plate in laboratory. Cultural isolates were identified on the basis of colony characteristics, Gram's staining and various biochemical tests. From the study on management of dairy animals, 14% were found on grazing system and rest 86% were on zero grazing system and 5% were with regular mineral and vitamin supplement. 30% (19) of the farmers had forage trees in their land, however, 70% (44) didn't have. Only 18 (29%) farmers had known about mastitis and 45 farmers (71%) didn't have any knowledge about mastitis. The average milk production of dairy animal was found to be 3.51lt ±1.47.In California Mastitis Test, on animal basis, 46.1% (29) were found positive & 53.9% (34) were negative. On quarter basis, 76(30.2%) were found to be positive and 176(69.8%) were negative. Culture report showed that prevalence rate was 28.6% on the basis of animal and 24.2% on the basis of quarter. Streptococcal mastitis has the highest prevalence rate (11.1%) followed by Coliform mastitis (9.5%) and Staphylococcal mastitis (7.9%). The research shows that, prevalence rate was the highest in left fore and right hind quarter (25.4%) followed by left hind (23.8%) and then right fore quarter (22.2%). Prevalence rate for Coliform & Staphylococcal mastitis was the highest in left fore quarter and right hind quarter respectively. In Chandreshwor VDC, Gentamicin, Amikacin and Norfloxacin were 100% sensitive, Tetracycline was 90% sensitive followed by Chloramphenicol (80%,) Ceftriaxone (60%) and Cephalexin (50%). In Archalbot VDC, Chloramphenicol, Amikacin, Norfloxacin, Tetracycline and Gentamicin were 100% sensitive, Cephalexin was 50% sensitive followed by Ceftriaxone to be 25% sensitive. The association between prevalence of subclinical mastitis & production of milk more than 3 lt per day was found highly significant($p<0.01$). The dairy animal management was found poor. Sheds were unhygienic, unscientific and people were unknown about mastitis.

Key words: *Subclinical mastitis, management, dairy animal, antibiotic sensitivity test, Lamjung.*

INTRODUCTION

Livestock production, one of the major components of Nepalese mixed farming system contributes 31% in the national Agricultural Gross Domestic Product (AGDP) (CBS, 2009). Buffalo milk production contributes nearly 72% (1,031,500 MT) and cattle milk contributes nearly 28% (413,919 MT) in total milk production of the country in the fiscal year 2008/09 (MOAC, 2008/09). Population of cattle in Lamjung district in the fiscal year 2009/10 was 37,272 compared to 37,170 in the previous

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year. Among them, 36,817 were local breed, 257 were cross-breed and 198 were exotic breed. The population of buffalo in the same year was 49,783 compared to 51,123 in the previous year. Among them, 44,623 were local breed, 4,479 were cross-breed and 681 were improved breed. The total number of milking cattle and buffalo in Lamjung district were 7,991 and 19,969 in the fiscal year 2009/10 (DLSO, Lamjung, 2066/067). Total milk production in Lamjung district in the year 2008/09 was 13099 MT with 3519 MT cow milk and 9580 MT buffalo milk (MOAC, 2008/09).

Mastitis is one among the top three threats faced by the farmers in terms of economic loss, our cattle and buffaloes could definitely not stay apart. Traditional farming knowledge has further made the conception of this disease narrow and eventually the losses go unnoticed. Approximately 10% of total value of milk sale is lost each year as a result of decreased milk production, increased milk replacement cost, discarded milk, drug costs, veterinary fees and labor costs (DeGraves & Fetrow 1993; NMC 1996).The cost of clinical mastitis has also been estimated to be $107 US per clinical episode with over 70% of the cost associated with decreased milk production and milk withheld from the market, over 20% with drugs, veterinary costs and replacement costs, and the remainder with labor (Smith & Hogan 1993; DeGraves & Fetrow 1993; NMC 1996). In Nepal, according to Dhakal & Thapa (2000), largest proportion of losses in milk results from decreased milk production i.e., Rs. 4287 or USD 63 per buffalo per lactation. Milk loss was found 11% of average total lactation yield. Expenditure in medicine accounts 34% of the total treatment and management cost.

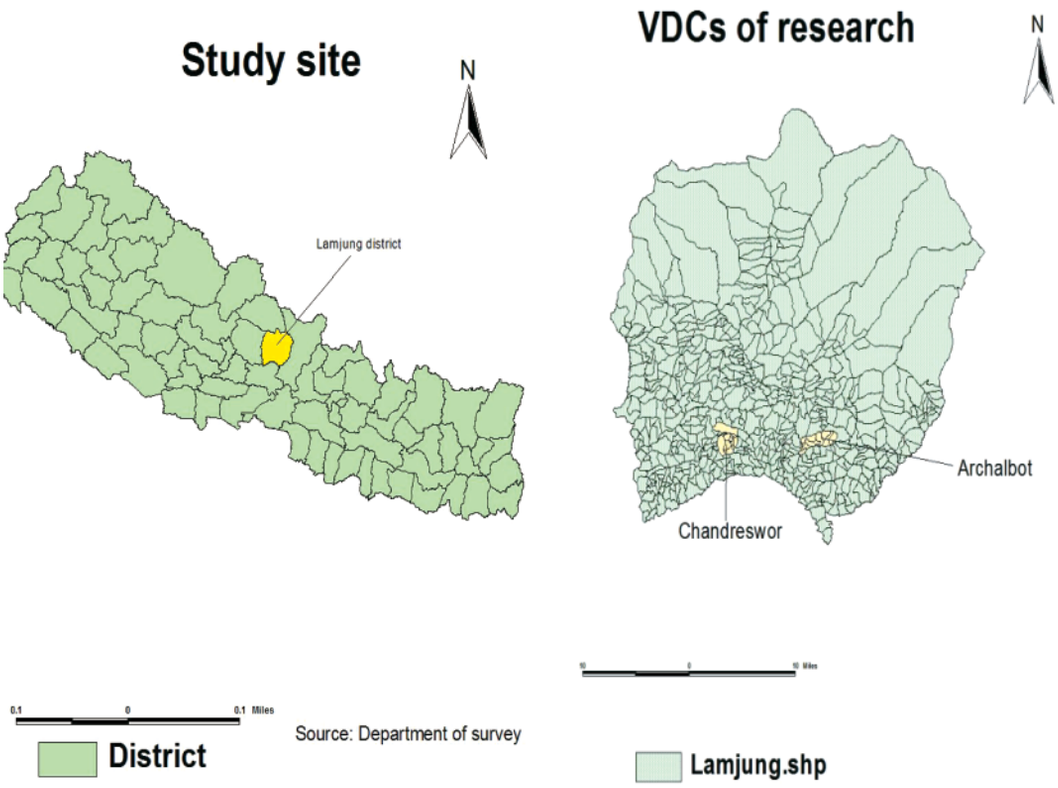
In most countries, surveys in dairy herds indicate that prevalence of mastitis is about 50% of cows and quarter infection rate of about 25% (Radostits *et al.*, 2010). The average annual incidence rate of clinical mastitis, calculated as the number of clinical quarter cases per 100 cows at risk per year including the dry period in individual herds ranges from 10-12% in most herds but higher values, ranging from 16-65% occur in some herd (Radostits *et al.*, 2010). Dhakal *et al* (2007) reported the maximum numbers (16%) of clinical mastitis in July, when temperature and humidity were highest. Coagulase negative *Staphylococci* (CNS) such as *Staphylococcus albus* and *S. epidermidis* were the predominant organisms associated with subclinical mastitis. However, CNS and *Coliforms* are predominant in clinical mastitis. Enrofloxacin had the highest sensitivity (91%) for all types of bacteria followed by Gentamicin (87%), Tetracycline (83%) and Chloramphenicol (82%). Adhikari *et al.* (2009) reported that prevalence of sub-clinical mastitis (SCM) in Chitwan district was 40% in cattle and 32% in buffalo. The predominant organisms causing mastitis in Chitwan district were Coliforms (37.7%) followed by Staphylococcus spp. (28.8%), *Streptococcus spp*. (13.3%) and *Pseudomonas spp*. (4.4%).

Animals raised by the farmers are facing constant risk due to poor management practices, lack of proper nutrition, disease outbreaks and several other factors. So, animal health and proper management practices are the most important components for rearing the livestock. The feeding system in Nepal differs from region to region. In the eastern hills, a cow was fed an average of 25 kg of green grass, 5 kg of dry grass, and 2.24 kg of concentrates per day. In the central hills, an adult animal is provided with 30 kg of grass and 25-30 kg of fodder leaves per day. In the western hills, forage is mostly home grown and the forest resources contribute only 3-14% according to season. Rice straw is the main crop by-product fed during winter and maize stovers during the rainy season. Lactating animals are given about 1.5 kg of homemade concentrate feed daily during the lactation period. Commercial feed was only purchased by those farmers who are rearing improved breeds (Tulachan *et al.*, 2002).

MATERIALS AND METHODS

Study site

This study was conducted in Archalbot and Chandreshwor VDCs of Lamjung district, which are pocket area of milk production. The Laboratory analysis of the collected milk samples was carried out at Bacteriology unit of the National Avian Laboratory (NAL) Bharatpur, and Veterinary Teaching Hospital (VTH), Chitwan.



Questionnaire Survey

To assess the management aspects and its possible impact on SCM, questionnaire survey was conducted with each farmer. Individual cattle & buffalo from farmers' shed were selected and other relevant information was recorded.

Sampling Technique and laboratory examination

Samples were collected from the dairy animals. Samples representing all the wards of Archalbot and Chandreshwor V.D.Cs were collected. Out of those, 30 animals from each V.D.C were selected using purposive random sampling method. About 10 ml of milk sample from each quarter was collected in a sterilized syringe. They were numbered and marked as left front (LF), left hind (LH), right front (RF) and right hind (RH) respectively. The diagnostic tools used for mastitis were California Mastitis Test (CMT), Cultural Examination and Biochemical Tests. All samples were subjected to cultural examination on Nutrient agar, Mac Conkey agar plate & EMB media. They were incubated at 37°C for 24 hours. Cultural isolates were identified on the basis of colony characteristics, Gram's staining and biochemical tests (Catalase test, Oxidase test etc). Antibiotic sensitivity test was done using Disc diffusion method as described by Chakraborty (2003).

RESULT

During this study period, out of 63 animals 46 were in early lactation and 17 were in late lactation period. Among them, 8 were of first parity, 16 of second parity and 39 were belonged to third parity or more. Most of the animals were found to be stall fed. Only 14% were found on grazing system and 86% were on zero grazing system. In total, only 5% of the animals were supplied with regular minerals and vitamins, 24% were with the occasional supplementation but 71% had never been provided with any kind of vitamins and minerals. Of total farmers, 19 (30%) were with fodder trees in their land, however, 44 (70%) were without. Those with fodder trees haven't been suffered from scarcity of feed during winter but those without were suffered. All of the households

were found to do deworming of their animals in every 6 month. The average milk production per lactation in dairy animals of the VDCs under study was found 3.99± 0.18 lt with highest frequency of 4 lt. Significant numbers of farmers were found to have any knowledge on mastitis (45 out of 63). Regarding SCM, the following table presents the prevalence.

Table 1: Prevalence of subclinical mastitis in dairy animals of Lamjung district.

CMT Result	Number of animals(63)			Number of quarters(252)		
Negative	19(55.8%)	15(51.7%)	34(53.9%)	81(69.8%)	95(69.8%)	176(69.8%
Positive	15(44.1%)	14(48.3%)	29(46.1%)	35(30.2%)	41(30.2%)	76(30.2%)
Total	34	29	63	116	136	252

The research shows that, prevalence rate was similar in all quarters. The geographic and quarter wise distribution of mastitis is demostrated below in figures.

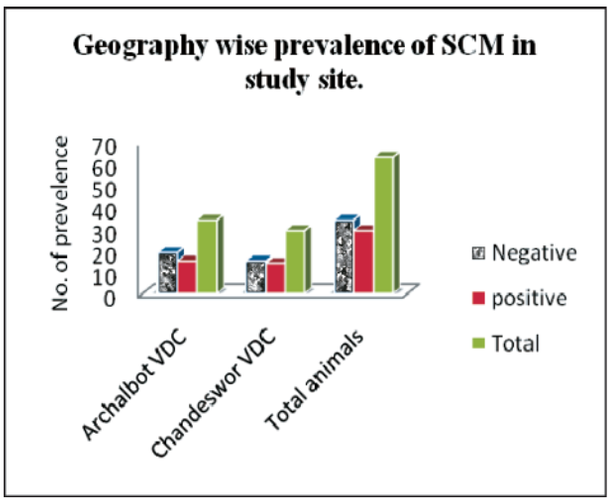


Fig. 1: Prevalence of SCM in two VDC of study site

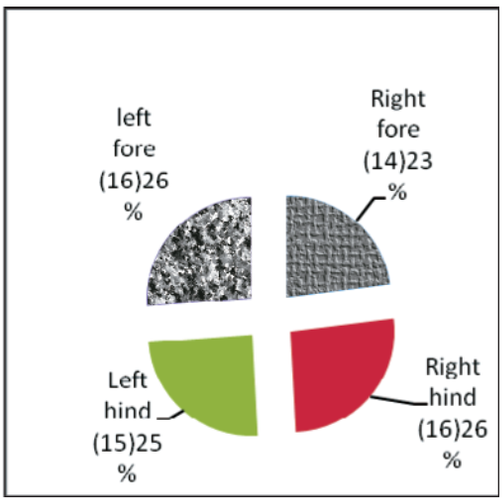


Fig.2: Quarter wise distribution of mastitis

The result of bacterial isolates showed that the highest prevalence was of Streptococcus (43%) followed by E. coli (33%) and Staphylococcus (33%). The quarterwise distribution of different kinds of mastitis were as below:

Table 2: Quarter wise distribution of different types of mastitis.

Culture Report	Number of animals (63)			Number of quarters (252)		
	Archalbot	Chandreswor	Total	Archalbot	Chandreswor	Total
Mastitis Negative	26(76.5%)	19(65.5%)	45(71.4%)	112(82.3%)	79(68.1%)	191(75.8%)
Coliform Mastitis	2(5.8%)	4(13.8%)	6(9.5%)	5 (3.7%)	15(12.9%)	20(7.9%)
Staphylo Mastitis	4(11.9%)	1(3.5%)	5(7.9%)	11 (8.1%)	4(3.5%)	15(5.9%)
Strepto mastitis	2(5.8%)	5(17.2%)	7(11.1%)	8 (5.9%)	18(15.5%)	26(10.3%)
Total	34	29	63	136	116	252

Table 3: Pattern of antibiotic sensitivity test for various antibiotics in two VDCs

Name of Antibiotic	Archalbot VDC		Chandreshwor VDC	
	No of Sensitive Isolates	% Sensitive Isolates	No. of Sensitive Isolates	% Sensitive Isolates
Chloramphenicol	8	100	8	80
Amikacin	8	100	10	100
Norfloxacin	8	100	10	100
Tetracycline	8	100	9	90

Name of Antibiotic	Archalbot VDC		Chandreshwor VDC	
	No of Sensitive Isolates	% Sensitive Isolates	No. of Sensitive Isolates	% Sensitive Isolates
Ceftriaxone	2	25	6	60
Gentamicin	8	100	10	100
Cephalexin	4	50	5	50

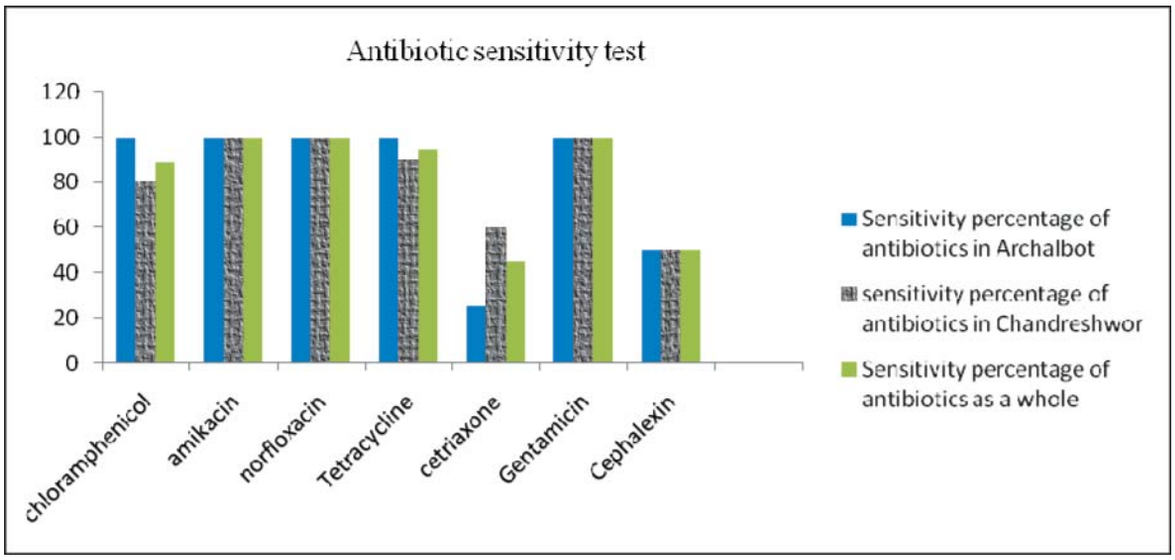


Fig. 3: Antibiogram of different of antibiotics

Relation between prevalence of sub clinical mastitis and amount of milk production

Prevalence of Subclinical mastitis was significantly higher (73%) among the dairy animals producing more than 3 litres compared to dairy animals producing 3 or less than 3 litres (7.7%). The pearson's chi-square value 29.194 gives p value less than 0.05 that signifies for high yielding animals (> 3 lts) are more susceptible to be infected with mastitis.

Relation between prevalence of sub clinical mastitis and stage of lactation

Prevalence of subclinical mastitis was higher (58.8%) among the dairy animals in late lactation compared to dairy animals in early lactation (41.3%).The chi square test (χ^2= 1.54, i.e. p=0.216 >0.05) showed the association between the stage of lactation and prevalence of mastitis is non- significant. This means that the stage of lactation could not be determining factor for mastitis in Lamjung.

DISCUSSION

The prevalence of SCM in cattle and buffaloes of Lamjung district was 28.6%% and 24.2% on animal basis and quarter basis respectively during cultural examination. The result obtained by Dhakal *et al.*(2002) showed the prevalence rate of SCM to be 56% of cattle with 35% of the quarters and 44% of buffaloes with 27% of the quarters which is far greater than the present result on animal basis but similar on quarter basis. Thapa (2006) has reported the prevalence rate to be 72% by cultural examination in Gitanagar VDC of Chitwan which is far greater than our result. Upadhayaya (2008) reported the prevalence rate of SCM to be 48.3% quarter and 68.3% cattle in Kathmandu district which is also higher than our result. However, Adhikari *et al.* (2009) reported the prevalence rate of SCM to be in 40% cattle and 32% buffaloes which is similar to our findings. On the other hand, CMT report shows that the prevalence rate of SCM in Lamjung was 46.1% on animal basis and 30.2% on quarter basis. Khakural(1996) reported the prevalence rate in Kathmandu valley to be 17.2% by CMT test which is less than our findings. However, our finding is comparable to the findings of Thapa(2006) who got it to be 36% in Gitanagar VDC.

In our study, we found that left fore quarters (26%) and right hind quarters (26%) had the highest incidence followed by left hind quarters (25%) and then right fore quarters (23%). This result is in contrast to the result

obtained by Dhakal *et al.* (1991) who found the incidence to be highest in left hind quarters (34.2%) followed by right hind quarters (31.6%). Our finding is also contrasting with that of Jha *et al.* (1994) who found that right fore quarters had highest incidence (33.9%) followed by left fore quarter (28.6%). However, Thilager *et al.* (1992) found the highest incidence in the left fore quarters which is similar to our result. Our findings are also contrasting with that of Upadhyaya (2008) who found the incidence to be highest in right fore quarters followed by left hind quarters.

In our research study, we got that Streptococcus were the most predominant pathogens with 11.1% prevalence rate followed by Coliform (9.5%) and Staphylococcus (7.9%). However, Coliform mastitis was the most frequent type followed by Staphylococcal mastitis in Chitwan in the study of Dhakal *et al.* (2002) and Adhikari *et al.* (2009) which is contrasting to our findings. Similarly, Balakrishnan *et al.* (2004) and Dhote *et al.* (1999) reported Staphylococcus species being the most predominant pathogens followed by Coliform species which is also contrasting to our result. Our result is also contrasting with the findings of Mandial *et al.* (1999). Dhakal *et al.* (2007) reported that Staphylococcal mastitis was most predominant which is also different from our findings. Upadhyaya (2008) also reported that Staphylococcal mastitis had the highest prevalence rate in Kathmandu district followed by E.coli, mixed infection and then Streptococcus species which is contrasting to our result. Our findings are also different from that of Adhikari *et al.* (2009) who found that Coliforms were most prevalent followed by Staphylococcus spp., Streptococcus spp. and Pseudomonas spp. However, in Archalbot VDC, Staphylococcus spp. was predominant which is similar to the findings of various researchers.

From the antibiotic sensitivity test report, we found that Gentamicin, Norfloxacin and Amikacin were highly sensitive (100%) followed by Tetracyclin(90%) and Chloramphenicol(80%). However, Ceftriaxone (60%) and Cephalexin(50%) were quite resistant. The resistance of these antibiotic may be due to their irrational use in the research site. This result is similar to the findings of Dhakal *et al.* (2002) who showed that sensitivity was the highest in Gentamicin followed by Chloramphenicol, Tetracycline and Ampicillin. Similarly, Dhakal *et al.* (2007) found that, Enrofloxacin had the highest sensitivity (91%) followed by Gentamicin (87%), Tetracycline (83%) and Chloramphenicol (82%) which is quite near to our findings.

Prevalence of SCM was higher in late lactation period (58.5%) compared to those in early lactation(41.3%) which is contrasting to the view of Radostits *et al.* (2006). Similarly, the prevalence rate was higher in high yielding animals (73%) as compared to low yielding (7.7%) which is similar to the view of Radostits *et al.*(2006).

In the research site, 71% of the dairy animals were of exotic breed, which is quite favourable condition for increase in milk production in Lamjung district. Similarly, all the respondents were involved in agriculture and all were literate. So, there is possibility of commercialization in dairy farming. In our study, we found that 86% of the animals were stall fed and remaining were free ranged animals. Similarly, 84% of the animals were provided with tap water which is free of contamination while others were provided with both tap and river water. Similarly, 56% of the animals were provided with both roughages and concentrate in balance but remaining were provided with dry straw only which is quite similar to the finding of Upadhyaya (2008). Rice straw and maize stovers were main crop by-product fed during winter and rainy season respectively. Lactating animals were given homemade concentrate feed daily during the lactation period. Commercial feed was only purchased by those farmers who are rearing improved breeds. This finding is quite similar to the finding of Tulachan et al (2002). Milking and pregnant animals were fed Khole (concentrate) in the morning and day time with very limited amount of green fodder during dry season which is similar to the finding of Regmi *et al.* (1999). Very few animals were provided with regular supplementation of minerals and vitamins. So, it is an obstacle to increase milk production in the district. Only 30% of the farmers had forage trees in their land. So, most of the farmers experienced scarcity of green grass in winter which is similar to the finding of Regmi *et al.*(1999). Almost all of the animals were dewormed regularly which is far better situation than that of Kathmandu district as reported by Upadhyaya *et al.* (2008).

CONCLUSION

The knowledge of mastitis in the people of Lamjung was found poor. The management of livestock is very unscientific. But, through the non formal education like trainings on the particular issues, can be the milestone in mastitis control and high milk production. Gentamicin, Norfloxacin and Amikacin were highly sensitive and should be used if needed but Ceftriaxone and Cephalexin were quite resistant so should not be used. The resistance of these antibiotics may be due to their irrational use in the research site. Every farmer should be distributed with the CMT paddle and its reagent so that after training, they can test the udder health condition and SCM in their farm regularly. The farmers should be guided about fodder production in their

degraded land and avoid feed scarcity in local level. Disinfection of the shed and hygienic and scientific shed is strongly recommended in Lamjung. The optimum utilization of local resources could be the best method for the shed.

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COMPARISION OF DIFFERENT TESTS TO IDENTIFY SUB-CLINICAL MASTITIS IN DAIRY BUFFALOES OF BAGLUNG DISTRICT

N. Paudyal[1] and S. Sapkota[2]

ABSTRACT

A survey cum lab analysis of the milk collected from the dairy farmers in Baglung district was undertaken for identifying the best farm side test to detecting the SCM in buffaloes. The survey showed that most of the farmers have poor farm hygiene and sanitation. Mastrip test and MWT indicated similar degree of SCM but none had good specificity and sensitivity. CMT and SCC seemed to have a positive linear relationship with each other. In the CMT score of trace (0), the mean SCC was 9.33x1.6x104, 9.33x1.6x104, 7.25x1.6x104 and 8.71x1.6x104 in LF, LH, RF and RH quarters respectively. Similarly for CMT score of weak positive (1), mean SCC was 20.75x1.6x104, 23.28x1.6x104, 21.2x1.6x104 and 23.42x1.6x104 for LF, LH, RF and RH quarter respectively. And for the CMT score of distinct positive (2) the SCC was 49x1.6x104, 60x1.6x104, 45x1.6x104, 55x1.6x104 for LF, LH, RF and RH quarter respectively. Prevalence of SCM was 22.03% on individual quarter basis (240 quarters) Left quarter only 21.66% (120 quarters), Right quarter only 22.5% (120 quarters), 3.33% buffaloes have SCM on all quarters. So it was recommended that farmers can use CMT as a farm side test for identifying the SCM in their dairy buffaloes.

INTRODUCTION

Several mastitis indicator tests such as California Mastitis Test (CMT), Modified Whiteside Test (MWT), Bromothymol blue (BTB) based on SCC of milk samples may not always give accurate diagnosis of subclinical mastitis. These tests denote a rough estimation of the cells, since positive reaction in all these tests depend upon higher concentration of the cells in the milk. Bacteriological examination of milk has not been fully reliable, as a single bacteriological examination of quarter milk samples does not necessarily identify all infected quarters. Bacterial colonization of the teat duct may be the reason for bacterial contamination of the milk. So it is a felt need to verify some farm side or cow side tests which can efficiently predict the presence of mammary abnormalities which may eventually develop to clinical mastitis.

GENERAL OBJECTIVES

- To assess the efficacy of Mastrip test, CMT, MWT and SCC to detect SCM in dairy buffaloes.

  The specific objectives were undertaken as follows:
- To estimate and compare the mean value of SCC and CMT of buffalo milk.
- To study the association of age, parity, stage of lactation and factors of management with the incidence of subclinical mastitis among the dairy buffaloes of Baglung disrtict.

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MATERIALS AND METHODS

Site Profile

Two hundred and fourty quarters milk samples from 60 apparently healthy buffaloes with history of poor production were randomly collected from Kalimati milk pockets of Baglung Municipality and Bihun VDC.

Sample collection

The samples were collected following the strictest of hygiene and sanitation procedure. After discarding first few streaks of milk, 10 ml milk sample from each quarter was collected in replicates in four separate, sterilized screw-capped test tubes. One sample used for CMT, Mastrip and MWT while the other was used for somatic cells counting. Conduction of tests was undertaken in the laboratory of DLSO, Baglung and final somatic cell count was done at HICAST Veterinary Teaching Hospital.

Tests performed

Mastrip test with Strip of mastrip paper (Dabur Ayurvet ®), California mastitis test (CMT) as per the procedure of Schalm and Noorlander (1957) employing the modified reagent of Pandit and Mehta (1969) and Modified white side test (MWT) were performed initially on all the samples. The SCC of the milk sample was performed as described by Schlam *et al.*, (1971) on all the milk samples after staining with Newman stain.

RESULTS

Results of field survey

Results show that most of the farmers kept the middle age animals (5 to 10 year) because middle age of the dairy animal is the most milk yielding age of its productive life. The proportion of various age groups of lactating buffaloes included in the study was young animals (<5 yrs) 10/60 (33.3%); middle aged (5 to 10 years) 32/60 (53.33%) and old (more than 10 years) 18/60 (30%).

It was also found that most of the animals included for this study were of earlier lactation. 48/60 (80 %) were of 1-5th lactation, 10/60 (16.67 %) were of 5th-10th lactation while only 2/60 (3.34 %) were of more than 10th lactation.

The analysis of housing pattern and style revealed that most of the shed roofing was done by stone slab. Most of the roofing 38/60 (60.33%) were done with stone slab were only 3/60 (5%) was done with straw and 19/60 (31.67%) sheds were roffed with glavaniged tin sheats. Majority of the sheds 50/60 (83.33%), had the stone slab floors, 9/60 (15%) floor were of RCC and 1/60 (1.67%) floor were of soil.

The distance between shed and manure pit was 41/60 (68.33%) have the distance 1 to 3 m, 13/60 (21.66%) sheds were at a distance of 3-5 m from manure pit, while 6/60 (10%) sheds were at a distance >5m from the manure pit.

Results of Laboratory Analysis

Nine of sixty (15%) sample of LF quarter, 4/60 (6.66%) sample of LH quarter, 8/60 (13.33%) sample of RF quarter and 10/60 (16.66%) sample of RH quarter are positive on mastrip test out of 240 samples.

Ten out of sixty (16.66%) sample of LF quarter, 11/60 (18.33%) sample of LH quarter, 9/60 (15%) sample of RF quarter and 11/60 (18.33%) sample of RH quarter are positive out of 240 samples on modified white side tests.

Fourteen out of sixty (23.33%) sample of LF quarter, 12/60 (20%) sample of LH quarter, 14/60 (23.33) sample of RF quarter and 13/60 (21.66%) sample of RH quarter are positive out of 240 samples on CMT.

Figure 1 shows a linear relationship between CMT score and SCC of the same. With increase in the CMT grading, a gradual increase in the SCC result is noted. In case where CMT was trace SCC ranged from 5 X 1.6 X 104 cells/ml to 12 X 1.6 X 104 cells/ml of the milk with a mean of 9.33 X 1.6 X 104 cells/ml. For CMT score 1, the number of the cells per ml milk ranged from 14 X 1.6 X 104 cells/ml to 26 X 1.6 X 104 cells/ml with a mean of 20.75 X 1.6 X 104cells/ml whereas for CMT score 2 the SCC was 49 X 1.6 X 104cells/ml.

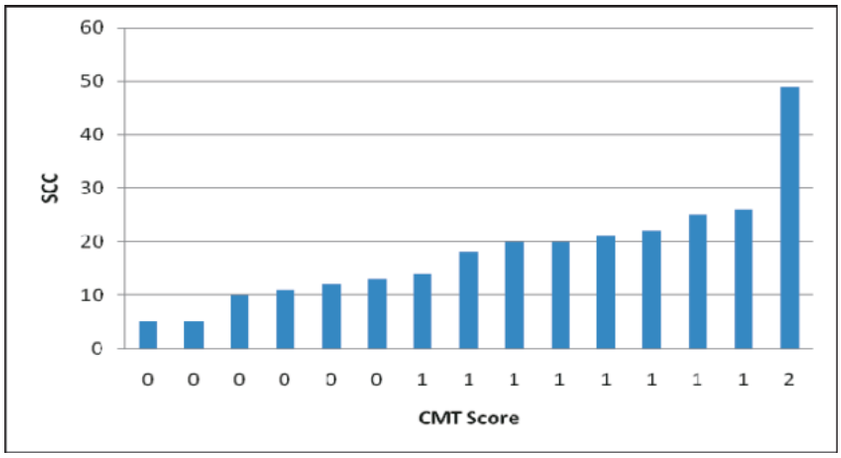

Figure 1: Relationship between CMT & SCC in LF Quarter

Figure 2 shows a linear relationship between CMT score and SCC of the same. Here also, with increase in the CMT grading a gradual increase in the SCC result is noted. In case where CMT was trace SCC ranged from 3 X 1.6 X104 cells/ml to 12 X 1.6 X 104 cells/ml of the milk with a mean of 9.33 X 1.6 X 104 cells/ml. For CMT score 1, the number of the cells per ml milk ranged from 14 X 1.6 X 104 cells/ml to 23.28 X 1.6 X 104 cells/ml with a mean of 20.75 X 1.6 X 104cells/ml whereas for CMT score 2 the SCC was 60 X 1.6 X 104cells/ml.

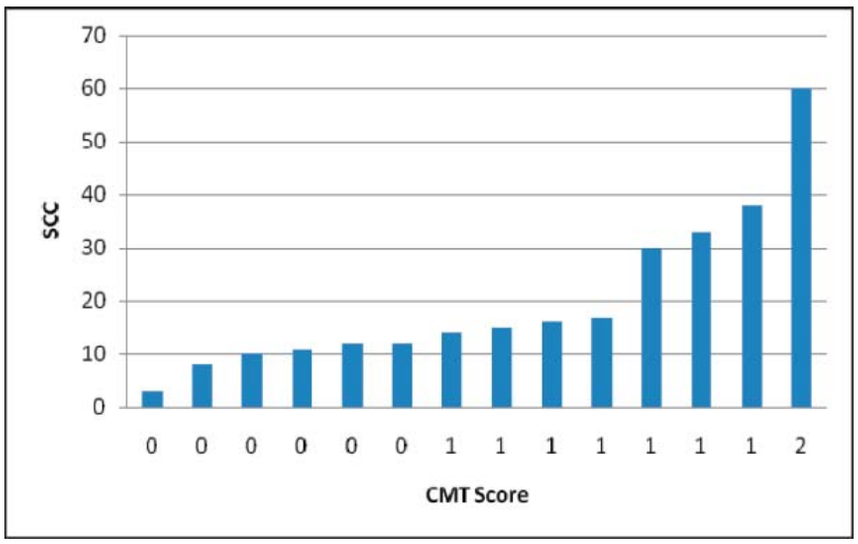

Figure 2: Relationship between CMT & SCC in LH Quarter

Figure 3 also shows a linear relationship between CMT score and SCC. With increase in the CMT grading a gradual increase in the SCC result is noted. For CMT score 0 the mean SCC was 7.25 X 1.6 X 104 cells/ml and it ranged from 2 X 1.6 X 104 cells/ml to 13 X 1.6 X 104. For CMT score 1 the

mean SCC was 21.2 X 1.6 X 104 cells/ml with a range of 14 X 1.6 X 104 cells/ml to 24 X 1.6 X 104 cells/ml. When CMT score 2 the SCC was 45 X 1.6 X 104 cells/ml.

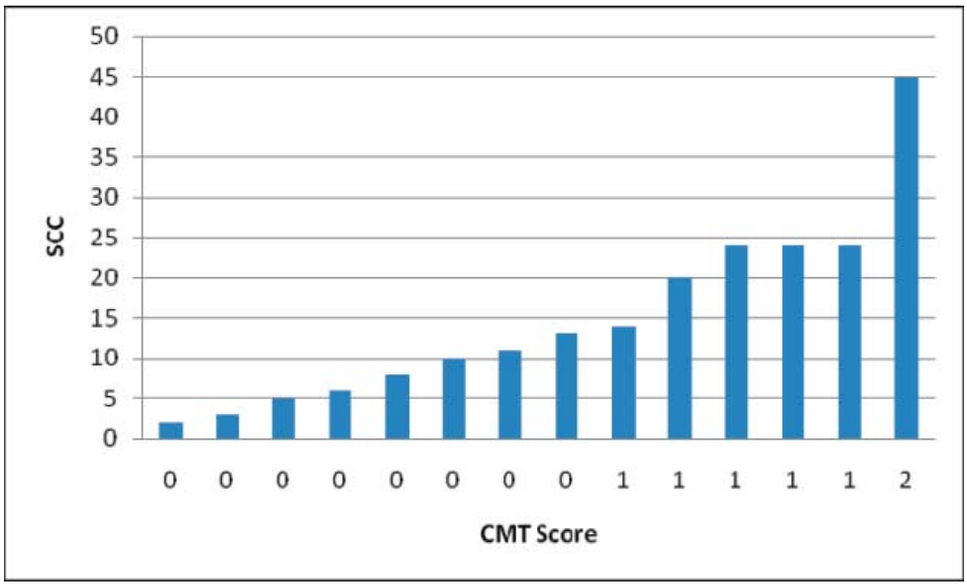

Figure 3: Relationship between CMT & SCC in RF Quarter.

Figure 4 also shows a linear relationship between CMT score and SCC. When CMT score is 0 the mean SCC was 8.71 X 1.6 X 104 cells/ml with a range of 5 X 1.6 X 104 cells/ml to 13 X 1.6 X 104.. When the CMT score is 1 the mean SCC is 23.24 X 1.6 X104 cells/ml with a range of 16 X 1.6 x 104 cells/ml to 33 x 1.6 x 104 cells/ml. For CMT score 2 the SCC was 55 X1.6 x 104 cells/ml.

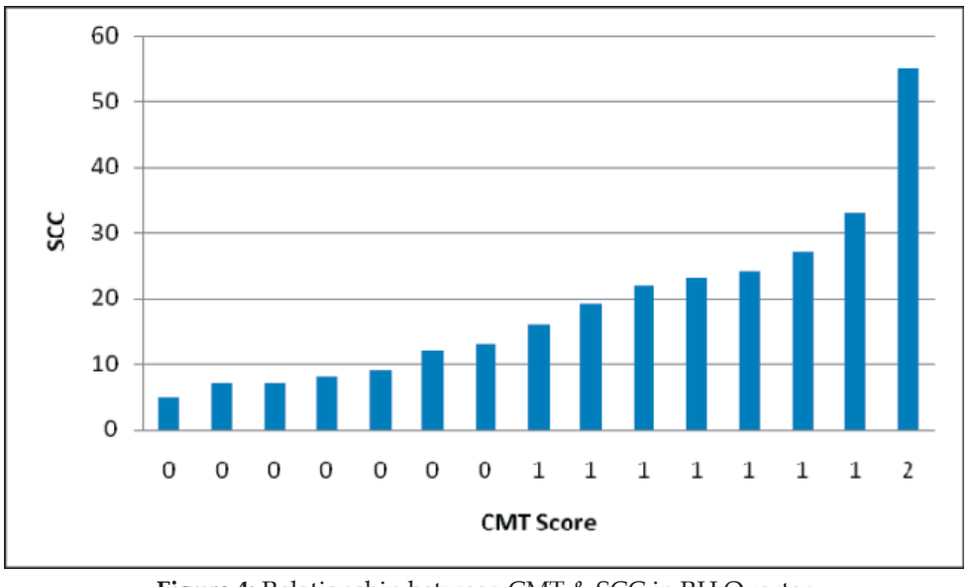

Figure 4: Relationship between CMT & SCC in RH Quarter

DISCUSSION

Epidemiological survey

Factors such as climate, housing, bedding and rainfall interact with the degree of exposure of teat and to the pathogens and cause mastitis. The results have shown that the maximum numbers of animal included in this were of the age group 5-10 years. But the incidence of CM and SCM was significantly lower as compared to other reports. Buffaloes in young age and initial lactation number with a good housing system might have cause the decrease incidence. As seen from the results, roofing was done by stone slabs in most cases and the floor was of stone slabs too. Ease of cleaning and the impervious nature of stone slabs might have decreased the chances of mastitis in this study. No other literature regarding the relationships of stone's roof and floor with mastitis could be found. However many literatures clearly indicate that with increase in ease of cleaning the incidence of mastitis decreases (Radostits et.al, 2000). It is easily deduced that the soil floor is difficult to clean and RCC for floor is expensive, so medium class families use stone slab that are easily available in the local areas of the research site to the maximum.

The distance between the shed and manure pit, in most cases manure pit was in a range of 1-3 m of the shed. It is a common practice to just let the urine flow on it's own to the urine pit and the dung is removed with shovel or by hand. So for this the farmers want the manure pit to be close by so as to facilitate the removal of dung. It may seem that manure pit close to shed might be unhygienic and lead to poor barn sanitation however no any relationship was found between the distance of shed and manure pit to the occurrence of mastitis. There might have been seasonal influence to decrease the fly population and hence decrease the disease transmission ratio.

Various other report like Dhakal (2007), Dhakal (1995), Dhakal (2004), have justified the role of various environmental factors and management factor predisposing to either SCM or CM, which is similar to the findings of this study.

Laboratory analysis

In buffaloes, overall prevalence of subclinical mastitis was 27%, clinical mastitis 4% and blind quarters 10%. In crossbred cows, subclinical mastitis was observed in 36%, clinical mastitis in 5.5% and blind quarters in 8% quarters (Khan and Mahomad 2005). The overall quarter wise prevalence was 58.75%, while animal wise prevalence was 77.98% in Pakistan (Bachaya, et al, 2007).

A study conducted during 1991/92 in Ilam and Dhankuta districts of the eastern hills of Nepal to determine the prevalence of mastitis in buffaloes has indicated that the prevalence of mastitis in buffaloes was 21.3% (Jha *et al.*, 1993). The result of investigation using sodium lauryl sulphate and white side test in the command areas of Lumle Agricultural Research Center has shown that the prevalence of sub clinical mastitis in buffaloes was 39.6% in the mid-hill area (Joshi and Joshi, 1997). Total SCC in milk is the most frequently used indicator of subclinical mastitis and milk quality (Radostis et.al, 2000). The SCC level under 200,000 cells/ml of milk is considered normal, but it may be less in the first lactation. Generally, SCC increases with the age and parity of infected buffalo but in non contaminated cases the SCC does not seem to vary with the age. In a study with the influence of herd size and milking management on the udder health status of buffaloes, using SCC, CMT, proportion of neutrophils and bacterial infection; a weaker correlation among the tests was observed in comparison to that of the bovine milk. In another similar study (Spencer and Simon, 1960) who evaluated SCC and CMT where CMT was found to be the most useful tool for field application. Similar reports have been provided by Dhakal (2007) and Dhakal (2004) who recommended that CMT can be efficiently used as a cow-side test for SCM. This research has

also found a linear relationship between CMT and SCC but no such relation could be analyzed from other tests ie mastrip and MWT. Regarding to mastrip test and MWT, theses test can only sometime indicate the presence of SCM with mastrip having more sensitivity and specificity then MWT (Dhakal, 2007). Kapur and Singh (1977) made a comparative study on SCC and other indirect tests namely, CMT and Modified Whiteside Test (MWT) for the diagnosis of subclinical mastitis in buffaloes and found a better accuracy of CMT i.e. 97.20 %. Similar opinion had been given by Sukla and Supekar (1982) where CMT yielded more sensitivity than the MWT. The relationship between SCC and CMT profiles and infectious status showed an accuracy of 65 percent (Suarez et al, 2002). Clements et al, (2003) evaluated the sensitivity and specificity of CMT for different threshold levels of SCC and bacteriological status in ewes. They suggested that CMT was a best diagnostic test for detecting subclinical mastitis (SCM). Sena and Sahani (2001) found a positive correlation between CMT and TLC as screening indicators for mastitis detection. Milk samples from clinically healthy camels were assessed by the CMT, SCC and bacteriological culture. Infected quarters showed significantly higher SCC and CMT score than non-infected quarters

The loss result from subclinical mastitis is more than the clinical mastitis. The study was conducted during 1991/92 in Ilam and Dhankuta districts of the eastern hills of Nepal to determine the prevalence of in cows and buffaloes .milk samples from 388 apparently healthy cows and 150 buffaloes examined using sodium lauryl sulfate and teepol (SLST) test. The SLST test positive milk samples were further subjected to total leukocyte count, bacteriological examination and antibiotics sensitivity testing. The results of the study indicated that the prevalence rates in cows and buffaloes were 18.8% and 21.3%respectively.the maximum prevalence rate in both the species was in single quarter. The effect of lactation no the prevalence of in cows was in an increasing trend as the lactation no. increased.

There was no significant difference in the prevalence rate due to the effect of age or stage of lactation in both the species. On bacteriological culture, the staphylococcus species (36.1%) followed by E. coli (17.3%), Streptococcus species (15.8%) where the most commonly encountered isolates (Jha *et al.*, 1993).

The criteria adopted by International Dairy Federation (IDF) for the diagnosis of subclinical mastitis is based on the isolation of pathogenic organism from aseptically collected milk samples and elevated SCC to more than 500,000 per ml. The milk samples from 85 apparently healthy cows and 363 apparently healthy buffaloes were tested with sodium lauryl sulphate and white side test in the command areas of Lumle Agricultural Research Center. The test positive milk samples were also subjected to total leukocyte count and bacteriological examination. The result of investigation showed that the prevalence of sub clinical mastitis in cows and buffaloes was 46% & 39.6% respectively in the mid-hill area (Joshi and Joshi, 1997). Dangore (2000) reported that modified CMT and MWT were efficient byre–side tests for the detection of subclinical mastitis. In another similar study Spencer and Simon (1960) evaluated SCC and CMT where CMT was found to be the most useful tool for field application. Kapur and Singh (1977) made a comparative study on SCC and other indirect tests namely, CMT and Modified Whiteside Test (MWT) for the diagnosis of subclinical mastitis in buffaloes and found a better accuracy of CMT i.e. 97.20, per cent. Similar opinion had been given by Sukla and Supekar (1982) where CMT yielded more sensitivity than the MWT. A controversy exists between those workers and Joshi *et al.*, (1976) where Whiteside test was found more effective and cheaper than CMT in detecting the traces of infection Clements et al, (2003). They suggested that CMT was a best diagnostic test for detecting subclinical mastitis (SCM). CMT score with a distinct gel formation and SCC thresholds greater than 1200x103 cells/ml would be appropriate, especially when low prevalence was expected (i.e. below 5 percent).

CONCLUSION

Among the four tests assessed in this study for detection of SCM namely- Mastrip test, Modified Whiteside Test, California Mastitis Test and Somatic Cell Count, first two test did not show any difference in detecting the presence of SCM and were not very reliable to be used at the farm level. However, CMT when compared with SCC showed a linear relationship in all of the four quarters for detection of SCM, which clearly indicates that CMT can be used as a good farm side test for detection of SCM in buffaloes.

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WILDLIFE AND COMPANION ANIMAL HEALTH MANAGEMENT

HAEMATOLOGY AND SERUM BIOCHEMISTRY ANALYSIS IN CAPTIVE HIMALAYAN GRIFFON VULTURES (GYPS HIMALAYENSIS)

S. Subedi[1], S. Paudel[2], D. K. Singh[3] and J. Thapa[4]

ABSTRACT

The Himalayan Griffon Vultures (Gyps himalayensis) are old world vultures of high hills and mountains and listed as Least Concern in IUCN redlist. The objective of this study was to analyze the blood and serum biochemistry of the captive vultures at Central Zoo as hematology and serum biochemistry parameters provides the health and immune status of birds. All four healthy birds were included in this descriptive study and hematological analysis was performed manually as per the protocol of Nollet and Pizzi (2010) for vulture conservation and breeding center. Serum biochemistry was performed with STAT FAX 3300 in Akla pathology lab. Results are presented as Mean ± SE. Since data on these species were unavailable, obtained results are compared with normal values for other vultures and raptors. PCV was lower but higher than the value to be considered as anaemic. No blood parasites were observed in blood film. All other hematological parameters were under normal range. The serum biochemistry values were within the normal range except SGOT level which probably is associated small enclosure in zoo and creatinine level whose interpretation is not clear in vultures. The Urea:Uric acid ratio, a more definite parameter for kidney function, was found within normal range despite a higher uric acid level. The obtained data can be a basis for medical evaluation in the field of conservation medicine and for the establishment of reference level for these species.

INTRODUCTION

There has been a devastating population decline in the vulture population in the last 15 years. The Indian subcontinent showed a devastating figure of more than 98% decline in the population of Oriental white-backed vulture (Gyps bengalensis), Slender billed vulture (Gyps tenuirostris) and long billed vulture (Gyps indicus) (Naido *et.al.*, 2008). The cause of decline has been stated as diclofenac, an anti-inflammatory drugs used in cattle, to be a major contributing factor apart from the habitat destruction and decrease in carrion (Green *et.al.*, 2004; Oaks *et.al.*, 2004). Vulture decline in the other continent, although slow and gradual, has shown declining figures in population of some Beared vultures in the Europe, Ruppell's Vulture and Lappet faced vulture in the Africa and the near extinction of California condor in the North America. These are usually linked with multi factorial causation from chemical poisoning, lead poisoning, persecution, in traditional medicine, poaching as well as habitat destruction and feed decline (Ogada *et.al.*, 2011).

Knowledge of the causes of death, illness and injury can improve and facilitate the application of the management measures for the conservation of endangered species. Clinical hematology and serum biochemistry are useful diagnostic tools in clinical practice and are especially important when only

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few overt signs of disease are shown. These are useful for evaluating the health of birds in wild and captive population. Thus clinical examination has to be combined with tests to diagnose and treat the sick birds and also for the rehabilitation of the injured individuals (Hernández & Margalida, 2010).

Himalayan Griffon vultures are the old-world vultures of the high hill region of Asia and listed as least concern in the IUCN redlist (Birdlife International, 2009). Though in the past studies these vultures having a fairly stable population, Acharya *et al.* (2009) showed a declining population and habitat of these vultures in Nepal (upper mustang area). The conservationists in Nepal are encountering sick Himalayan Griffon vultures. But relatively few studies in these vultures and unavailability of the reference values for these species have posed a difficulty in the medical evaluation and treatment of these birds. So a study was carried out to evaluate the hematology and serum bio-chemistry of healthy Himalayan Griffon (Gyps himalayensis) vultures of Central Zoo. This study will encourage and motivate for the further study on the blood values of other birds of the same species so that a reference range for these species could be prepared in the near future.

MATERIALS AND METHODS

Blood Collection

The study was carried out with the Himalayan Griffon vultures of Central Zoo, Jawalakhel, Lalitpur, Nepal from September to December, 2011. With the ethical approval from the NTNC-Central zoo, all four Himalayan griffon vultures were included in the study. These vultures were deemed healthy by Veterinary officer of the zoo based on the physical evaluation, normal behavior, normal appetite, of average body weight and being under supervision in captivity before and after two weeks period of blood collection in the study period. Blood collection was done on the day of fasting to avoid vomition while and after handling of these birds. These birds were manually restrained by zoo keepers with a net and towel. A 23-gauge 5ml disposable sterile syringe was used to venipuncture the medial tarsal vein and blood as put in the commercial serum and EDTA vacutainer for the serum and blood samples respectively. These birds were then tagged with the feet collar to identify the respective blood samples. Serum was separated by centrifugation. Samples were evaluated immediately but were refrigerated before lab examination. Two time period study (21 days interval) was used to increase the replication and see if there are significant differences in the mean values in these studies.

Laboratory Analysis

The hematological technique for this study has been carried out as per Nollet and Pizzi (2010) in their lab manual for vulture conservation and breeding center. Thin blood smears were prepared by using slide on slide techniques with the fresh blood without anticoagulant. These blood smears were then fixed immediately and stained with Rapid Romanowsky stain as per the protocol of Nollet and Pizzi. These were then microscopically evaluated in the microscope under 100X objective oil immersion. Graduated eye-piece calibrated with the 0.01 mm etched stage micrometer (Erma, Japan) was used for determining cell morphology, sizes and characterization. Moreover the smears were also evaluated for the presence of blood parasites. Polychromasia and anisocytosis were estimated to access the erythropoietic activity and presence of anemia. Differential Leukocytic count (DLC) was performed by examining about 200 white blood cells and then categorized into heterophils, lymphocytes, eosinophils, monocytes and basophils on the basis of the staining characteristics and morphology. No counting of total immature RBCs and differentiation of large and small lymphocytes were done.

Manual blood cells count was performed on the double ruling improved Neubauer hemocytometer (Marienfeld, Germany and Fein-Optik Blankenburg, GDK). Total erythrocytic count (TEC) was performed with the normal saline and Total leukocytic count (TLC) was performed with the 1%

ammonium oxalate solution. The hemoglobin was estimated by cyanomethemoglobin method in Alka pathology lab. Packed cell volume (PCV) was estimated by standard microhematocrit method with centrifugation at 10,000 rpm for 5 minutes. The MCV, MCH and MCHC were calculated from the formula as described by Samour (2006) in his literature. Blood, after coagulation, was centrifuged at 5000 rpm for 10-15 minutes for complete separation of serum from the gel in commercial serum vacutainer. The serum was then collected into Ependorf tube, marked and sent to the laboratory for biochemistry analysis. STATFAX 3300 Chemistry Analyser (Awareness Technology Inc., Florida) was used for the biochemistry analysis using specific kits.

Statistical analysis

A descriptive study was carried out with the results of lab analysis. Data were entered in to MS-EXCEL 2007. Arithmatic Mean, standard deviation(SD) and standard error (SE) are calculated. Data are presented as Mean±SE.

RESULT AND DISCUSSION

The data for morphometrics of the blood cells are given in the Table 1. The hematology and serum biochemistry results are presented in Table 2 and Table 3 respectively. The obtained results are discussed with the results obtained for other raptor and vulture species.

In the rapid romanowsky stain, the red blood cells (RBCs), are elongated oval shaped cells with mean dimensions (se) of 13.72 (0.26) X 7.21 (0.11) µm with a centrally positioned dark basophilic nucleus of 6.42 (0.10) X 2.34 (0.09) µm. This finding is in accordance with the results obtained for Griffon vulture, Egyptian vulture, Imperial Eagle and Golden eagle (Polo, 1992) but slightly lower in length than that of Gyr falcons (Samour, 2005). The nucleus is uniformly condensed and darkly basophilic within the clear uniformly eosinophilic cytoplasm. Few erythroplastids, immature cells and polychromatic erythrocytes were also observed were observed in the blood films along with the presence of smudge RBCs. The heterophils were irregularly round cells, 11.27 (0.34) µm with lobulated nucleus; nucleus less basophilic than that of eosinophils. The cytoplasm has fine granules and more dark red, often seen as reticulated type in the clear cytoplasm and occasionally vacuolar cytoplasm. Eosinophils were also irregularly round cells 10.81 (0.24) µm diameter mostly with the bilobed nucleus and small orange-pink granules scattered uniformly obscuring the faint bluish cytoplasm. The eosinophils and heterophils in the present study were smaller in size than that Samour (2005) reported for Gyr falcons and different in the staining characteristics due to the different staining protocol. The basophils identified in the study were irregularly round with deep basophilic non-lobular and bean like nucleus. The granules were very large as transparent vacuoles in the deep blue cytoplasm and often scanty cytoplasm. Since only few basophils were reported in the smear, the size of these cells is not determined in the present study though they were smaller than other granulocytes and larger than the most lymphocytes. Lymphocytes are round cells, 8.77 (0.22) µm mean diameter, with high nuclear:cytoplasmic ratio. They have faintly bluish scanty cytoplasm, basophilic nucleus with coarsely condensed pattern. Small azurophilic granules were observed in few lymphocytes. Monocytes were largest of the white cells with mean diameter of 13.88 (0.27) µm. Cells were variable in size from round to star shaped with cytoplasmic projections. The nuclear cytoplasmic ratio was less. Cytoplasm was faint blue, often granular. Nucleus was variable in shape ranging from typical kidney shape to irregularly round and even indented in some cases. Vacuoles were observed in the cytoplasm of some monocytes. Thrombocytes were small oval shaped cells with mean dimensions of 7.94 (0.10) X 4.64 (0.08) µm. These cells had highly basophilic and condensed nucleus of dimensions 5.46 (0.11) X 4.47 (0.10) µm. The cytoplasm was transparently clear and some cells contained large single terminal vacuole.

Table 1: Normal blood cells morphometrics in Captive Himalayan Griffon Vultures (*Gyps himalayensis*)

Blood Cell	Dimension	Measurements (µm) Mean (SE)
Red blood cells (n=50)	Length	13.72 (0.26)
	Width	7.21 (0.11)
RBC Nucleus (n=50)	Length	6.42 (0.10)
	Width	2.34 (0.09)
Heterophils (n=50)	Diameter	11.27 (0.34)
Eosinophils (n=25)	Diameter	10.81 (0.24)
Lymphocytes (n=50)	Diameter	8.77 (0.22)
Monocytes (n=25)	Diameter	13.88 (0.27)
Thrombocytes (n=25)	Length	7.94 (0.10)
	Width	4.64 (0.08)
Thrombocyte nucleus (n=25)	Length	5.46 (0.11)
	Width	4.47 (0.10)

Hematological analysis in Table 2 showed the mean PCV (se) to be 37.75 (0.56) % which is lower than the lowest range for the normal PCV range obtained for the Beared vulture (Hernández & Margalida, 2010), the value obtained for captive white rumped vulture (Paudel, *et.al.*, 2011) and hematocrit value obtained for the African captive white-backed vultures (Naidoo *et.al.* 2008) but this obtained value is above the range for the vultures to be suspected of anaemia (Nollet & Pizzi, 2010). The obtained mean is in the lower range or the PCV obtained for the Egyptian vulture (Samour, 2006). The hemoglobin values obtained are in accordance to the normal range obtained for various birds (Polo et.al., 1992; Naidoo, *et.al.*; Hernández & Margalida, 2010). Though the normal mean is little lower than those obtained for beared vulture adult and African white backed vulture adult but is in accordance with the values obtained for the White rumped vulture (Paudel *et al.*, 2011). The RBCs in the blood smears of these vultures examined also did not have more than 1% immature RBCS and falls in "Index-2" of the Polychrompatophilic Index (PI) in the morphologic criteria (Pendl, 2006) of the RBCs with only few irregular and polychromatophilic cells. The low PCV in these birds might be the physiological adaptation of these birds or the role of K3EDTA in the PCV or due to the incomplete mixing of the blood before drawn into capillary tube for micro centrifugation. The mean total erythrocytic count (TEC) is $2.09\pm0.06 \times 10^{12}$/L was within the range obtained for other vulture and raptor species.

The differential WBC count has been the parameter that is highly variable in many falconiformes and birds of prey. In the present research, Heterophils were the most common white blood cells in the blood cell examination followed by the Lymphocytes, Eosinophils and the Monocytes. Basophils were extremely rare and only seen few. Total leukocytic count (TLC) and differential leukocytic count (DLC) in both time period study were in accordance to the ranges given for other vulture species but higher than those of Egyptian vulture and common buzzard (Samour, 2005) while lower than the range obtained for Saker falcon (Samour, 2006). There was significant variation in the mean values for the monocyte and eosinophil count in the above time period difference. The mean MCV value was within the range obtained for the range of beared vultures and other birds of prey while the MCH and MCHC values were little higher than these findings. The higher values of these parameters in this research could not be clarified but might be due to the manual hematology analysis which has more chances to be less precise and less accurate than the automatic analysis.

Table 2: Hematological parameters in Captive Himalayan Griffon vultures

Parameters	Day 1(n_1=4) Mean±SE	Day 21(n_2=4) Mean±SE	Total (n_1+n_2) (Mean±SE)
PCV %	37.00±0.82	38.50±0.65	37.75±0.56
Hemoglobin (g/dl)	15.10±0.25	15.00±0.25	15.05±0.16
TEC (10^9/L)	2.05±0.05	2.12±0.11	2.09±0.06
TLC (10^9/L)	15.00±1.12	13.96±1.18	14.48±0.78
MCV (fl)	180.44±2.51	182.35±6.38	181.40±3.19
MCH (pg)	73.71±1.64	71.07±2.71	72.39±1.55
MCHC (g/dl)	40.86±0.97	38.97±0.24	39.91±0.58
Heterophils (10^9/L)	8.99±0.67	9.04±1.01	9.01±0.55
Eosinophils (10^9/L)	1.12±0.07	1.74±0.16	1.43±0.14
Lymphocytes 10^9/L)	3.53±0.54	2.42±0.2	2.99±0.35
Monocytes (10^9/L)	1.31±0.16	0.69±0.09	1.01±0.15
Basophils (10^9/L)	0.05±0.03	0.07±0.05	0.06±0.02

Table 3: Serum bio-chemistry results of the captive

Parameters	Day 1(n_1=4) Mean±SE	Day 21(n_2=4) Mean±SE	Total (n_1+n_2) (Mean±SE)
Blood Sugar (mg/dl)	306.75±17.40	314.25±14.61	310.5±10.61
Total Serum Protein (g/dl)	4.02±0.15	4.17±0.17	4.1±0.11
Serum Albumin (g/dl)	1.27±0.06	1.22±0.08	1.25±0.05
Serum Globulin (g/dl)	2.75±0.16	2.95± 0.22	2.85±0.13
Calcium (mg/dl)	9.72±0.43	8.65±0.47	9.19±0.36
Phosphorus (mg/dl)	4.15±0.17	4.05±0.14	4.1±0.10
S. Bilirubin – Total (mg/dl)	1.02±0.11	0.90±0.09	0.96±0.07
S. Bilirubin – Direct (mg/dl)	0.30±0.04	0.25±0.03	0.27±0.025
SGPT(ALT) U/L	41.00±3.41	47.25±3.20	44.12±2.47
SGOT (AST) U/L	223.00±16.04	304.75±36.84	263.87±24.18
ALP (U/L)	67.50±3.10	72.25±12.75	69.87±6.14
Sodium (meq/l)	154.00±1.87	157.00±2.08	155.5±1.41
Potassium (meq/l)	2.62±0.26	3.52±0.53	3.07±0.32
Urea (mg/dl)	15.50±1.44	16.75±1.10	16.12±0.87
Uric Acid (mg/dl)	7.95±2.07	6.97±1.54	7.46±1.21
Creatinine (mg/dl)	0.65±0.06	0.65±0.03	0.65±0.03
Urea:Uric Acid	2.22±0.36	2.72±0.50	2.47±0.30

The obtained mean serum protein levels, 4.1±0.11 g/dl, are in accordance with the levels obtained for various vultures and raptors while slight higher than the mean values obtained for the beared vultures and African white backed vultures (Naidoo *et al.*, 2008; Hernández & Margalida, 2010). This might be due to the different analytical methods used for estimation of protein in serum. The serum enzyme levels for SGPT and ALT are in accordance with the values obtained for various vultures and raptor species although these is an increased level of the SGOT level above the range described. Naidoo *et al.* (2008) obtained a very significant level of this enzyme in the serum of the African white-backed which he described it to be a character associated with the free-ranging that are more prone to muscular activity and damage that resulted an increased level of these enzymes. A similar correlation can be connected to the vultures in this study, although being under captivity, the confinement in a small space and a congested enclosure factor might resulted in the muscle

damage associated with fright while undergoing for feeding, cage cleaning and blood sampling. The other factor might be due the hemolysis of the sample (Harr, 2006). The mean serum urea concentration is both time periods studies are in accordance with the range given for other raptor species. The mean uric acid level seemed a little higher in which is due to the higher level of uric acid in sample from a bird. Though the uric acid level was higher, the Urea:Uric acid ratio, a more precise parameter of renal function (Naidoo *et al.*, 2008), seemed to be within the range given. A higher creatinine level, 0.65±0.03 mg/dl, is obtained in the present study than the beared vultures but the interpretation of this parameter could not be defined.

CONCLUSION

Blood cell characterization, hematological and serum biochemical evaluation were done in the himalayan griffon vultures of central zoo. Through this research, a clinical correlation was done to identify if these birds were healthy or not. It was found that these birds were doing well in the captivity and were healthy with the absence of anemia or other blood parasites. Though the clinical correlation did not reveal any major abnormality, a little deviation of some of these parameters as compared to other raptor and vultures were found. This can be assumed that it might be physiological modification and response to captivity problems or also due to the species specific difference in those parameters for which the data to compare could not be found due to the unavailability of the reference range specific for these species. The data obtained through this research could be used in conservation medicine for medical evaluation and treatment, more specifically for these species in the zoo. However further studies on other different parameters are still needed. This research could take an important step becoming as a foreground for the further research of these type in these vulture species in the future.

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PICTURE INDEX

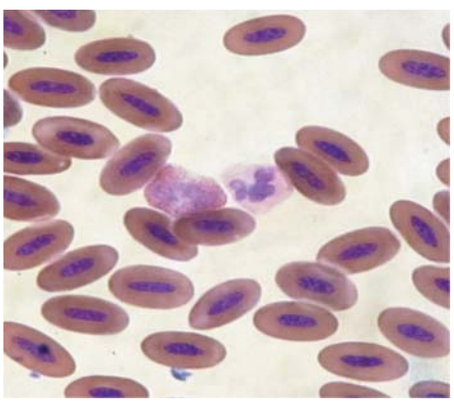

Fig 1: Eosinophil (right) and Heterophil (left) surrounded by

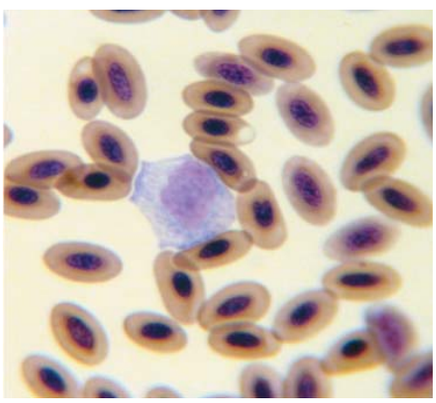

Fig 2: Lymphocyte(top), Eosinophil (left) and Heterophils

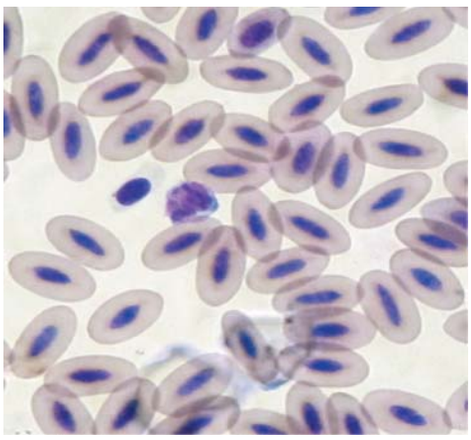

Fig 3: A thrombocyte (left) and lymphocyte (right)

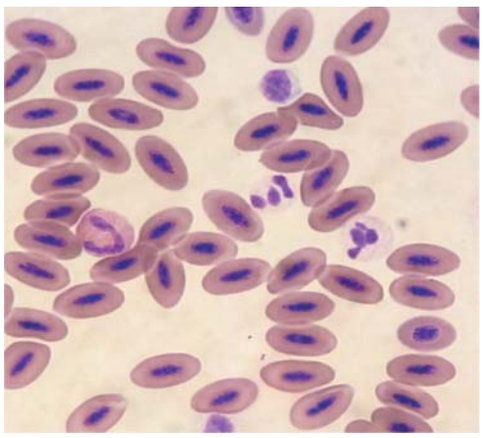

Fig 4: monocyte (center)

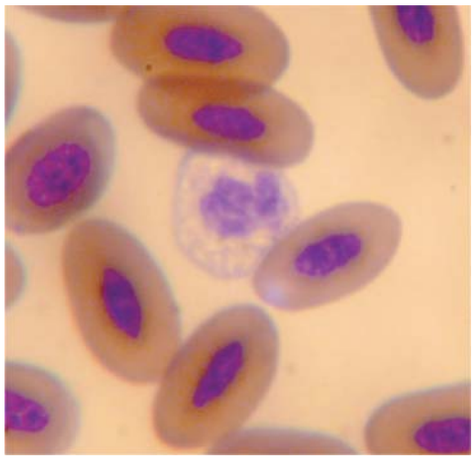

Fig 5: A basophil

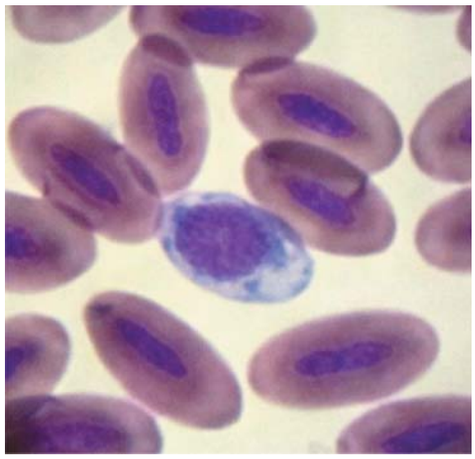

Fig 6: An immature erythrocyte

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RETROSPECTIVE STUDY ON PREVALENCE OF GASTROINTESTINAL HELMINTHS IN CAPTIVE ELEPHANTS

K. Shrestha[1] and B.R. Thapa[2]

ABSTRACT

The retrospective study was carried out to assess the prevalence of parasitic infestations in captive elephants of various Wildlife camps and Safari in Chitwan National Park. Clinical records of 73 Asian Elephants of various Wildlife camps and Safari treated by the vets for a period of 20 years (1991- 2011) were examined to determine the percent incidence of gastrointestinal helminthes particularly Roundworms, Liverfluke and Paramphistomes in relation to seasonal and geographical distribution. The parasitic examination performed in different time and season of the year were tabulated accordingly. Infected elephants were summarized and calculated as a percentage of the total number of elephants as per season and the location. Perusal of available records of elephants indicated that the overall incidence of infection was 82.67 percent, infectious being predominant during summer (82.94%) followed by Autumn (71.18%) and spring (62.59%). Overall parasitic infestation in elephants of Machan Wildlife camp, Chitwan Jungle Lodge, Gaida Wildlife camp, Narayani Safari, Tiger Tops and Temple Tiger are 100%, 79.71%, 100%, 58.92%, 64.20% and 93.20% respectively. Geographical-wise the incidence was highest in the elephants of Western side of the Park relative to the Eastern side. However, the result of high parasitic prevalence in Machan Wildlife camp and Gaida Wildlife camp is due to very limited data. Infections of Roundworms were observed almost in all the elephants followed by Liverfluke and Paramphistomes. Almost all the elephants in captivity in Chitwan are prevalent with the gastrointestinal helminthes from the past decades. Future work should aim for better management practices to limit parasitic problem and transmission.

INTRODUCTION

Elephants in captivity

The term 'captive elephants' means those caught in the wild and trained for human use. Likewise, 'domestic' means elephants are bred and raised by the humans. Historically, many Terai households kept captive elephants but there are none now (Yonzon, 2003). Earlier government elephant stables began with captive animals and now they straddle between captive and domestic animals between 1898 and 1970, there were 31 government stables for captive elephants, which stretched from Jhapa, east Nepal to Kanchanpur, far west Nepal.

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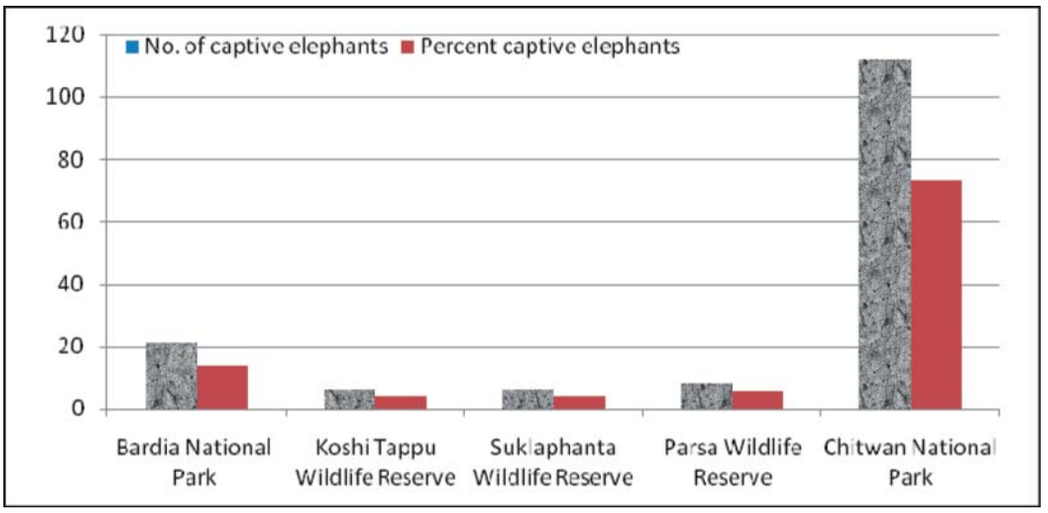

Fig. 1: Captive elephants in five Terai protected areas.

The six concessionaires inside the Park have 46 elephants, government agencies37, NGO 5 and outside the park, 24 privately – owned elephants are rented for tourists who view wildlife in the buffer zone (DNPWC, 2009).

Table 2: Captive/domestic elephants in chitwan national park and its buffer zone.

Location of trained elephants	Status	Adult	Calf
Elephant breeding center	Government	10	18
Saurah Haathisar	Government	6	3
NTNC	NGO	4	1
Tiger tops	Hotel	13	-
Temple tiger	Hotel	6	3
Island jungle	Hotel	4	-
Machan wildlife	Hotel	5	2
Chitwan jungle	Hotel	6	1
Gaida wildlife	Hotel	5	1
Privately owned elephants in buffer zone	Rental	23	1
Total		82	30

Elephants and the Parasites

Most free-living organisms harbor parasites of several species, which can adversely affect host health, fecundity and foraging, and may also modify host behavior to facilitate parasitic transmission (Vanitha et al, 2011). Parasitism has been shown to directly both the evolution and ecology of hosts through processes such as sexual selection or parasite-mediated competition, which can lead to a reduction in population size, or the extinction of one host (Price *et al.* 1986). Asian elephants Elephas maximus are susceptible to gastrointestinal parasitic infection in the wild and in captivity (Watve 1995; Dharmarajan 2000; Vidya & Sukumar 2002). Elephants in captivity are often confined to small enclosures and/or maintained in isolation (Vanitha 2007) in damp unhygienic conditions that may result in enhanced susceptibility to parasitic diseases (Dhungel *et al.* 1990; Chandrasekaran *et al.*1995; Suresh *et al.* 2001).

METHOD AND METHODOLOGY

Site of study

Temple Tiger Jungle Lodge, Tiger Tops Jungle Lodge, Narayani Safari Lodge, Chitwan Jungle Lodge, Gaida Wildlife Camp, Machan Wildlife Resort are the finest hotels located inside the Chitwan National Park ranging from West to the East which harbours a major share of elephants population in CNP. The National Park covers an area of 932 km2 and is located in the subtropical Inner Terai lowlands of south-central Nepal. Altitude ranges from about 100 meters (330 ft) in the river valleys to 815 meters (2,674 ft) in the Churia Hills. In the north and west of the protected area the Narayani -Rapti river system forms a natural boundary to human settlements. Adjacent to the east of Chitwan National Park is Parsa Wildlife Reserve; contiguous in the south is the Indian Tiger Reserve: Valmiki National Park.

Duration of study

The study was conducted from July to December of the year 2011.

Research Design

The research is of the retrospective study design.

METHODOLOGY

The available clinical case sheets and records of Asian Elephants belonging to different Wildlife camps and safaris for a period of 20 years (1991-2011) were examined in order to assess the percent incidence of Roundworn, Liverfluke and Paramphistome in relation to season and geopgraphical distribution. The diagnoses were made by the senior veterinary officer Dr. Balaram Thapa (Vet of all the camps). The parasites were classified into Trematode, Cestode and Nematodes, basically Paramphistomes, Liverfluke and Roundworm are taken into study. Infected elephants are summarized and calculated as a percentage of the total number of elephants.

Regarding the method of fecal examination, nearly 100 gm of feces were collected from outer covering of each ball (separately) from the pool sample of 5-7 balls making in total nearly 500 gm. Samples were then diluted to 5 litres of water in a bucket, made into solution like form and stained as large amount of fibres were present in their feces. The prepared fecal solution was kept stagnant for 12 hours which results in sedimentation. The Supernatant was discarded and sediment was taken in test tubes which were examined under low power (10x) microscope. Only identified eggs as described by Soulsby, 1988 and Thienpont et al, 1994 were examined and noted.

Statistical Analysis

The data obtained was analyzed using the Microsoft Excel.

RESULTS

Perusal of available records of 73 elephants of different Private Hotels and camps of CNP regarding Roundworm, Liverfluke and Paramphistome from the years 1991-2011were performed.

Parasitic prevalence in elephants of different safari camps and lodges (Location wise)

The study revealed that the overall prevalence of helminths in Temple tiger was 93.20%, in Tiger tops 64.2%, in Narayani safari 58.92%, Gaida Wildlife camp 100%, Chitwan Jungle Lodge 79.71% and Machan Wildlife camp 100%. Geographical-wise the incidence was highest in the elephants of Western side of the Park relative to the Eastern side. However, the result of high parasitic prevalence in Machan Wildlife camp and Gaida Wildlife camp is due to very limited data. Infections of Roundworms were observed almost in all the elephants followed by Liverfluke and Paramphistomes.

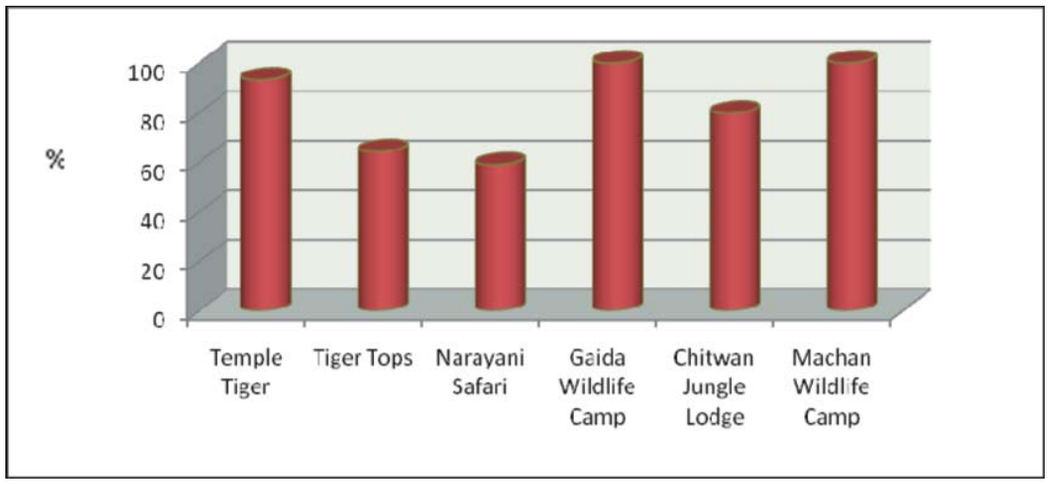

Fig. 2: Percentage parasitic prevalence in elephants of different safari camps and lodges.

Prevalence of Parasitc infestation in elephants of various hotels

The overall incidence of infection was 82.67 percent. Roundworm, Liverfluke and Paramphistome were prevalent in elephants of all the Hotels. In Machan wildlife camp Roundworm(RW), Liverfluke (LF) and Paramphistomes (Param) were found to be 69.44%, 8.33% and 33.33% respectively, Likewise in Chitwan Jungle Lodge the Roundworm, Liverfluke and Paramphistomes were 68.21%, 3.47% and 9.72% respectively while in Gaida wildlife camp RW, LF and Param were found to be 75%,12.5% and 16.5% respectively. Similarly, in Narayani Safari RW, LF and Param were 22.08%, 20.33% and 16.91% respectively. In Tiger tops RW, LF and Param were 27.13%, 32.58% and 4.53% respectively and in Temple Tiger RW, LF and Param were 53.02%, 36.25% and 4.44% respectively.

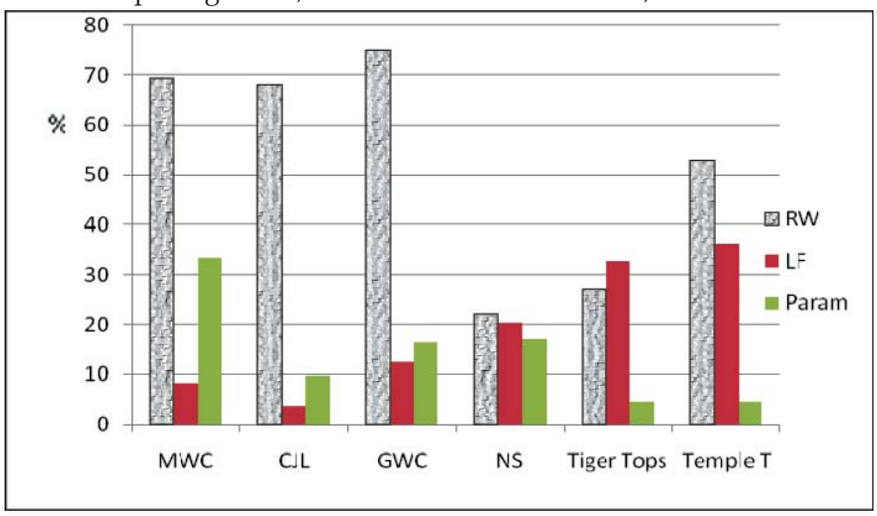

Fig. 3: Percentage prevalence of Parasitc infestation in elephants of various hotels.

Prevalence of parasitic infestation according to season.

The overall infection was found to be predominant during summer (82.94%) followed by Autumn (71.18%), spring (62.59%) and Winter (47.54%). The prevalence of helminths were found to be highest in Summer season. In Machan wildlife camp the helminths in Summer, Autumn and winter were found to be as 100%, 75% and 33.33% respectively. Similarly, in Chitwan Jungle Lodge, in spring, summer, Autumn and Winter the helminths were prevalent as 100%, 87.49%, 75.23% and 21.42% respectively. In Gaida wildlife camp, in spring and summer the helminths were prevalent as 50% and 100% respectively. In Narayani Safari, in Spring, Summer, Autumn and Winter the helminths were prevalent as 27.77%, 73.33%, 60.41% and 53.33% respectively. In Tiger tops, in Spring, Summer, Autumn and Winter the helminths were found as 45.21%, 70.58%, 69.04%, 43.26%.

In temple tiger, in Spring, Summer, Autumn and Winter the helminths were prevalent as 90%, 66.27%, 76.25%, 86.36% respectively.

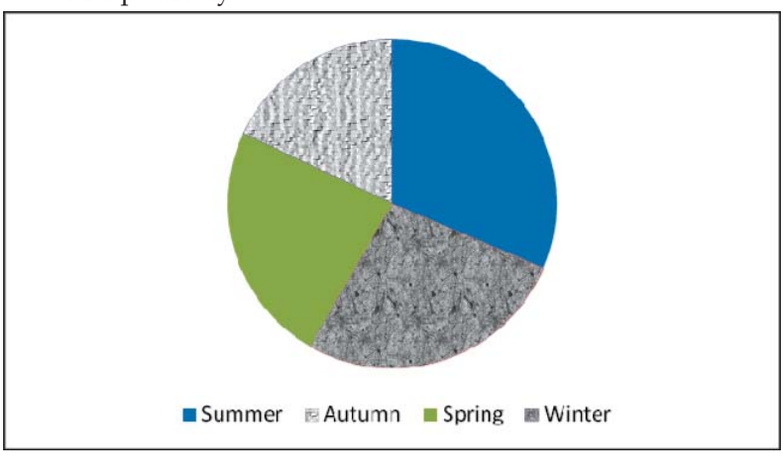

Fig. 4: Prevalence of parasitic infestation according to season.

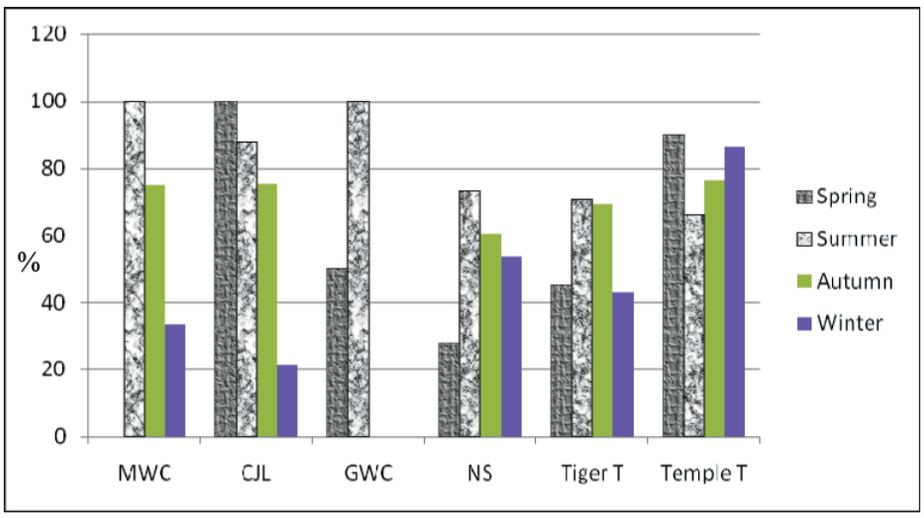

Fig. 5: Percentage prevalence of parasitic infestation according to season.

Number of elephants of Machan wildlife camp with parasitic prevalence in different years

The data of parasitic examination of elephants were available of the years 1992, 1993 and 1995 which shows most of the individuals were infected.

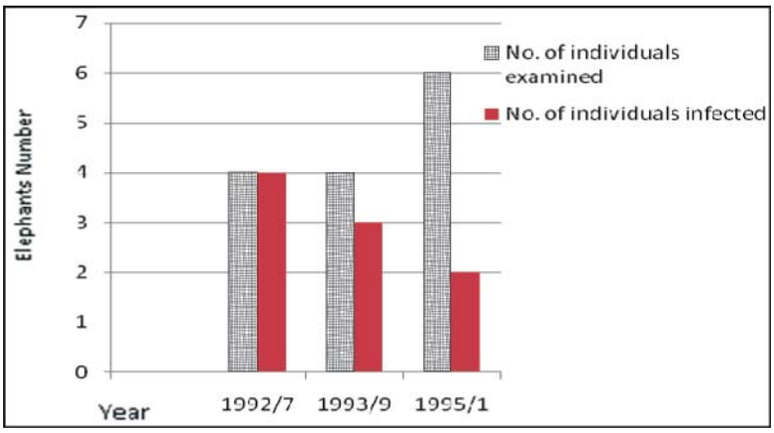

Fig. 6: Trend of parasitic infestation in elephants of machan wildlife camp in different years.

Number of elephants of Gaida Wildlife camp with parasitic prevalence in different years.

All the elephants were infected in 1993 while half of the elephants were infected in the year 2001.

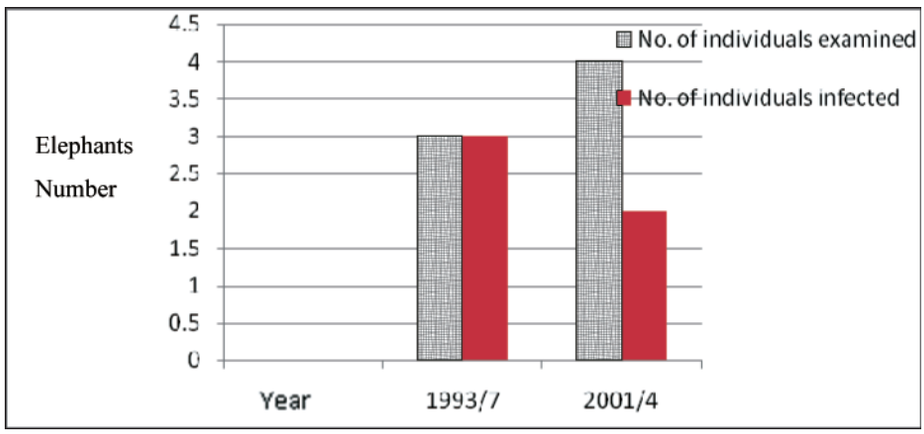

Fig. 7: Trend of parasitic infestation in elephants of Gaida wildlife camp in different years.

Number of elephants of Tiger Tops lodge with parasitic prevalence in different years

The elephants are dewormed so the infestation rate gradually declines while again it rises with the absence of periodic checkup. In 1990, 9 elephants were infected out of 16 and after 8 months only 4 elephants were infected out of 18 elephants. There is similar trend in 2001 November, 2004 July and 2005 January. Almost all the elephants are infected in the years 2005 and 2006. No parasite was found in April 2009.

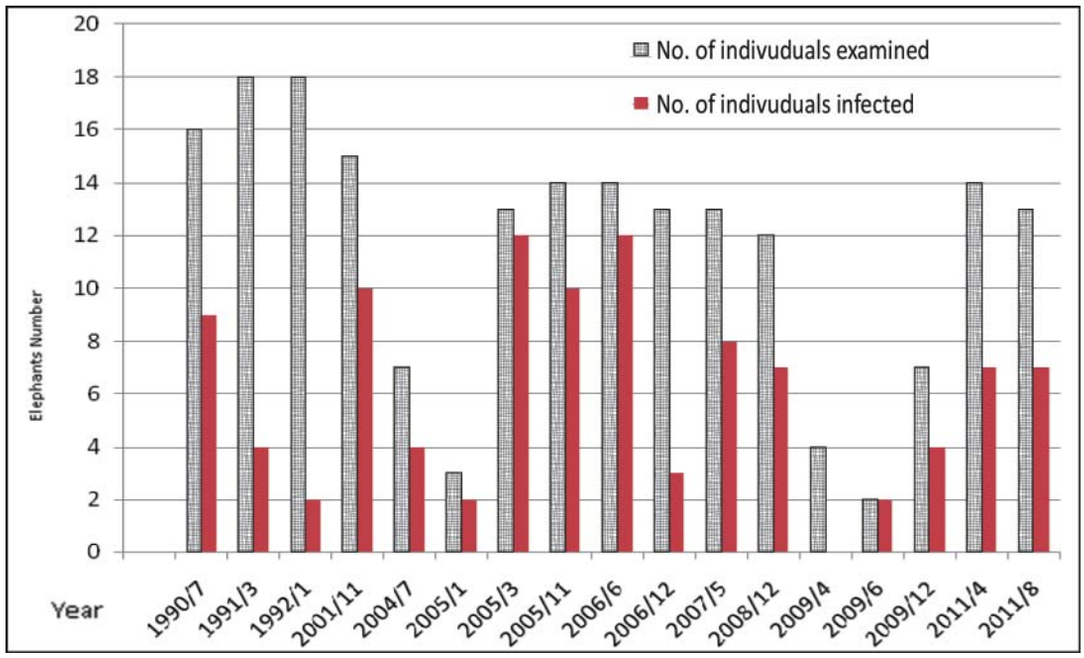

Fig. 8: Trend of parasitic infestation in elephants of Tiger tops lodge in different years.

Number of elephants of Chitwan Jungle lodge with parasitic prevalence in different years

The parasitic infestation is observed in almost all the elephants despite the periodic deworming. Two elephants were found positive out of 5 elephants examined in November 2001. Only 1 elephant was examined in April 2004 and that was found positive. All the eight elephants were infected in the April 2005 while 6 out of 7 elephants were infected in November of the same year. 6 elephants were examined out of which 5 elephants were positive for parasitic examination in the month

of July 2006. However, only 1 elephant was found positive out of 7 after five months. Again the parasitic positive elephants increased to 4 out of 6 examined in June 2007. 6 out of 6 elephants examined were found positive for fecal examination in November 2007 and June 2008. Only 2 out of 7 elephants examined were positive in December 2009 while all the 3 elephants examined were positive in April 2010. In June 2011, there were 7 elephants examined which all of them were positive.

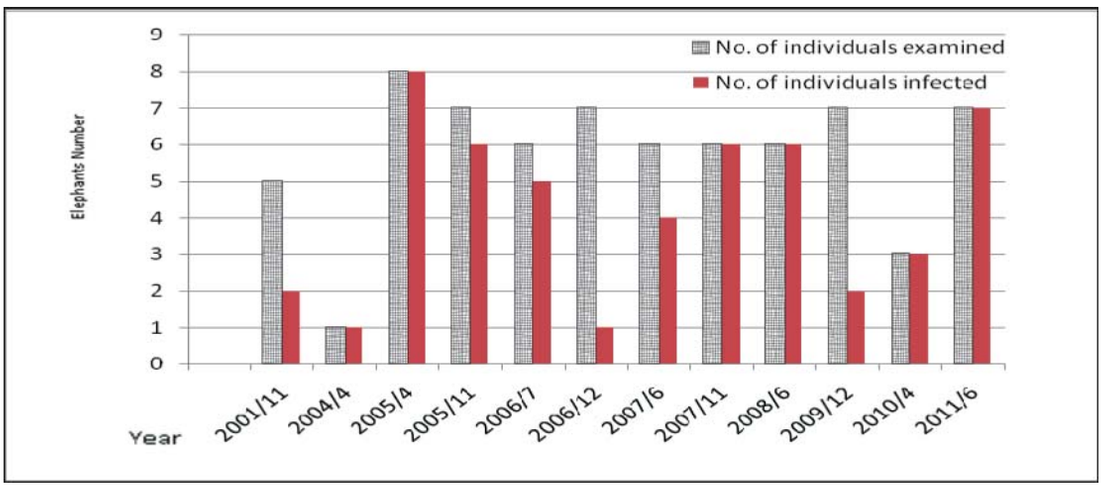

Fig. 9: Trend of parasitic infestation in elephants of Chitwan Jungle Lodge in different years.

Number of elephants of Temple Tiger lodge with parasitic prevalence in different years

Maximum numbers of elephants are observed to be infected from the year 2005.

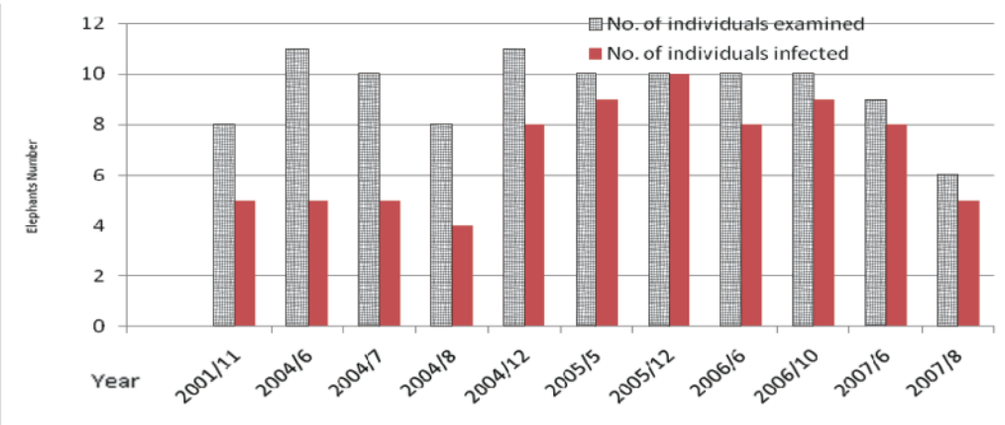

Fig. 10: Trend of parasitic infestation in elephants of Temple tiger lodge in different years.

Number of elephants of Narayani Safari lodge with parasitic prevalence in different years

In 1991, 3 out of 5 elephants are infected whereas only 3 out of 8 elephants are infected in the year 2001. The infection decreased to only 1 elephants out of 6 in May of the year 2006 but again the infestation increased to 6 out of 6 in the month of July the same year. From 2008, the infestation appears to be in decreasing order.

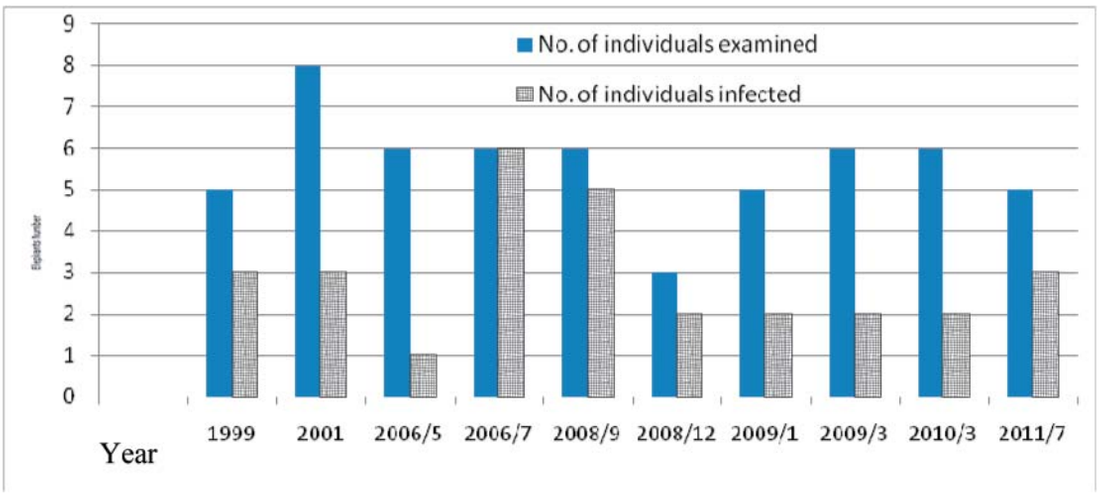

Fig. 11: Trend of parasitic infestation in elephants of Narayani safari camp in different years.

DISCUSSION

Every year before the start of summer season, elephants used to eat soil- 'mattine' and followed by 'shooting diarrhoea, some even died. The case of mud eating may be due to high parasitic load in the elephants. The one interesting thing about mud eating is that if the elephant eats the dirty stable mud then, it makes her more sick but if it eats of the jungle then it does not represent so (as per observation by the elephant keepers). Elephants infected with gastro-intestinal helminthes were generally anaemic with frequent colic accompanied by foul smelling diarrhea. Mud eating and oedematous swelling on the lower part of the jowl, neck, brisket and abdomen are indicative of fairly high infection with helminthes.

Potential factors determining the transmission of parasites include environmental conditions that affect the viability and behaviour of parasite propagules, and feeding, movement, and defecation patterns of the host, which determine the parasites encountered. Higher parasite loads in the dry season compared to the wet season, which has also been reported by Vidya et. al (2002), may occur due to reasons like transmission, diet and difficulty in collection of sample in winter. The lower prevalence of parasites in the wet season could be indicative of lower transmission. Transmission through water may play an important role since elephants often defecate near or in water and congregate at the limited water sources during summer. Experiments to assess the numbers of infective stages in stagnant and running water during different seasons, and examining the utilization of different water sources and patterns of feeding near them, may help elucidate this.

Host-parasite interactions can be very complex, with several factors acting in a varied fashion to produce patterns that may not be easily attributed to any one factor. This could be especially so when the parasite has intermediate stages, or the host is a predator sharing parasites with its prey, in which case, the predator's parasite loads could be determined by several factors related to prey dynamics.

Although the regular deworming is done in 4-6 month interval there is high parasitic problem in the elephants. This might be because elephant had free access to park and social communities creating situation of close contact and intermingling among wild animals and domestic livestock and presence of abundant snail in pastures. Wildlife-livestock competition for grazing is regarded as a major issue in protected area management in Nepal (Basnet, 2002). Preference of the elephants for water bodies and habit of soil licking might be the reason for higher parasitic prevalence. Apart from this the migration of elephants from one place to other for draught purpose and for other ceremony work could have favored the mixed parasitic infection.

Interesting thing is some elephants are always positive despite the Antihelminthic treatment and some are always negative which may be due to genetic cause.

Wild animals are the natural reservoir of various diseases and parasites unfortunately, assessing the risk of diseases transmission between wildlife and livestock is extremely difficult. Elimination of intermediate hosts mainly insects by burning pastures in dry season will help to break the parasitic lifecycle. Removal of snail from swampy area will also limit fluke prevalence. Before we come up with the simplest idea of deworming, it is mandatory to consider epidemiological pattern and life cycle of parasite and plan strategic mass deworming accordingly. It would be more fruitful to continue parasitic assessment in Chitwan National Park as well as other Wildlife Reserves and Parks of the country more extensively taking more disease parameters including highly infectious viral and bacterial diseases like Herpes virus.

Various ecological factors that potentially influence intestinal parasite loads must be studied like season, habitat also the effect of physical condition, age, and sex must be investigated.

CONCLUSION

Large numbers of elephants in captivity in Chitwan are susceptible to a number of ailments, particularly parasitic diseases. Gastrointestinal helminthes are very common and this high infection rate will decrease their productivity, growth and reproductive efficiency. Better management practices can be developed to limit parasitic problem and transmission. The management of livestock husbandry is one of the possible ways to limit parasite burden on pasture which will stop transmission of highly infectious protozoan, bacterial and viral diseases between the livestock and wild animals.

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PREVALENCE OF HELMINTH PARASITES IN DEER OF MRIGASTHALI

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ABSTRACT

A study was conducted as a part of internship research of Kathmandu District from October to March 2011 to assess the prevalence of helminth parasites in the deer of Mrigasthali. A total of 100 fecal samples were taken for the study. The samples were examined qualitatively by sedimentation and floatation method and quantitatively by Mc Master Method and larvae culture. The laboratory work was executed at Department of parasitology in Himalayan College of Agricultural Sciences and Technology (HICAST), Bhaktapur and Central Veterinary Laboratory, Tripureshwor. The study showed the overall prevalence of helminths to be 41%. The prevalence in barking deer, spotted deer and black buck was 78.57%, 42.5% and 28.26% respectively. The parasites found were Oesophagostomum, Haemonchus, Trichuris, Trichostrongylus and Paramphistomum. The findings provided the baseline idea on parasitic burden in deer and help to formulate appropriate strategies to mitigate the endoparasitic problem in deer in semi captive state.

INTRODUCTION

Nepal is remarkable for an abundance and variety of wildlife which have been famous throughout the world for their uniqueness, gameness, strength, agility and beauty. The mammals of Nepal are intrinsic and of biological value. The life of many valuable mammals including swamp deer and blackbuck is in peril in Nepal. Many pristine forms are now on the verge of extinction. Nepal, with one of the richest legacies of mammalian fauna, is guilty of abusing these gifts through centuries. The anthropogenic activities such as hunting, poaching, poisoning, bush fires, slaughter and the wholesale destruction of wild herds under the pretext of endemic diseases, have all contributed to the serious depletion of this once abundant wildlife in the Himalayas (Shrestha, 1997).

Wildlife population is declining and few are in constant threats of extinction because of habitat destruction, poaching, environmental pollution, introduction of exotic species, over exploitation, trade of items of wild origin, climatic change etc. Conservation measures are vital for the existence of wild animals. The measure may include establishment of situ conditions (habitat management, parks and reserves), enforcement of act, breeding in captivity and re introduction, mass education, and protection in assemblage (Thapa, 1999).

MATERIALS AND METHODS

The study area was Mrigasthali which lies in Kathmandu district. There were altogether 165 deers in Bankali forest which included 14 barking deer, 65 spotted deer and 87 black bucks. Deer in Mrigasthali are under the care of Pashupati Development Trust (PDT) and are in semi-captive state. Opportunistic fresh faecal samples were collected (Table 1).

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Table 1: Number of samples from the site

Site	Spotted deer	Black buck	Barking deer	Total
Pasupati	40	46	14	100

The qualitative parasitic investigation was carried out through Direct Smear, Sedimentation and Differential Flotation methods (Soulsby, 1978) and the eggand larval identification was made as per the atlas of (Soulsby, 1978).

RESULT

Overall prevalence of helminths

A total 100 samples were taken for the study. The number of sample from spotted deer was 40, black buck was 46 and barking deer was 14. Out of 100 samples examined for helminths, 41 samples were positive for at least one helminthes, showing the overall prevalence of 41 %.

Prevalence of helminths according to deer species

The number of samples taken for the study was 100. Out of 14 samples taken from the barking deer, 11 samples were positive for at least single parasite showing the parasitic prevalence of 78.57 %. Regarding spotted deer, 17 out of 40 were positive for at least one parasite showing 42.5 % prevalence. In case of Black Buck, 13 samples out of 46 were positive for at least one parasite showing 28.26 % prevalence.

Types of helminths in deer

Out of 100 deer; 2 deer showed Trichuris (2%), 6 showed Haemonchus (6%), 10 showed Oesophagostomum (10%), 10 showed Paramphistomum(10%) and 13 showed Trichostrongylus (13%).

Types of helminths in barking deer

The fecal sample was examined by sedimentation as well as floatation methods. No eggs were observed in floatation technique. Out of 14 samples, 1 sample was positive for Trichuris (7.14 %), 3 samples each were positive for Paramphistomumand Trichostrongylus (21.43 %) respectively and 4 samples were positive for Oesophagostomum (28.57 %). The overall prevalence in barking deer was 78.57 % (11/14).

Types of helminths in black buck

Out of 46 samples, 2 samples were positive forHaemonchus (4.35 %), 3 samples were positive forOesophagostomum(6.52 %) and 4 samples each were positive for Paramphistomumand Trichostrongylus (8.70 %) respectively. The overall prevalence in black buck was 28.26 % (13/46).

Types of helminths in spotted deer

Out of 40 samples, 1 sample was positive for Trichuris (2.50 %),3 samples each were positive forOesophagostomumandParamphistomum (7.50 %) respectively, 4 samples were positive for Haemonchus (10 %) and 6 samples were positive for Trichostrongylus (15 %). The overall prevalence in spotted deer was 42.50 % (17/40).

DISCUSSION

Thapaand Thapa (2007) found the prevalence of endoparasites to be 44.4 % in spotted deer of Shuklaphanata Wildlife Reserve and Fasciola(22.2 %), Strongyles(11.1 %), Paramphistome (5.5 %),

Ascaris (5.5 %), Trichuris(5.5 %), Strongyloides (16.6 %), Macrocanthus (5.5 %) were the prevalent helminths. This study showed 42.5 % parasitic infection in spotted deer which is similar to this finding.

Thapa and Adhikari (2008) observed 62.5 % prevalence of helminthes in deer of CNP buffer zone. Overall prevalence rate of parasite in livestock and wild animals was 72.49 % and 76.16 % respectively in the buffer zone of CNP. The higher rate of prevalence than this finding is attributed to their existence in wild state may be due to lack of deworming and sharing the common pasture with domestic animals.

Out of 60 Samples of deer from Maharajbag Zoo, Nagpur, 30 were positive for eggs and larvae of helminthic parasites. The encountered parasitic species were Haemonchus spp., Dicrocoeliumspp., Paramphistomum spp., Oesophagostomum spp. and Bunostomum spp. etc. Direct smear method together with sedimentation technique were used for the purpose (Borghare*et al.*, 2009). The higher rate of prevalence than this finding may be due to improper managemental practices and untimely deworming.

Ninety two roe deer (CapreoluscapreolusLinnaeus) from 9 regions of Ukraine were examined by the partial helminthological dissection. Prevalence of roe deer infection with helminths was 92.4%. Sixteen helminth species were found. Setariacervi (10.9%) was found in visceral cavity. Dictyocauluseckerti (6.9%) and D. capreolus(2.3 %) was found in lungs. Taeniahydatigenalarvae (2.3%) were found in mesentery. Paramfistomumcervi (10.9%), Haemonchuscontortus (57.6%), Ashworthiussidemi (40.2%), Marshallagiamarshalli(15.2%), Nematodirusoiratinus(1.1%), Trichostrongylusaxei (3.3%) were found in stomach. Moniesiaexpansa (1.1%), Bunostomumphlebotomum (10.9%) were found in small intestine. Trichocephalusovis (18.5%), Oesophagostomumvenulosum (7.6%) and O. dentatum (1.1%) were found in caecum. Chabertiaovina (28.3 %) was found in large intestine. Forty-four helminth associations were separated in the roe deer examined (Kuzmina*et al.*, 2010). The higher rate of prevalence than this finding may be due to the fact that the study covers a wide range of area and their existence in the wild state.

CONCLUSION

The findings of this study have concluded that there is prevalence of helminths in the deer of Mrigasthali, Kathmandu and the wardens are not aware of this situation. Due to the habit and habitat of deer, wardens cannot manage this situation which may make these animal species endangered on the near future. So, there is a need of wildlife habitat improvement, health improvement, preservation of biological diversity, protection of natural environment and monitoring of pollution, which is the fundamental of conservation. Parasitic infection decreases the production and productivity in the animals mainly in the reduction of body weight or failure to gain weight or sometime even mortality in acute cases. This baseline idea on gastro intestinal parasitic loads in deer might be useful in formulating strategy to control disease and mortality in deer of study site as well as other habitat and may be new avenues to conservation regarding loss from disease.

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EQUINE NUTRITION: SOME IMPORTANT CONSIDERATION

M. P. Sah[1]

ABSTRACT

Equine nutrition deals with the nutrient requirement and feeding of horses, ponies, mules, donkeys, and other equines. Equines are monogastric animal but can use forages effectively due to the presence of the microbial population in the hindgut and so also called "hind gut fermenter". Horses have a small stomach (with a capacity of only 15 liters) in terms of relative size compared to other classes of livestock. This makes the rate of passage of feed through the stomach relatively fast. Horses are physically unable to vomit or belch. Consequently, overfeeding and rapid rates of intake are a potential problem. Horses do not have a gall bladder, so bile flows constantly, an adaptation to a slow but steady supply of food. Like all animals, equines require five main classes of nutrients to survive: water, energy, proteins, vitamins, and minerals. Nutritional requirements depend on the body weight, growth rate, work type, pregnancy and lactation stage. As with other species of livestock, access to good quality water is important to ensure optimum health and performance. Energy requirement during the last trimester of pregnancy and lactation increased upon the maintenance requirement by 11%-20% and 25-40% respectively. Similarly, during heavy work (like racing) energy requirement may increase by 100%. Likewise, growing horses have a higher need for protein (14-16% of total ration) than mature horses (8- 10% of total ration). Fetal growth during the last third of pregnancy increases protein requirements somewhat (10-11% of total ration), and lactation increases requirements still further (12-14% of total ration). Lysine is the first indispensable amino acid for young horses. Generally vitamin and minerals are supplied by good quality green grasses and hay, otherwise 1-2 % vitamins & mineral mixture should be added in ration. For feeding of horses, dry matter intake varies from 1.5 % to 3 % of body weight depending on work types and production stages but good quality roughages must be fed at least 1 % of the body weight /day to ensure normal digestive function. So, long-stemmed forages (hays or straw, green fodder) or pasture grasses are necessary in the diet. When concentrates are fed, consideration should be given to grain processing, frequency of feeding, the amount of concentrate necessary and dental soundness. Colic, choke, and laminitis are the life-threatening illness of horse related to improper feeding.

INTRODUCTION

Equine nutrition deals with the nutrient requirement and feeding of horses, ponies, mules, donkeys, and other equines. Equines are non-ruminant herbivores animal. They eat the fibrous feeds (roughages) but do not have a rumen with bacteria for fermentation to utilize the fiber. They have relatively large cecum and colon with useful bacteria for fermentation to utilize the fiber so also called hind gut fermenter (Hintz, H. F., 1977). Nutrient requirement and feeding management of equine differs from that of other domestic livestock, primarily because of differences in digestive anatomy and physiology like- absence of gall bladder, more capacious hind gut compare to fore gut, inability to regurgitate food, and microbial fermentation in cecum and colon (Lardy, et al, 2001) etc.

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DIGESTIVE TRACT OF THE EQUINE

For domestic livestock, horses have a peculiar digestive tract. Functionally, the digestive tract can be divided into two components (foregut and hindgut). Components of the foregut include the mouth, esophagus, stomach, and small intestine; while the hindgut includes the cecum, large colon, small colon, and rectum. The foregut of the horse accounts for approximately 35 to 40 percent of the relative capacity of the digestive tract. When compared to the relative capacity of the foregut in pigs (60 to 65 percent) and cattle (85 to 90 percent); the uniqueness of the horses' digestive anatomy becomes apparent. Similarly, the hindgut of the horse accounts for approximately 60-65% percent of the relative capacity of the digestive tract which is higher compare hindgut capacity(10- 15%) of cattle.

Table 1: Major differences of Digestive Tract of the Equine with Cattle

	Horse	Cattle
Foregut (Relative capacity)	35 -40 %	85-90 %
Hind gut (Relative capacity)	60 – 65 %	10 – 15 %
Stomach (Average Capacity)	15 lit.	150 – 230 lit
Small intestine	15 to 21 m & 40 – 45 lit.	40 m & 42-26 lit
Large intestine	7- 8 m & 115 – 125lit	10 – 11m
Cecum	1.2 m & 25- 30 lit	0.75 m & 7- 8 lit.
Colon	6.5 – 7 m & 90 – 95 lit	10 m & 25 – 30 lit

Source: Primary Veterinary Aantomy

Digestive physiology

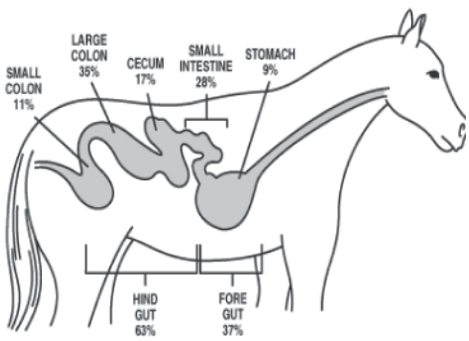

Fig 1: Anatomy of the horse digestive tract with relative sizes.
Source: NDSU; www.ag.ndsu.edu

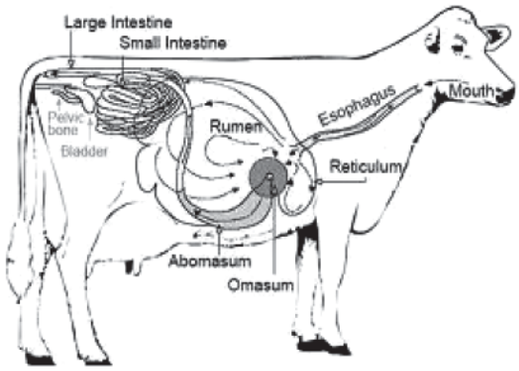

Fig 2: Anatomy of the cow digestive tract.
Source: www.articlesweb.org.cow

Horse require small amount of feed frequently because his stomach is small compare to body size. So, horse grazes most of time at ground or eats hay or straw in the stable and rest only for short period. They do not have a gall bladder, so bile flows constantly, an adaptation to a slow but steady supply of food, and another reason for providing fodder to horses in several small feedings. Horses are physically unable to vomit or belch. Consequently, overfeeding and rapid rates of intake are a potential problem.

Digestion and absorption of soluble CHO (e.g. Starch) takes place in stomach and small intestine as non- ruminant but the digestion and absorption of non-soluble CHO (e.g. cellulose) takes place in cecum and colon by microbial fermentation like ruminant animals. Digestion of average quality grass hay in horse is only about two- thirds as efficient as the ruminant. Volatile fatty acid produced in hind gut supply about one-fourth of horse energy need (Reddy, 2009). Rate of passage of feeds is also fast in horse (65-70 hrs.) compare to cattle. (> 90 hr. in cow) on hay- grain ration. Digestion and absorption of protein, fat, vitamins and minerals primarily takes place in small intestine. Similarly, absorption of water primarily takes place though cecum and colon. Some of major factors affecting digestion of feed are: processing of feeds, level of intake, frequency of feeding, work, individuality, associative effects, time of watering etc. (Hintz, H. F.,1977).

NUTRIENTS AND NUTRITIONAL REQUIREMENTS

Like all animals, equines require five main classes of nutrients to survive: water, energy (carbohydrates and fats), proteins, vitamins, and minerals. Nutritional requirements depend on the body weight and functions like: growth, work, pregnancy and lactation.

Water

Water makes up between 62-68% of a horse's body weight and is essential for life. It is important for horses to have access to a fresh, clean, and adequate supply of water. Water requirements depend largely on environment, physical activity being performed, and nature of the feed and physiologic status of the horse. The minimal daily water requirement of an adult horse is 33 ml/ kg body wt. /day with average intakes of ~50 ml/kg body wt. /day. Lactation or sweat losses, however, may increase the needs by 50-200%. An average 450 kg horse drinks 45 litter of water per day (Merck Veterinary Manual).

Energy (Carbohydrates and Fat)

Nutritional sources of energy are carbohydrates and fat. Carbohydrates, the main energy source in most rations, are usually fed in the form of hay, grass, and grain. Fat in the form of oil may use for energy in heavy working and lactating horse. Energy requirements may be classified into those needed for maintenance, growth, pregnancy, lactation, and work. Its requirement is increased over the maintenance requirement during pregnancy, lactation and heavy work by 20-30%, 40-50%, 50-100% respectively (Reddy, 2009). If a horse has too much energy in diet and not enough exercise, it can become too high-spirited and difficult to handle. Table 1. and Table 2.show the daily nutrient requirements of growing and mature horses and ponies. However, the need for energy differs considerably among individuals and digestibility of feeds often differs greatly from published values.

Protein

Horses actually have an amino acid requirement rather than a requirement for protein. Protein is needed as a source of indispensable amino acids and of nitrogen for synthesis of dispensible amino acids. Lysine is an indispensable amino acid for the young horse. Methionine and threonine may also be the indispensable amino acids for the horse under certain conditionsn (Reddy, 2009). Soybean meal, gram and Alfalfa / other legumes hay are good sources of protein that can be easily added to the diet. Protein requirement depends on the age and working condition. Weanlings require 2.1 g and yearlings 1.9 g of lysine/Mcal DE/day. Requirements for other dietary amino acids have not been established; however, the crude protein recommendations given in Table 3. and Table 4. for growing and mature horse and ponies (NRC,1986).

Table 2: Recommended crude protein level in total diet (Cunha, 1991)

S.N.	Stage of life cycle	Percentage of protein in the total diet
1.	Creep feed and feed for foals	16-18
2.	Weaned foals to 12 months	14-16
3.	Yearling, long-yearling, and two-year-olds	12-14
4.	Mare (gestation and lactation) and breeding stallions	12-14
5.	Mature horses (idle and at work)	9-11

Vitamins

Vitamins are organic compounds needed in trace amounts that regulate a multitude of bodily functions. Fresh and green grasses are good source of vitamins.Horses that are not subjected to hard work usually have more than adequate amounts of vitamins in their diet if they are receiving fresh, green, leafy forages. Sometimes a vitamin supplement is needed when feeding low-quality

hay, if a horse is under stress (illness, traveling, showing, racing, and so on), or not eating well. Grain has a different balance of nutrients than forage, and so requires specialized supplementation to prevent an imbalance of vitamins and minerals. Generally 1-2% vitamin mixture is given for medium type work, pregnancy and lactation. Similarly during growing and heavy work vitamin requirement is increased by 20 -40% (Merck Veterinary Manual).

Minerals

Minerals are inorganic compounds needed as components of body tissue and as facilitator of various body processes. Minerals are commonly found in most good-quality feeds. Normally, if adult animals at maintenance levels are consuming fresh hay or are on good pasture, they will receive adequate amounts of minerals in their diet, with the exception of sodium chloride (salt), which needs to be provided, preferably free choice. Some pastures are deficient in certain trace minerals, including selenium, zinc, and copper, and in such situations deficiency diseases may occur if horses' trace mineral intake is not properly supplemented. Foals and young growing horses through their first three to four years have special nutritional needs and require feeds that are balanced with proper calcium: phosphorus ratio and other trace minerals. Calcium and phosphorus are needed in a specific ratio of between 1:1 to 2:1. Adult horses can tolerate up to a 5:1 ratio, foals no more than 3:1. A total ration with a higher ratio of phosphorus than calcium is to be avoided. Hard work increases the need for minerals. Therefore, supplementation with electrolytes may be required for horses in intense training, especially in hot weather (Reddy, 2009).

Table 3: Daily Nutrient Requirements of Ponies (200 kg mature body weight), NRC (1989).

Animal	Weight (kg)	Daily Gain (kg)	DE (Mcal)	Crude Protein (g)	Lysine (g)	Calcium (g)	Phos-phorus (g)	Magne-sium (g)	Potas-sium (g)	Vitamin A (10^3 IU)
Mature horses										
Maintenance	200		7.4	296	10	8	6	3.0	10.0	6
Stallions (breeding season)	200		9.3	370	13	11	8	4.3	14.1	9
Pregnant mares										
9 months	200		8.2	361	13	16	12	3.9	13.1	12
10 months			8.4	368	13	16	12	4.0	13.4	12
11 months			8.9	391	14	17	13	4.3	14.2	12
Lactating mares										
Foaling to 3 months	200		13.7	688	24	27	18	4.8	21.2	12
3 months to weaning	200		12.2	528	18	18	11	3.7	14.8	12
Working horses										
Light work[a]	200		9.3	370	13	11	8	4.3	14.1	9
Moderate work[b]	200		11.1	444	16	14	10	5.1	16.9	9
Intense work[c]	200		14.8	592	21	18	13	6.8	22.5	9
Growing horses										
Weanling, 4 months	75	0.40	7.3	365	15	16	9	1.6	5.0	3
Weanling, 6 months										
Moderate growth	95	0.30	7.6	378	16	13	7	1.8	5.7	4
Rapid growth	95	0.40	8.7	433	18	17	9	1.9	6.0	4
Yearling, 12 months										
Moderate growth	140	0.20	8.7	392	17	12	7	2.4	7.6	6
Rapid growth	140	0.30	10.3	462	19	15	8	2.5	7.9	6
Long yearling, 18 months										
Not in training	170	0.10	8.3	375	16	10	6	2.7	8.8	8
In training	170	0.10	11.6	522	22	14	8	3.7	12.2	8
Two year old, 24 months										
Not in training	185	0.05	7.9	337	13	9	5	2.8	9.4	8
In training	185	0.05	11.4	485	19	13	7	4.1	13.5	8

Table 4: Daily Nutrient Requirements of Horses (500 kg mature body weight), NRC (1989)

Animal	Weight (kg)	Daily Gain (kg)	DE (Mcal)	Crude Protein (g)	Lysine (g)	Calcium (g)	Phos-phorus (g)	Magne-sium (g)	Potas-sium (g)	Vitamin A (10^3 IU)
Mature horses										
Maintenance	500		16.4	656	23	20	14	7.5	25.0	15
Stallions (breeding season)	500		20.5	820	29	25	18	9.4	31.2	22
Pregnant mares										
9 months	500		18.2	801	28	35	26	8.7	29.1	30
10 months			18.5	815	29	35	27	8.9	29.7	30
11 months			19.7	866	30	37	28	9.4	31.5	30
Lactating mares										
Foaling to 3 months	500		28.3	1,427	50	56	36	10.9	46.0	30
3 months to weaning	500		24.3	1,048	37	36	22	8.6	33.0	30
Working horses										
Light work[a]	500		20.5	820	29	25	18	9.4	31.2	22
Moderate work[b]	500		24.6	984	34	30	21	11.3	37.4	22
Intense work[c]	500		32.8	1,312	46	40	29	15.1	49.9	22
Growing horses										
Weanling, 4 months	175	0.85	14.4	720	30	34	19	3.7	11.3	8
Weanling, 6 months										
Moderate growth	215	0.65	15.0	750	32	29	16	4.0	12.7	10
Rapid growth	215	0.85	17.2	860	36	36	20	4.3	13.3	10
Yearling, 12 months										
Moderate growth	325	0.50	18.9	851	36	29	16	5.5	17.8	15
Rapid growth	325	0.65	21.3	956	40	34	19	5.7	18.2	15
Long yearling, 18 months										
Not in training	400	0.35	19.8	893	38	27	15	6.4	21.1	18
In training	400	0.35	26.5	1,195	50	36	20	8.6	28.2	18
Two year old, 24 months										
Not in training	450	0.20	18.8	800	32	24	13	7.0	23.1	20
In training	450	0.20	26.3	1,117	45	34	19	9.8	32.2	20

TYPES OF FEED

Equine can consume approximately 2-2.5% of their body weight in dry feed each day. Foals less than six months of age eat 2-4% of their weight each day. Feed for horses can be divided into four categories: pasture, hay, concentrates & other supplements.

Pasture and green grass

The most natural food for horses is good quality pasture. Good pasture provides both nutrients and the opportunity to exercise. Most mature horses doing light work will do well on pasture alone if they have sufficient grazing. However, horses are selective grazers and need a large area to meet their nutritional needs. Rotational grazing is good as it give the grass a chance to grow back and can pick up the manure. The pasture should be kept free of weeds if possible by regular mowing or clipping. A legume-grass mixture offers the advantages of good nutrient supply, persistence, and durability. Ideal mixes vary with region, and local recommendations from specialists should be followed. Pasture grass such as dub (cynodon dactylon), pergola, timothy and orchard grasses are popular. Similarly, Lucerne, berseem, cowpea, oats and maize green fodders are excellent for horses (Reddy, 2009).

Hay and straw

Hay is the basic food of domestic horses. Hay should be good quality and free from dust and mould. Feeding moldy hay can cause colic and dusty hay can cause respiratory problems. The type of hay available varies according to the area. Common types of hay used to feed horses include both grass hays (such as oat, timothy, orchard grass) and legumes hays (such as alfalfa, berseem, clover. Straws are used primarily for bedding but clean paddy, oat and wheat straw can be used as

a filler roughage. Oat straw is more palatable.

Concentrates

Concentrates are high in energy and protein. Hay alone cannot provide enough nutrition for hard-working horses, pregnant and nursing mares, or growing youngsters. They need concentrates to supplement the hay. Basically, there are two type of concentrate: energy rich (oats, barley, maize, wheat, wheat bran, rice bran, molasses and oils) and protein rich (soybean meal, groundnut cake & gram).

Oats, barley and maize are the principal cereal grains for horses. Of the three, Oats preferred over other cereals because starch of oats has high intestinal digestibility than others. This is due to different morphology of starch granules of oats and higher amylase activity in the jejunum. It also contain high fibers which prevent metabolic disturbance (like colic). It is soft compare to other grain. So it can fed as whole grain because it is easily rupture during mastication. Similarly, it content higher percentage of oil which help to maintain health and shiny hair coat. It also has good palatability and better protein quality (lysine content) compare to other cereals (Hintz, H. F.,1977).

Wheat and rice bran are byproduct supplements that are commonly fed to horses. However, both are very high in phosphorus (>1.2%), and so the proper calcium: phosphorus ratio should be maintained when any form of bran is added to the diet. Wheat bran by tradition is much favored feed for horses. Rice bran is a high-fat product that is added to rations of horses that need extra calories.

Fats and molasses may be added to the diet to increase the energy density. Usually 2 – 5% oil or molasses is recommended in horse ration to reduce the dustiness and increase energy content. Corn and vegetable oils are commonly used. Oils or molasses should be introduced slowly to the ration to avoid diarrhea.

Soybean meal is the good protein concentrate feed for horse. Soybean meal is a palatable protein supplement with good amino acid balance for use with grains. It may be fed when pastures or hay are low in protein and are of poor quality or when protein requirements are greatest, such as during early growth or lactation. Groundnut cake is the alternative. Linseed meal or cottonseed meal should not be used as a protein supplement for young foals due to their low lysine content, but they are adequate for adult horses. Linseed seeds are toxic too and must be boiled in water before feeding.

Bengal gram is the most popular protein supplement feed for horses in the Indian subcontinent. It is usually fed as a single concentrate feed after soaking in water overnight. It also fed as crushed and mixed with other grains. It can provide the requirements of protein alone at maintenance level when good quality roughage is not available. For better utilization, it can be soaked in water preferably for 5-6 hours. However, excessive soaking is likely to cause fermentation (Reddy, 2009).

Other supplements

Beet pulp, a byproduct of the sugar beet industry, is added to horse rations as both a source of calories and fiber. It contains moderate amounts of calcium and protein and can be safely fed on a daily basis in larger amounts than the bran products. Beet pulp should be soaked in water before feeding to horses.

Succulent Feeds like carrots and apples are commonly used as treats. A daily allowance of 0.5-1.5 kg is common. Similarly, silage or haylage of good quality is highly nutritious succulent forage. However, horses are extremely sensitive to mold in silage, and its use is not recommended, especially in hot, humid climates.

Salt (NaCl) should be provided in a block or in granular form ad lib. It may be desirable to use a trace mineralized salt that contains added iodine, iron, copper, cobalt, manganese, zinc, and selenium. The need for these additional minerals varies with the locality.

Vitamin and mineral should be provided in adequate amount. If the feed is insufficient in vitamin and minerals, commercially available vitamin and mineral supplement should be added in concentrate diet.

FEEDING PRACTICES

Horses are fed a variety of forms and types of feeds. Most horses are fed forage in the form of hay or pasture in combination with a grain mix, salt and water. The maximal dry matter intake in 24 hr is only 3-3.5% of a horse's body wt., and many horses voluntarily consume only 2-2.5% of their body wt. The choice of feed is influenced by the horses' requirements, availability of pasture, availability and cost of commercially prepared feeds, what traditionally has been fed, and how the horses are used and managed. Nutrients should be supplied in the amount, form and method that safely and efficiently meet requirements. Correctly supplying nutrients to horses requires the knowledge of requirements, feeds and nutritional management (Freeman, 2009).

Some important consideration of good feeding:

- Feed according to work, condition and temperament.
- Feed the horse a little and often.
- Feed plenty of roughage. (at least 1% of body weight)
- Horses should not be offered >0.4% of their body weight in concentrates (textured grain, pellets, or extruded feed) in a single feeding. For more concentrate, divide the feed into 3-4 times.
- Make any change gradually.
- Keep to the same feeding time every day.
- Feed something succulent every day.
- Green grasses should not cut before 45 days and should be free from poisonous grasses.
- Leave 1 hour after feeding before work.
- Water before feeding.
- Keep utensils clean
- Reduce the amount of feed on the horse's rest day

Feeding the Foal

Young horses require a nutrient dense diet because of small size of their digestive system. Foals must receive a diet adequate in energy, protein, vitamins and minerals in order to grow properly and achieve their full genetic potential. Feeding of foal start with colostrum for first 2-3 days. Milk requirement of the foal is about 10 % of the body weight. Foals will meet their nutritional needs in their first two to three months with mare's milk as long as the mare is milking properly. In the third month of lactation, the mare's milk production drops while the foal's nutritional needs keep increasing. Then start creep feed at a rate of 0.5 to 1.0 per cent of the foal's body weight per day up to a maximum 1.8 to 2.2 kg. Creep feed should contain CP 18 to20 , Ca 0.80%, P 0.6 %, Lys 0.7 % and Met 0.75% (Hintz, H. F.,1977).

Table 5: An Example of creep feed (Cunha, 1991)

Feed	Percent in diet
Oats (rolled /flaked)	35
Maize, barley, sorghum, (rolled or combination Of them rolled or fleked)	35.75
Soybean meal	15
Dried skim milk	05
Molasses	05
Dicalcium phosphate	02
Ground limestone	00.75
Trace mineral salt	01
Vitamin supplement	00.50

Feeding the Weaning Horses (6 months to 1 year of age)

One of the most critical times in the life of a growing horse occurs between weaning and about 1 year of age. During this post-weaning period fed a balanced high-quality ration with a good hay and green grasses as available. The feeding rate is 1- 1.5 kg concentrate and 1 kg forage per 100 kg body weight. The concentrate ration should provide 16- 18 % CP, 0.85 % Ca and 0.75 % P. The concentrate ration constitutes 65 to 70 % of the total ration (Reddy, 2009).

Feeding the Yearling Horses (1-2 years old)

During this period, the horse should be placed on a feeding program of 1 to 1.5 kg of forage and 1 – 1.5 kg concentrate mixture per 100 kg of body weight. Concentrate mixture should contain 14- 16 % CP, 0.8 % Ca and 0.65% P (Reddy, 2009). It is advisable to increase the oat percentage and decrease the corn percentage and adjust soybean meal/ gram percentage to meet CP % compare to the weaning horse ration. The level of feeding can vary considerably depending on how the horses are to be used, the kind and quality of ration, and the response of the horse to the feeding program followed.

Feeding the Working Horse

For light and medium work, energy requirement is increased by 25-50 %, so concentrate ration should be increased by 20 -40 depending on hay and green grass quality and availability. For High-level Performance Horse/ Race Horse energy requirement is increased by 50-100 % .The concentrate mixture should be at a level of 50 to 70 % of the total feed intake. The concentrate ration should contain 18 % CP, 0.95% Ca and 0.85 % P and this level can be increased during heavy training or racing. Roughage should be of high quality hay/ pasture. The concentrate part should be cut the night before the rest day to reduce the risk of 'tying-up' (Azoturia) and then reintroduced over 2 days once work has resumed. It is advisable to add 5-10% fat to the diet with increased level of vit. E or molasses 2 to 5% to fulfill the energy requirement (Hintz, H. F.,1977).

Feeding the Pregnant Mare

When mares are entering or are about to enter the last trimester of their pregnancy, this time 60% of fetal growth occurs and the mare's energy requirements will increase by around 20% meaning that mare is likely to need a change of feeding regime. During this time concentrate should be form 35% of the total ration. The gestation diet should have 12-14 % protein, 1% Ca & 0.9 % P. Some important consideration for feeding the broodmare at this stage are: monitor body weight, ensure a balanced diet, feed little and often, use the best quality sufficient forage as available (Reddy, 2009).

Feeding of Lactating Mare

Nursing makes the greatest nutritional demands as hardworking performance horses. One mare produce 15 -20 kg milk per day and each kg milk required. 792 Kcal DE. The mare's energy needs are double over the maintenance. Without sufficient calories in her diet, a lactating mare's hipbones and ribs sometime seem. So it is advisable to increase concentrate mixture at a level of 50 to 70 % of the total feed intake. The concentrate ration should provide 14% CP, 0.95% Ca and 0.85 % P. Roughage should be of high quality hay/ pasture. It is advisable to add 5-10% fat to the diet with increased level of vit. E or Molasses 2 to 5% to fulfill the energy requirement (Reddy, 2009).

Feeding the Stallion

The stallion's nutrient requirements are very similar to the nutrient requirements for maintenance. The primary difference is the need for increased energy in breeding season. Therefore, good quality forages is needed for breeding stallion. If grain is needed, a good rule is no modified a diet higher in energy to maintain a desirable body condition score and behavior. High-energy dense grains such as corn or oils at 10 to 20 percent can be added to the diet to increase the energy density, if necessary. However, individual stallions will respond differently to increased energy (Reddy, 2009).

Feedign the Mature horse

The energy requirement of mature horse at maintenance is low and can be met by feeding good quality roughage. However, salt and a balanced mineral supplement need to be provided free- choice. If good quality roughages are not available, some concentrate (10 to 20%) are fed to meet the nutritional requirements (Reddy, 2009).

Special Feeding Issues For Ponies, Mules And Donkeys

Ponies, mules and donkeys are usually easy keepers and need less feed than full-sized horses. This is not only because they are smaller, but also, because they evolved under harsher living conditions than horses and use feed more efficiently. Ponies easily become obese from overfeeding and therefore are at high risk for colic, and laminitis. Fresh grass is a particular danger to ponies; they can develop laminitis in as little as one hour of grazing on lush pasture.

Forage with water and a salt and mineral block can fulfill the nutrient requirement for maintenance and medium type work. Only hard-working pony needs concentrates, a ratio of no more than 30% concentrates to 70% forage is recommended. Concentrates designed for horses, with added vitamins and minerals, will often provide insufficient nutrients at the small serving sizes needed for ponies. Therefore, if a pony requires concentrates, feed and supplements designed especially for ponies should be used. Donkeys and mules need less protein and more fiber than horses. They do best when allowed to consume small amounts of food over long periods, as is natural for them in an arid climate. They can meet their nutritional needs on 6 to 7 hours of grazing per day on average dry land pasture that is not stressed by drought (Hintz, H. F.,1977).

Illnesses related to improper feeding

Colic, choke, and laminitis can be life-threatening when a horse is severely affected, and veterinary care is necessary to properly treat these conditions. Other conditions, while not life-threatening, may have serious implications for the long-term health and soundness of a horse like growth disorders, heaves, tying up (azoturia), lactation tetay, wood chewing etc(Hintz, H. F.,1977).

CONCLUSION

Horses are monogastrics but can use forages effectively due to the presence of the microbial population in the hindgut. They have a unique digestive tract in comparison to other species of livestock and because of this, proper feeding management is important to ensure the nutritional needs of the horse.

Long-stemmed forages such as hays or pasture are necessary in the diet to ensure normal digestive function. Hays that are excessively dusty, moldy, weedy or have blister beetles present should not be fed. When concentrates are fed, consideration should be given to grain processing, frequency of feeding, the amount of concentrate necessary and dental soundness. Ration changes should always be made gradually. As with any species of livestock, access to good quality water is also important to ensure optimum health and performance.

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PREVALENCE OF BLOOD PARASITES IN HYPERTHERMIC DOGS OF KATHMANDU VALLEY

S. Subedi[1] and M. N. Shrestha[2]

ABSTRACT

Investigation on the prevalence of blood-parasites in the hyperthermic pet dogs of Kathmandu Valley was carried out from September 2009 to December 2009. A total of 50 blood samples were collected from different clinics and hospitals of Kathmandu Valley and tested for blood parasites. The RBC, WBC, PCV, Hb, DLC of each sample were also assessed and analyzed. Data was analyzed to determine prevalence of various species of blood parasites to establish the correlation of these infections with age, sex and breed. An overall prevalence of blood-parasites was recorded as 14 %; Babesia canis, B. gibsoni and Ehrlichia spp being 8.00%, 2.00% and 4.00 % respectively. The percentage of infection was greater in males (12%) than females (2%). High occurrence was encountered in German shepherd breed of dog (85.71%) and rest (14.29%) was found in Japanese Spitz. The prevalence of blood parasites was found to be greater in the higher age group of canine population.

INTRODUCTION

The dog (Canis lupus familiaris) is a domestic subspecies of the wolf, a mammal of the Canidae family of the order Carnivore. Dogs are not only "man's best friend," but they can also be trained to be man's best helpers in many ways. Guide dogs are trained to guide blind people through their daily activities. Dogs can also be taught to act as "ears" for the deaf or perform specialized police work. Of course they are best at being family companions. Dogs are susceptible to various diseases, ailments, and poisons, some of which affect humans in the same way, others of which are unique to dogs. Dogs, like all mammals, are also susceptible to heat exhaustion when dealing with high levels of humidity and/or extreme temperatures. There is an estimated dog population of 1,849,106 in Nepal. Presently total dogs have been categorized in three groups in both urban and rural area in Nepal viz. pet dogs, community dogs and street dogs (Joshi *et al.* 2002). The dog population in Central Development Region is around 0.6 million and around 90 thousands in Kathmandu valley. The dog population within 35 wards of Kathmandu Metropolitan City is around 61 thousands. Out of which 52% are pet dogs, 20% are community dogs and 28% are stray dogs (Joshi *et al.* 2002). Stray dog population is 25,000 in Kathmandu valley (KAT Centre 2007).

Endoparasitic infestations have always been a major health problem in animal species. There may be different types of endoparasites parasitizing in different parts and systems of an animal body. Among these, blood parasites are also the major ones causing severe disease conditions. These blood parasites affect the blood vascular system, which may be the intra-erythrocytic parasite, the intraleukocytic parasite or those living freely.

The common blood parasites include protozoas such as Babesia spp., Theileria spp. and Trypanosoma spp. and rickettsial such as Anaplasma spp. and Ehrlichia spp.

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Babesia, Theileria and Anaplasma are intra-erythrocytic parasites whereas Trypanosoma are extracellular parasites and Ehrlichia is mainly found in leukocyte. The common diseases caused by blood parasite in dogs are Babesiosis, Trypanosomasis and Ehrlichosis.

MATERIALS AND METHODS

This study was carried out from September 2009 to December 2009 during the internship period at various veterinary clinics of Kathmandu.

Selection of dogs

Blood samples were purposively taken from 50 dogs with hyperthermia (temp.> 103°F) and history of tick infestation. These samples were randomly selected (irrespective of age, sex and breed) from different veterinary clinics of Kathmandu. All other information like: owner's name, dog's name, age, sex, body weight, temperature, clinical signs and all other important information were taken.Collection of blood, preparation of slide and staining, RBC and WBC count, DLC, PCV and Hemoglobin determination was performed as per Benjamin (2001).

Data processing

The collected data and information were subjected to appropriate statistical tool, SPSS 11.5 and was interpreted accordingly. The analysis was done using Students T test as per Pravakaran (Pravakaran, 2008).

RESULTS AND DISCUSSIONS

Study population

This research work was performed in total of 50 dogs selected purposively. The number of different breeds of dog was German shepherd (GS)- 18, Japanese spitz (JS)-9, Mongrel-12, Laborador (Lb)-5, Tibetan mastiff ™-2, Lasa apso-1, Dalmation-1, Great Dane-1 and Pomerian-1. There were 33 male and 17 female.

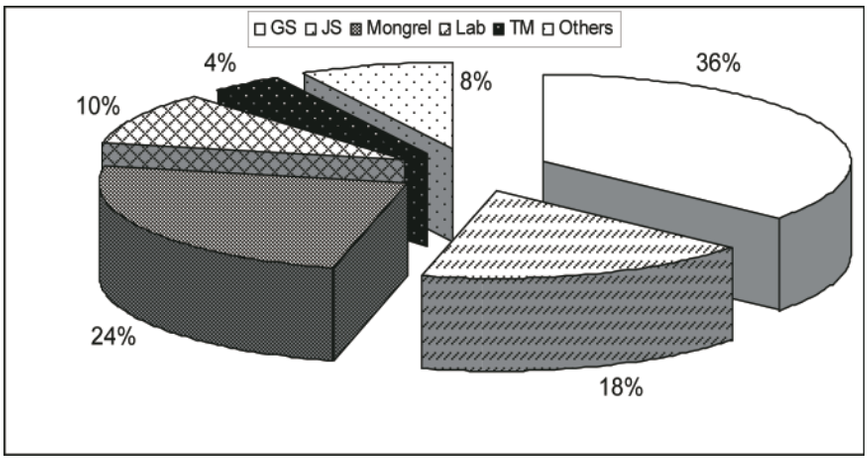

Fig. 1: Breed of dogs under study

This shows the preferences of German shepherd breed and male dogs in people of Kathmandu valley. Since sample size was very small and also unequal, so this finding cannot be said to be totally correct which is a limitation of this study.

Prevalence of blood parasites

In the study done in dogs with hyperthermia and history of tick infestation, 14% of the total cases were found to be caused by blood parasites as presented in Fig 2.This result is nearly similar to that reported by Manandhar and Rajawar (2006) (17.14%). Whereas, Bashir *et al.* (2009) had reported the prevalence to be 2.62% in Lahore, which is markedly below than our findings. This may be due to the methodology adopted for our study, selecting the dogs only with hyperthermia and history of tick infestation.

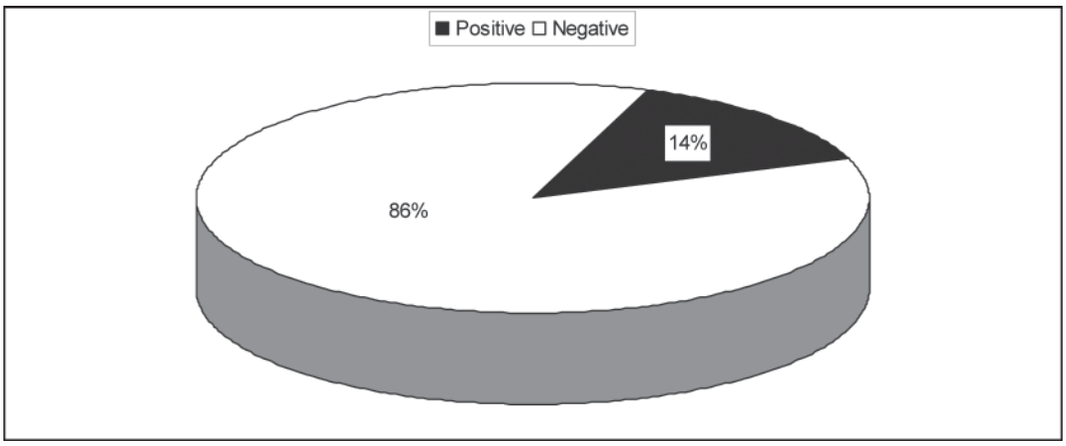

Fig. 2: Prevalence of blood parasites

Among the 50 samples tested, 4 samples (8%) were found to be positive for Babesia canis, 1 sample (2%) was found to be positive for B gibsoni and 2 samples (4%) were suspected to be positive for Ehrlichia spp. Gadahi *et al.* (2008) has reported prevalence of B. canis to be 5.00%, which is slightly less than our findings. This again may be due to the methodology of sample selection.

We didn't find the morulae of E. canis in the peripheral blood smear though the dogs were showing the characteristic clinical signs and symptoms as well as hematological reports were indicating E. canis infection. This holds true to the fact that in Ehrlichia infection very small to negligible number of organisms are found in blood or other tissues. For confirmatory diagnosis serological tests could have been applied, but the time and funds were the major limitations. Also, out of the total 5 positive cases of Babesiosis 4 (80%) were caused by the species Babesia canis and 1(20%) were by Babesia gibsoni.

Clinical signs

The clinical signs of Babesiosis observed during the present studies were; high temperature (104°F - 107°F), anemia, icteric mucus membrane, anorexia, weight loss, dehydration, labored breathing.

The major clinical observation in Ehrlichiosis* in our study was epistaxis, weight loss fever, anorexia, anemia etc.

Table 1: Result of blood examination

			Frequency	Incidence	Male	Female	Mean body temp.
Positive	Babesiosis	B. canis	4	8%			
		B. gibsoni	1	2%	6 (12%)	1(2%)	105.42 0F
	Ehrlichiosis		2	4%			
Negative			43	86%	27 (54%)	16 (32%)	103.78 0F
Total			50				

* suspected cases

Table 2: Details of positive cases

Breed	Sex	Age (yrs)	Temp (0F)	Result	RBC (×106/µl)	TLC (×103 /µl)	DLC				Hb (gm%)	PCV (%)
							N	L	M	E		
JS	F	1	106	B. gibsoni	5.4	8.500	77	20	3	0	9	28
GS	M	3	104	B. canis	4.5	1.200	26	64	10	0	9.8	27
GS	M	4	107	B. canis	1.5	0.200	20	80	0	0	3.4	10
GS	M	5	105	E. canis *	2.4	6.200	61	33	5	1	5.2	19
GS	M	3	106	B. canis	3.5	19.500	59	33	7	1	7	19
GS	M	0.83	104	B. canis	10.2	10.275	75	15	3	7	15	45
GS	M	5	106	E. canis *	2.5	6.100	60	35	4	1	5	21
Average		3.12	105.42		4.28	7.42	54	40	4.57	1.43	7.77	24.14

*suspected cases

Sex wise prevalence of blood parasites

High prevalence (12%) of blood parasites was found in male dog compared to the females (2%) as presented in figure 3. This holds true with the findings of Bashir *et al.* (2009) that the male dogs were more prone to disease than female dogs (3.39 vs. 1.32%).

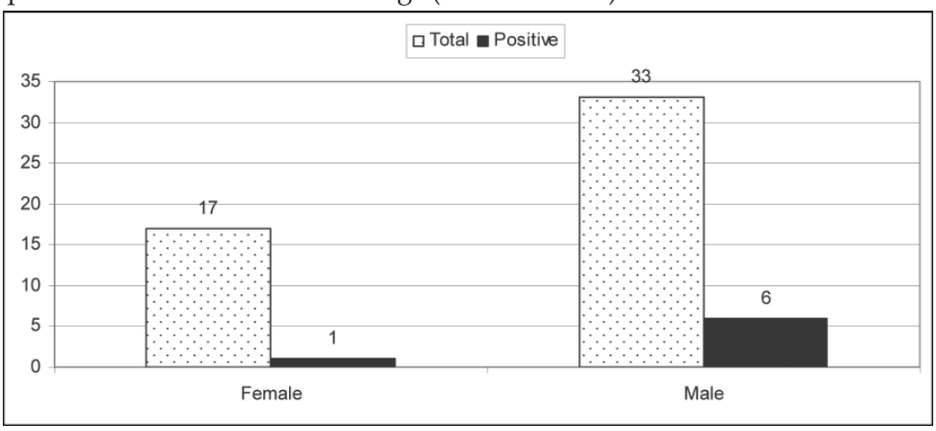

Fig. 3: Sex wise prevalence.

Breed wise prevalence of blood parasites

Out of the 7 total positive cases including 2 Ehrlichiosis suspected cases, majority (6 cases) i.e. (85.71%) were encountered in German Shepherd breed of dog as stated in figure 4. Rest 1 case i.e., 14.29% was found in Japanese spitz. This holds true for the fact presented by Chakrabarti (2007) that due to inadequate immunogenic response dogs like German Shepherd use to suffer more than other variety of dogs.

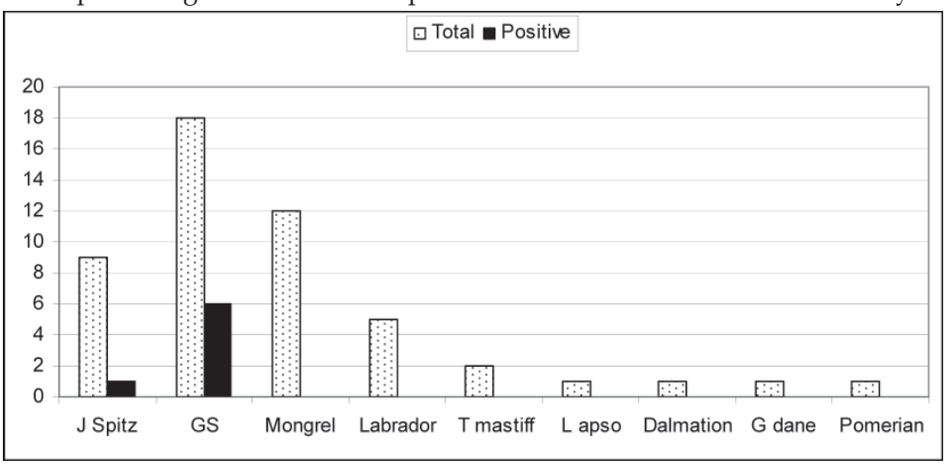

Fig. 4: Breed wise prevalence.

Temperature

The mean temperature of the positive cases caused by blood parasites infection was found to be 105.42 0F, whereas for the negative cases it was 103.78 0F as shown in figure 5. Our finding closely resembles with the findings postulated by Manandhar and Rajawar (2006).

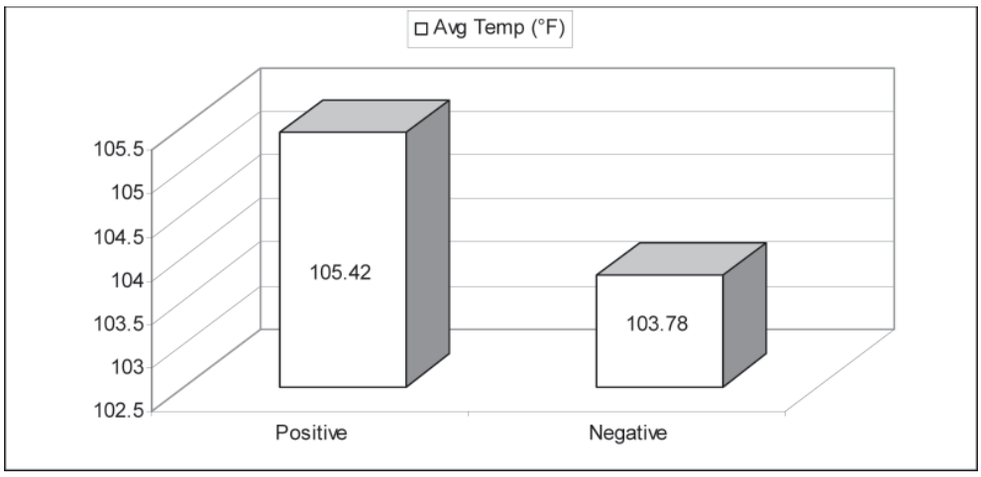

Fig. 5: Average temperature of positive Vs negative cases

Mean RBC, PCV and Hb count

Table 3: Comparison of hematological values of positive cases with the normal.

Cases		RBC (× 106 / µl)	Hb (g %)	PCV (%)	TLC
Positive	Mean	4.286	7.771	24.143	7.424
	± S.E.	± 1.106	± 1.479	± 4.143	± 2.436
	S.D.	2.927	3.91	10.961	6.445
Normal mean		6.25	14.5	45.5	13

Significant at P value: 0.05 & 0.01

Statistically significant decrease in value of Hb, PCV and TLC was found in this study.

Anemia was the most consistent finding in positive cases during hematology in our study with the mean RBC count being 4.28 ×106 / µl, mean PCV and Hb being 24.14 % and 7.77 g % respectively as shown in figure 6. This data justifies the presence of marked anemia in blood parasite infections as stated by Irwin and Hutchinson (1991), Freeman *et al.* (1994), and many others.

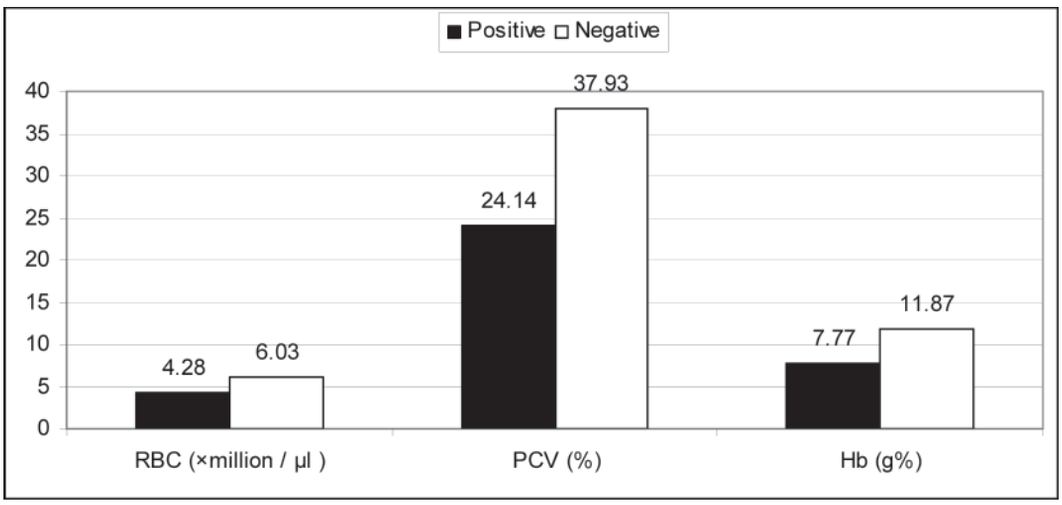

Fig. 6: Mean RBC, PCV and Hb count

Total leukocyte count

The mean total leukocyte count was found to be 7.42 $\times 10^3/\mu l$ for the positive cases. Whereas, it was $9.32 \times 10^3/\mu l$ for the negative cases. The data is presented in figure 7. The normal range is 8.35 $\times 10^3/\mu l$. Considering this as the normal reference, we can say that there was a slight leucopenia in positive cases and leukocytosis in negative cases. This data slightly agrees with that of Irwin and Hutchinson (1991) suggesting marked leucopenia. Whereas, Manandhar and Rajwar (2008) has reported the mean TLC of $8.48 \times 10^3/\mu l$ in positive cases and $8.15 \times 10^3/\mu l$ in negative cases.

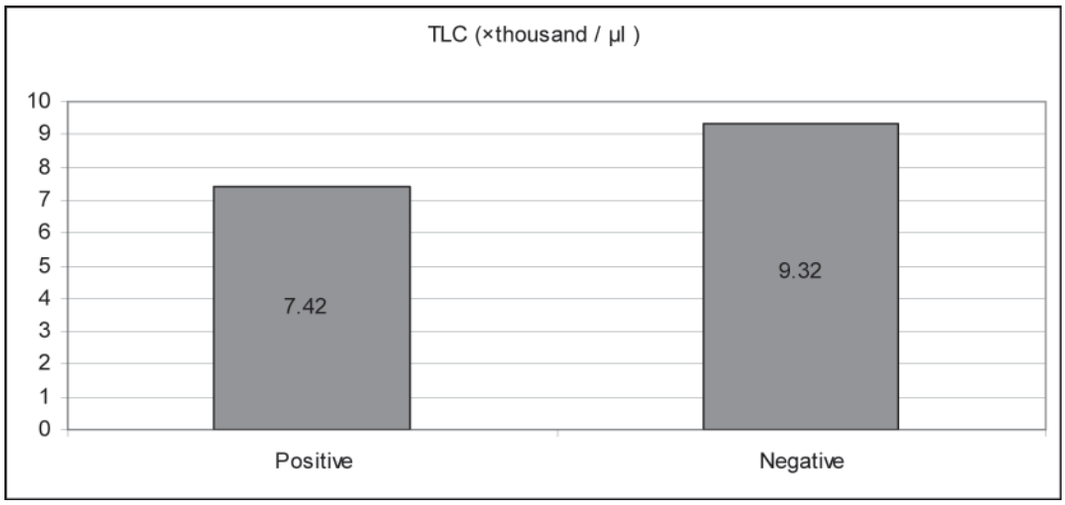

Fig. 7: Mean TLC count

Average DLC

The mean neutrophil count was found to be 54% in positive cases and 67.3% in negative cases. Similarly lymphocyte was found to be 40% and 29%, monocyte 4.57% and 2.93% and eosinophil 1.43% and 0.58% for positive and negative cases respectively.

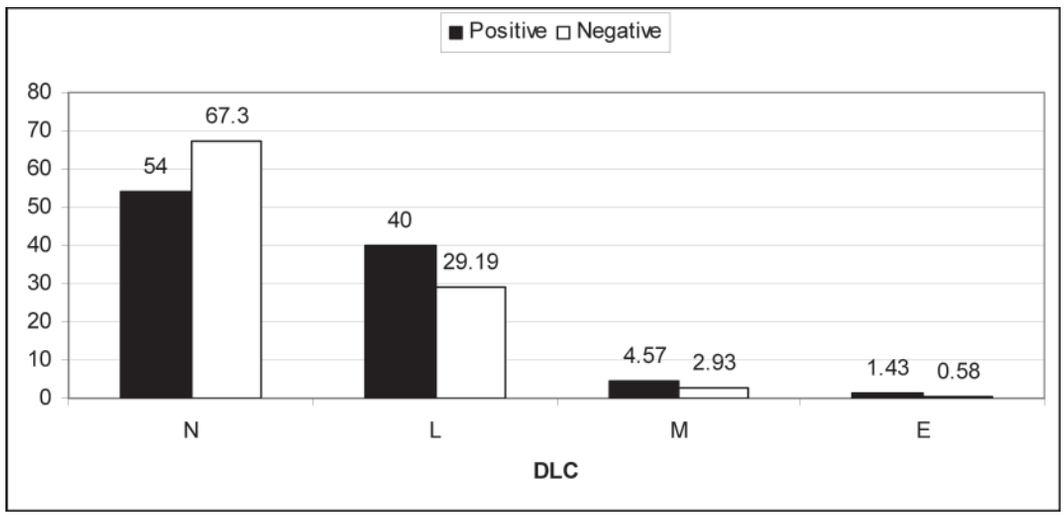

Fig. 8: Average DLC

Age wise prevalence of blood parasites

The prevalence of blood parasites was found to be greater in the higher age group as shown in figure 9 and 10. The same fact of higher prevalence in higher age group has been presented by Manandhar and Rajawar (2006).

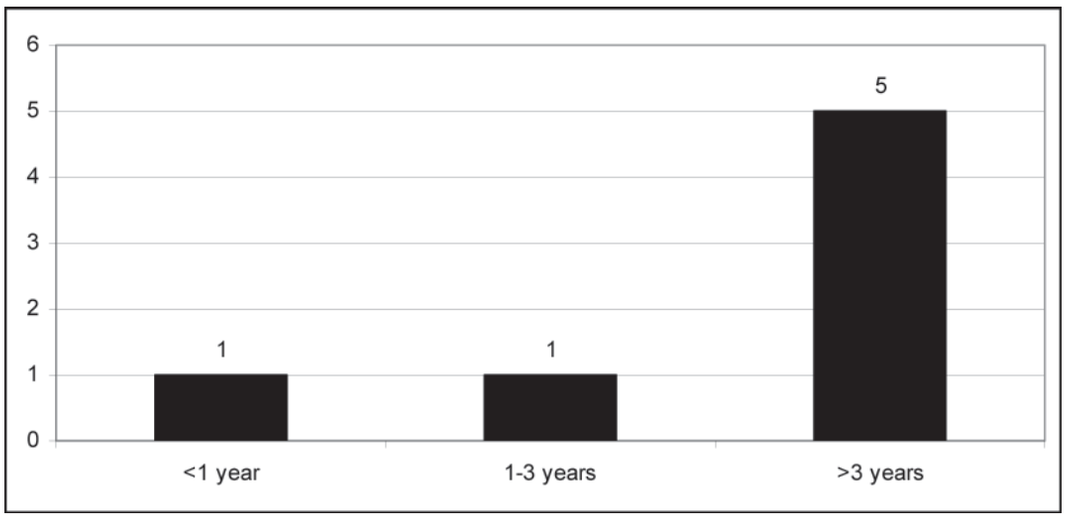

Fig. 9: Prevalence of blood parasites age-wise

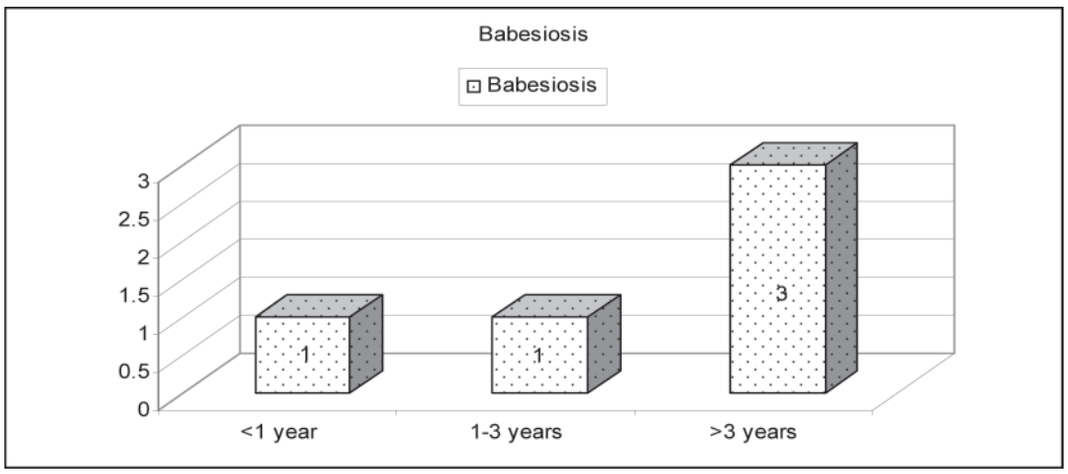

Fig. 10: Prevalence of Babesiosis age-wise

CONCLUSION

The study focused towards the investigation on the prevalence of blood-parasites in the hyperthermic pet dogs of Kathmandu Valley from September 2009 to December 2009. Fifty blood samples were collected and tested for blood parasites. Different hematological parameters like RBC, WBC, PCV, Hb and DLC of each sample were also assessed and analyzed. Data was analyzed to determine prevalence of various species of blood parasites to establish the correlation of these infections with age, sex and breed.

An overall prevalence of blood-parasites was recorded as 14 %; Babesia canis, B. gibsoni and Ehrlichia spp* being 8.00%, 2.00% and 4.00 % respectively. The percentage of infection was greater in males (12%) than females (2%). High occurrence was encountered in German Shepherd breed of dog (85.71%) and rest (14.29%) was found in Japanese Spitz. The prevalence of blood parasites was found to be greater in the higher age group of canine population.

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PREVALENCE OF GASTRO-INTESTINAL HELMINTH PARASITES IN DOGS OF KATHMANDU VALLEY

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ABSTRACT

A coprological study was conducted to determine the prevalence of gastro-intestinal helminths parasites in dogs of Kathmandu valley during September 2010 to Februrary 2011. A total of 207 samples were taken for the study, of which 105 samples were taken from domesticated dogs brought at Mobile Veterinary Consultancy Service (MVCS), Jawalakhel and 102 samples were taken from stray dogs brought at Animal Nepal, Chovar (48 samples) and Kathmandu Treatment Centre (KAT) Centre, Chapaligaun (54 samples), Kathmandu. The samples were examined microscopically by direct, sedimentation and flotation method. The laboratory work was performed at MVCS, Jawalakhel. The study showed the overall prevalence of gastro-intestinal helminths parasites to be 45.41% with high prevalence in stray dogs (68.63%) than in domesticated dogs (22.86%). In the study, eight different parasitic species were observed, of which Toxocaracanis (14.49%) showed the highest prevalence followed by, Ancylostomacaninum (11.11%), Trichurisvulpis (7.73%), Taenia/Echinococcus spp. (7.25%), Spirometra spp. (3.86%), Dipylidiumcaninum (3.38%), Diphyllobothriumlatum (1.93%) and Toxascarisleonina (0.97%). Out of positive samples, 81.91% showed single parasite infection, 18.09% showed mixed (more than two) infections. The sex-wise prevalence showed higher prevalence in females (55.06%) than males (34.69%). In domesticated dogs, the breed-wise prevalence of helminth parasites showed 23.94% in pure breed, 18.51% in mongrels, and 28.57% in cross breeds. Similarly, the age-wise prevalence showed 33.33% in puppies, 18.18% in young and 18.0% in adults.

INTRODUCTION

Gastrointestinal helminths of dogs pose serious impact both on the host and human beings. They impede the successful rearing of dogs and result in loss¬es that are manifested by lowered resistance to infectious diseases, retarded growth, reduced work and feed efficiency and general ill health (Soulsby, 1982). Parasitized animals show a variety of symptoms, depending on the parasite species and density. These signs are attributed to intestinal obstruction, ir¬ritation, maldigestion, malabsorbtion, and protein losing gastroentropathy in¬duced by the parasites (Dunn, 1978). Severe cases could be fatal (Barutzki&Schaper, 2003)

Animals may swallow certain objects that resemble parasite forms. These are known as pseudoparasites. They include such things as pollen grains, plant hairs, grain mites, mold spores and a variety of harmless plant and animal debris. Spurious parasites are encountered in feces. For example, parasite eggs or cysts from one species of host may be found in few feces of a scavenger or predator host as the result of coprophagy (Sloss, 1970).

The common gastro-intestinal parasites of dogs are Ascarid worm (Toxocaracanis, T. leonina), Hookworm (Ancyclostomacaninum, Uncinariastenocephala), Whipworm (Trichurisvulpis), Tapeworm (Diphyllobothriumlatum. Dipylidiumcaninum, Echinococcusgranulosus.

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Taeniahydatigena, T. psisiformis, T. taeniaeformis, Spirometra spp. etc.), and protozoal worms are Coccidia (Isoporabigemina, I. canis, I. revolta, Eimeriacanis), Giardia (Giardia canis), Amoeba (Entamoebahistolytica), etc. (Chakraborty, 2006).

MATERIALS AND METHODS

Sample collection site

Samples were collected from three different places of Kathmandu valley. Fecal samples from domesticated dogs were collected from Mobile Veterinary Consultancy Service, Jawalakhel. Similarly, fecal samples from stray dogs were collected from Animal Nepal, Chovar and Kathmandu Animal Treatment (KAT) Centre, Chapaligaun, Kathmandu.

Sample size

A total of 207 samples were collected. 105 samples were collected from the dogs brought at Mobile Veterinary Consultancy Service and 102 samples were taken from the stray dogs brought at Animal Nepal and KAT centre. Laboratory work was performed at Mobile Veterinary Consultancy Service, Jawalakhel, Lalitpur.

Sample collection technique

The dogs were restrained with the help of muzzle. The samples were collected per-rectum using the plastic gloves and turned inside out. All samples were clearly labeled with the help of permanent marker indicating the breed, age and sex of the animal. .

Transportation of sample

The shipment of the samples to the laboratory was done by keeping them in the cool box. Immediately after the arrival in laboratory, the samples were examined.

Direct smear technique

This technique was used when small quantities of material were available or when the fecal examination was to be completed in a short period of time. This procedure involved mixing a small amount of fecal material in a drop or two of saline placed on a slide and examined under microscope (Kirk &Bistner, 1969).

Qualitative concentration methods

Sedimentation method

About 2-3 gm of fecal sample was grinded and placed in 100 ml in beaker and water was added. The mixture was poured through a tea sieve and collected in a beaker. The material left in the sieve was discarded. After 10 minutes approximately 70% of supernatant was discarded and that was refilled with fresh water until the supernatant was cleared. Then sediment left in bottom was examined under microscope (Soulsby, 1978).

Floatation method

The basis of any floatation method is that when worm eggs are suspended in a liquid with a specific gravity higher than that of eggs, the latter will float up to the surface. Nematode and cestode eggs floats in a liquid with a specific gravity of between 1.10 and 1.20; trematode eggs which are much heavier, require a specific gravity of 1.30 – 1.35. The floatation solutions used for nematode and cestode ova are sodium chloride (sp.gr. 1.20) and magnesium sulphate (sp.gr. 1.28), for trematode eggs, saturated solution of zinc chloride or zinc sulphate (sp.gr. 1.18) are used (Bhatia *et al.*, 2006).

1-5 gm of strained faecal sample was mixed with ordinary water in 15 ml centrifuged tube and centrifuged for 1-2 min at 1500- 2000 rpm. The supernatant solution was discarded and centrifugation was repeated. The faecal sediment at the bottom of the tube was mixed with saturated sodium chloride solution and filled the tube to its brim, mixed properly and covered with a clean round cover slip. After centrifugation the tube was kept in vertical position in the tube stand for 2 minutes. The cover slip was picked up gently and put over a slide for examination (Bhatia *et al.*, 2006).

Identification of parasitic eggs

Identification of parasitic eggs found in the fecal samples were done on the basis of their shape, shell contents, colors and characteristic external features with the help of microscope under 10X and 40X objective lens. The identification of eggs was carried out according to Foreyt, 2001; Bhatia *et al.*, 2006 &Thienpont*et al.*, 1986.

Data analysis

The data was collected from both primary and secondary sources. The data analysis was done by using Microsoft Excel program.

RESULTS

Overall representation of sample

Out of 207 fecal samples examined, 105 samples were collected from domesticated dogs and 102 samples were collected from stray dogs. Out of 102 samples from stray dogs, 48 samples were taken from Animal Nepal, Chovar and 54 samples were taken from KAT centre, Chapaligaun, Kathmandu.

Overall prevalence of sample

Prevalence of positive and negative samples

On laboratory examination of 207 fecal samples, 94 samples were found to be positive for at least one parasite, representing the prevalence of 45.41%.

Parasitic species isolated in positive samples

In a total of 94 positive samples, eight different parasitic species were observed, of which 28.57% were T. canis, 21.90% were A. caninum, 15.24% were T. vulpis, 14.29% were Taenia/Echinococcus spp. 7.62% were Spirometra spp., 6.67% were D. caninum, 3.81% were D. latum and 1.90% was T. leonina. Paragonimus spp., a lung fluke was also observed in one sample.

Pattern of parasitic infection

Out of 94 positive samples, 77 samples (81.91%) showed single parasite infections, 17 samples (18.09%) showed mixed (more than two) infections.

Sex-wise prevalence of parasites

Out of 207 samples, 98 were males and 109 were females. Among 98 samples from males, 34 (34.69%) were found positive and out of 109 samples from females, 60 (55.06%) were found positive.

Prevalence of parasites in domesticated dogs

Prevalence of positive and negative samples

Out of 105 samples examined, 24 (22.86 %) samples were found positive. Among 24 samples, all samples had single infection.

Parasitic species identified in positive samples

In a total of 24 positive samples, five different parasitic species were observed, namely T. canis(37.50%), D. caninum(29.17%),A. caninum(16.76%),Taenia/Echinococcus spp. (12.50%), and T. vulpis(4.17%).

Breed-wise prevalence of parasites

Breeds were divided into three groups; pure breed, mongrel and cross breed. In a total of 105 samples, 71 samples were from pure breed, 27 samples were from mongrel and 7 samples were from cross breed. Out of 71 samples from pure breed, 17 (23.94%) were found positive. Similarly, out of 27 samples from mongrel, 5 (18.51%) samples were positive and out of 7 samples from cross breed, 2 (28.57%) were found to be positive.

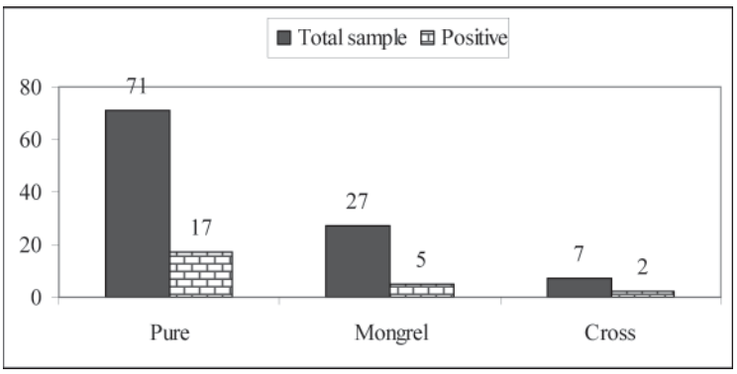

Fig. 1: Breed-wise prevalence of parasites in domesticated dogs.

Age-wise prevalence of parasites

Dogs up to 6 months of age were classified as puppies, from 6 months through one year of age were referred to as young dogs while adults were dogs above one year of age. Out of 33 samples from puppies and 22 samples from young dogs, 11 samples (33.33%) and 4 samples (18.18%) were found positive respectively. Similarly, out of 50 samples from adults, 9 samples (18.0%) were found to be positive.

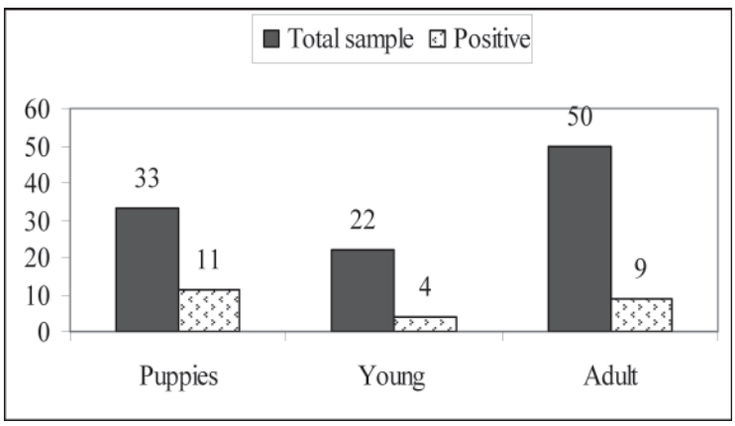

Fig. 2: Age-wise prevalence of parasites in domesticated dogs.

Age-wise prevalence of parasitic species

The following parasitic species were observed according to age-wise.

Table.1: Age-wise prevalence of parasitic species.

S.N.	Parasites	Age group		
		Puppy (n=33)	Young (n=22)	Adult (n=50)
1.	Toxocaracanis	7	-	2
2.	Dipylidiumcaninum	2	2	3
3.	Ancylostomacaninum	-	2	2
4.	Taenia/Echinococcus spp.	2	-	1
5	Trichurisvulpis	-	-	1

Note: n = total number of examined samples

Sex-wise prevalence of parasites

Out of 105 samples, 66 were from male and 39 were from females. Out of 66 male samples, 14 (22.73%) were found to be positive and out of 39 female samples, 10 (25.64%) were found to be positive.

Prevalence of parasites in stray dogs

Prevalence of positive and negative sample

Out of 102 samples examined, 70 samples (68.63%) were found to be positive.

Parasitic species isolated in positive samples

In a total of 58 positive samples, seven different parasitic species were observed, namely T. canis(25.93%),A. caninum(23.46%),T. vulpis(18.52%),Taenia/Echinococcus spp. (14.81%), Spirometra spp. (9.88%), D. latum (4.94%) and T. leonine (2.47%).

Pattern of parasitic infection

Among 70 samples, 53 (75.71%) samples were found to have single infection, 17 samples (24.29%) were found to have mixed infection.

Sex-wise prevalence of parasites

Out of 102 samples, 32 were from male and 70 were from females. Out of 32 male samples, 20 (62.50%) were found to be positive and out of 70 female samples, 50 (71.42%) were found to be positive.

DISCUSSIONS

A total of 207 fecal samples were examined to determine the prevalence of gastro-intestinal helminth parasites in dogs (both domesticated and stray) of Kathmandu valley. The overall prevalence was found to be 45.41%. The prevalence of intestinal parasites in dogs brought at Central Veterinary hospital, Tripureswor was reported 41.33% by (Giri, 2009) and 41.55% by (Ghimire, 2002) which is quite similar. This showed parasitic problem persists in canines of Kathmandu valley.

The prevalence obtained through this study is lower than that reported by (Karki, 2003) in Kathmandu valley who encountered canine parasitic general prevalence of 64.90%. It might be due to sampling variation, seasonal variation, diagnostic protocols etc.

A lower prevalence of parasitic infections in dogs comparative to my study was reported as 20.5% in Dutch (Nobel *et al.*, 2004), 35.5%, in Venezuela (Ramirez-Barrios *et al.*, 2004), 34.8% in USA (Kirkpatrick, 1988), 36.1% in Belgium (Vanparijs*et al.*, 1991), 35.9% in USA (Blagburn*et al.*, 1996) and 24% in Santiago, Chile (Lopez *et al.*, 2006). The prevalence of parasites in Enugu State, Nigeria, was 68.5% (Anene*et al.*, 1996), Southern Spain 74.33% (Martı´nez-Moreno *et al.*, 2007), Ambo, Ethiopia 52.86% (Zewdu*et al.*, 2008), and in Botucatu, Sa˜o Paulo State, Brazil 54.3% (Katagiri& Oliveira-Sequeira, 2007) which are higher than my study. These variations might be the result of

the difference in geographical location and climatic conditions of country, demographic factors, poor management practices, sampling protocols, controlling strategy followed by government and frequent mixing of pets with stray dogs which might have the infections etc.

In this study the prevalence of parasites in domesticated dogs were observed 22.86% which is in accordance to the findings in pet dogs of Australia, which was 23.9% (Palmer *et al.*, 2008), 20.4% in household dogs of Northen Belgium (Caerebout*et al.*, 2009), in owned shepherd and hunting dogs in Serres Prefecture, Northern Greece which was 26 % (Papazahariadou*et al.*, 2007), and 22.4% in military personnel and military dogs, Thailand (Leelayoova*et al.*, 2006).

In the study, the prevalence of gastro-intestinal helminths parasites in dogs was higher in stray dogs which support the findings of (Khante*et al.*, 2008; Minnaar*et al.*, 2002), which might be due to various reasons like no de-worming of dogs, scavenging meat and fish offal, frequent contact with contaminated soil, dirt etc.

Concurrent infections with two or more parasites were recorded in 47% of the cases (Anene*et al.*, 1996) and 62.16% (Zewdu*et al.*, 2008) which is higher than my study which is 18.09% only. Dogs harbouring one parasite were more common (31.4%) than thoseharbouring two (18.5%), three (3.2%) or four (1.2%) (Katagiri& Oliveira-Sequeira, 2007) which quite supports this study. Single parasite infections (85.7%) were more common than mixed infections (Sowemimo&Asaolu, 2008) which support my findings 81.91% single parasitic infection. Similarly, dogs harbouring one-parasite eggs were more common (73.8%) than those harbouring two or three (Swai*et al.*, 2010) which also agreed my findings.

The predominant parasites encountered in this investigation were T.canis and A.caninum which supports the findings of (Giri, 2009; Khante*et al.*, 2008; Swai*et al.*, 2010; Gingrich *et al.*, 2009; Sowemimo, 2009; Anene*et al.*, 1996). The common gastro-intestinal helminth parasites observed were T. canis, A. caninum, T. vulpis, D. caninum, Taenia/Echinococcus spp., which supports the findings of many researches in different region of the world (Giri, 2009; Anene*et al.*, 1996;Oliveira-Sequeira*et al.*, 2002; Katagiri& Oliveira-Sequeira, 2007; Dubna*et al.*,2007; Khante*et al.*, 2008; Zewdu*et al.*, 2008; Umar, 2009&Sowemimo, 2009).

Dyphyllolobothriumlatum in this investigation was found to be 3.92% in stray dogs. It is the common parasite of fish and occasionally seen in the dogs. In this investigation this much incidence may be due to the stray dogs which feed near fish market and these results are in partial accordance with (Giri, 2009; Khante*et al.*, 2008; &Pulola*et al.*, 2006). The prevalence of Spirometra spp. was found to be 3.86% which merely supports the findings of (Yamamoto *et al.*, 2009).

In domesticated dogs, the study shows high prevalence of parasites in dogs below one year of age which showed similar finding of (Giri, 2009; Swai*et al.*, 2010) which might be due to underdeveloped immune system of young pups and also because of maternal milk, the major route of infection.

The investigation showed that pups were more susceptible to T. canis, infections than adult dogs, which was also shown in other studies (Nobel *et al.*, 2004; Pullola*et al.*, 2006; Martı´nez-Carrasco *et al.*, 2007; Palmer *et al.*, 2008; Swai*et al.*, 2010).Regarding sex-wise prevalence, female dogs encountered more parasitic infections than males which support the findings of (Umar, 2009; Swai*et al.*, 2010).

CONCLUSION

A coprological study was conducted to determine the prevalence of gastro-intestinal helminths parasite in dogs of Kathmandu valley. The study showed the overall prevalence of gastrointestinal helminths parasites to be 45.41% with high prevalence in stray dogs (68.63%) than in domesticated dogs (22.86%). In the study, eight different parasitic species were observed, of which Toxocaracanis (14.49%) showed the highest prevalence followed by, Ancylostomacaninum (11.11%), Trichurisvulpis (7.73%), Taenia/Echinococcus spp. (7.25%), Spirometra spp. (3.86%), Dipylidiumcaninum (3.38%), Diphyllobothriumlatum (1.93%) and Toxascarisleonina (0.97%). Paragonimus spp., a lung fluke

was also observed in one sample.

Results obtained in this investigation and previous ones (Giri, 2009; Karki, 2003; Ghimire, 2002) suggest gastrointestinal helminths infections are prevalent in Kathmandu valley and is still a significant problem. Base-line information of parasitic infection in dogs of other regions of Nepal needs further studies. Additionally, the present study has provided further evidence that both domesticated and stray dogs are reservoirs for zoonotic intestinal helminth parasites and should be considered important to public health. It is imperative for human to avoid faecal contamination at home and its premises, in streets, open place, public gardens and parks. Thus, concerted efforts should therefore be made to educate dog keepers/owners and a public person to embrace dog's parasitic disease control programs, specifically the need for routine de-worming of dogs. Moreover, health education provided to the affected population and affected area could enable to protect both humans and dogs against infection. So, we can conclude that there is high prevalence of parasites in stray dogs which are harmful to human as well as dogs themselves which ought to be control strategically.

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STUDY OF HAEMATO-BIOCHEMICAL CHANGES ON EHRLICHIA CANIS POSITIVE DOGS OF KATHMANDU VALLEY

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ABSTRACT

Improved breeds of dogs were getting popular in the Kathmandu Valley. In the mean time they are also dying with anemia. It was suspected that canine ehrlichiosis is the primary cause of the death. Thus, the study was carried out from September 2010 to February 2011 at Mobile Veterinary Consultancy Services, Jawalakhel, Lalitpur to determine the haemato-biochemical changes on Ehrlichia canis positive dogs of Kathmandu valley. The hematological study revealed statistically significant (at both 0.05% and 0.01%) decrease in the value of PCV, RBC and Hb level.Statistically significant increase in neutrophil (at both 0.05% and 0.01%) count was observed, whereas the lymphocyte count was found to be within the normal range.The biochemical study for the liver function tests revealed statistically significant (at both 0.05% and 0.01%) increase in the value of total bilirubin and direct bilirubin was found in the study. The biochemical study for the kidney function tests revealed statistically non-significant (at 0.01%) increase in the value of BUN. Whereas, the test was significant at 0.05%. Similarly, statistically significant (at both 0.05% and 0.01%) increase in the creatinine value was observed in the study. Local bred dogswere found to be less critical than the exotic breed dogs. Thus early monitoring of hemato-biochemical changes in the dogs will help to help to diagnose and treat canine ehrlichiosis, which will help to prevent this silent killer from dogs of Kathmandu Valley.

INTRODUCTION

There is an estimated dog population of 1,849,106 in Nepal. Presently total dogs have been categorized in three groups in both urban and rural area in Nepal viz. pet dogs, community dogs and street dogs (Joshi *et al.* 2002). The dog population in Central Development Region is around 0.6 million and around 90 thousands in Kathmandu Valley. The dog population within 35 wards of Kathmandu Metropolitan City is around 61 thousands. Out of which 52% are pet dogs, 20% are community dogs and 28% are stray dogs (Joshi *et al.*, 2002). Stray dog population is 25,000 in Kathmandu valley (KAT Centre 2007).

Endoparasitic infestations have always been a major health problem in animal species. There may be different types of endoparasites parasitizing in different parts and systems of an animal body. Among those, blood parasites are also the major ones causing severe disease conditions. These blood parasites affect the blood vascular system, which may be the intra-erythrocytic parasite, the intraleukocytic parasite or those living freely (Ettinger 1983).

The common blood parasites include protozoas such as Babesia spp., Theileria spp. and Trypanosoma spp. and rickettsial such as Anaplasma spp. and Ehrlichia spp. Babesia, Theileria and Anaplasma are intra-erythrocytic parasites whereas Trypanosoma are extracellular parasites and Ehrlichia is mainly found in leukocyte. The common diseases caused by blood parasite in dogs are babesiosis, trypanosomasis and ehrlichosis (Ettinger 1983).

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People of Kathmandu are keeping pure breed dogs. Many animals were found to be dying of anemia. Dr.Thapa stated that canine ehrlichiosis could be the primary cause (personal communication with Dr. Thapa). It was suspected that this canine ehrlichiosis would be one of the important reasons for the silent death of the improved breeds of dogs. The haemato-biochemistry of this rickettsial disease in the context of canine population of Kathmandu Valley is still unknown or underknown. Similarly the prevalence of canine ehrlichiosis in the local breeds of the country has not been studied and reported till date.

To prevent death from this silent killer of the improved bred dogs, early diagnosis and treatment is the must important factor. Hence hemato-biochemical change in the E. canis positive dog was studied to monitor the disease early and to prevent the death. This kind of study in the dogs of Kathmandu Valley is itself a new and will help to find out the changes occuring in E. canis infection in our own geographical area. This study will also try to find out the difference between local and improved breeds regarding ehrlichiosis.

MATERIALS AND METHODOLOGY

This study was carried out from September 2010 to February 2011 during the internship period at Mobile Veterinary Consultancy Services, Jawalakhel, Lalitpur. Blood samples were purposively taken from 55 dogs suspected of E. canis and tested positive by the rapid test kit. These samples were randomly selected (irrespective of age, sex and breed) from Mobile Veterinary Consultancy Services, Jawalakhel, Lalitpur. All other information like: owner's name, dog's name, age, sex, body weight, temperature, clinical signs and all other important information were taken.

Collection of blood, preparation of slide and staining, RBC count, DLC, PCV and Hemoglobin determination was performed as per Benjamin (2001). E. canis rapid test was performed as per the instructions of VAL Vetall laboratories (2008). Liver function tests and kidney function tests were performed by using biochemical analyser by using the reagent given by DiaSys and Centronic manual. The observed hematological and biochemical parameters were compared with the normal ranges as given by Willard *et al.* (1994).

The collected data and information were subjected to appropriate statistical tool, SPSS 11.5 and was interpreted accordingly. The analysis was done using Students T test as per Pravakaran (2008).

RESULT AND DISCUSSION

Haematological findings

Table 1: Statistical representation of hematological findings

	Packed cell volume (PCV) (%)	Red blood cell (RBC) (X106/cu.mm.)	Hemoglobin (Hb) (g/dl)
Mean	26.10	4.48	8.79
Median	25	4.4	8.66
Mode	22	5	7.3
SD	9.22	1.42	2.88
±SE	1.24	0.19	0.38

PCV

The mean PCV of the study population was found to be 26.1 %, which is less than the normal range (37 – 55 %).

Breedwise

The mean PCV was found to be 36% for Terrier, followed by 31% Boxer, 31% mongrel, 30% Labarador, 29% Dalmation, 26.34% German shepherd, 24% Dobermann, 23% L. apso, 20.25%

mastiff and 6% J. spitz. The mean PCV of all the breeds is lower than the normal range.

Among exotic and local breeds

The mean PCV for local breeds was found to be 31%, whereas that of exotic breeds was found to be 23.32%. The mean PCV for both the local and exotic breeds is lower than the normal range.

Sexwise

The mean PCV for female dogs was found to be 29.6%, whereas that of male dogs was found to be 24.66%. Both these values are lower than the normal range.

Age-groupwise

The mean PCV was found to be lowest (16%) for the dogs below 1 year of age, which was subsequently followed by 23%, 25.77% and 30.28% in the age group greater than 10 years, 1 – 5 years and 5 – 10 years respectively. All of these findings are lower than the normal range.

RBC count

The mean RBC of the study population was found to be 5.34 X 106, which is lower than the normal range (6.15 – 8.7 X 106).

Breedwise

All the breeds had the mean RBC count lower than that of the normal range. The mean RBC count was found to be lowest (1.2 X 106 / cu. mm.) in Japanese spitz breed, which was subsequently followed by mastiff – 3.475 X 106 / cu. mm., Boxer- 3.5 X 106 / cu. mm., Lhasa apso- 3.8 X 106 / cu. mm., Dobermann – 4 X 106 / cu. mm., German Shepherd – 4.56 X 106 / cu. mm., Dalmation – 5 X 106 / cu. mm., Labarador- 5 X 106 / cu. mm., mongrel – 5.2 X 106 / cu. mm. and Terrier – 6 X 106 / cu. mm.

Among exotic and local breeds

The mean RBC count was found lower than that of normal range for both local and exotic breeds. Among them the mean RBC count was found lower in the exotic breeds (3.7 X 106 / cu. mm.) than that of the local breeds (5.7 X 106 / cu. mm.).

Sexwise

The mean RBC count was found lower than that of the normal range in both males and females. Among them, males had lower RBC count (4.12 X 106 / cu. mm.) than that of the females (5.37 X 106 / cu. mm.).

Agewise

All the age groups of the dog had lower RBC count than the normal range. Among them dogs of <1 years had lowest mean RBC count (3.2 X 106 / cu. mm.) followed by >10 years (3.8 X 106 / cu. mm.), 5 – 10 years (5.01 X 106 / cu. mm.) and 1 – 5 years (5.8 X 106 / cu. mm.) respectively.

Hb level

The mean Hb level of the study population was found to be 8.79 g/dl, which is less than the normal range.

Breedwise

All the breeds had lower mean Hb level than the normal range. Among them Japanese spitz had the lowest mean Hb level (3 g/dl) followed by Mastiff (6.65 g/dl), Lhasa apso (7.6 g/dl), Dobermann (8 g/dl), German shepherd (8.7 g/dl), Dalmation (9 g/dl), Boxer (10 g/dl), Labarador (10 g/dl), Mongrel (11.11 g/dl) and Terrier (12 g/dl) respectively.

Among exotic and local breeds

Both the exotic and local breeds had lower mean Hb level than the normal range. Among them exotic breeds had lower mean Hb level (7.75 g/dl) than that of local breeds (11.11 g/dl).

Sexwise

Both the male and female dogs had lower mean Hb level than the normal range. Among them males had lower mean Hb level (8.15 g/dl) than that of females (10.35 g/dl).

Agewise

All the age groups had lower mean Hb level than the normal range. Among them, dogs below 1 years of age had the lowest mean Hb level (5.35 g/dl) followed by dogs of age group >10 years (7.6 g/dl), 1-5 years (8.74 g/dl) and 5 – 10 years (10.07 g/dl) respectively.

Differential leukocyte count (DLC)

Table 2: Statistical representation of DLC

	Neutrophils (N) (%)	Lymphocytes (L) (%)	Monocytes (M) (%)	Basophils (B) (%)	Eosinophils (E) (%)
Mean	82.03	16.72	0.21	0.07	0.4
Median	82	17.36	0	0	0
Mode	82	18	0	0	0
SD	10.84	8.55	0.61	0.36	1.05
±SE	1.46	1.15	0.08	0.04	0.14

The mean neutrophil count was found to be 82.03% and the mean lymphocyte count was found to be 16.72%. The mean neutrophil count was found to be little bit higher than the normal range.

Biochemical findings

Liver function tests

Table 3: Statistical representation of liver function tests

	TP	Al	G	AST	ALT	TB	DB
Mean	7.29	3.05	4.24	40.41	24.22	1.97	1.54
Median	7.7	2.8	4.4	34	22.25	2	1.6
Mode	8.6	2.2	4.4	1.8	16.4	3.3	0.2
SD	1.81	1.10	1.82	30.80	17.96	1.19	1.17
±SE	0.24	0.14	0.24	4.15	2.42	0.16	0.15

Total protein and albumin

The mean value of total protein level (7.29 g/dl) was found to be within the normal range. Whereas, the mean value of albumin level (3.05 g/dl) was found to be lower than the normal range.

Breedwise

Among the breeds of dogs, boxer (3.9 g/dl) and Dobermann (3.9 g/dl) had the value of mean total protein less than normal range (5.3 – 7.6 g/dl). Similarly, mean total protein level of mongrel (6.46 g/dl) and mastiff (7.075 g/dl) was found to lie within the normal range. The mean total protein level of Dalmation (7.7 g/dl), German Shepherd (7.81 g/dl), Labarador (8 g/dl), Lhasa apso (8.6 g/dl), Japanese spitz (9 g/dl) and Terrier (9 g/dl) were found to greater than the normal range.

The mean value of albumin level was found to be lower than the normal range (3.2 – 4.7 g/dl) in Boxer (2.2 g/dl), Dobermann (2.2 g/dl), Labarador (2.7 g/dl), mastiff (2.75 g/dl), Japanese spitz (2.8 g.dl), Terrier (2.8 g/dl) and German Shepherd (2.9 g/dl). The mean value of albumin level was

found within the normal range for Dalmation (3.3 g/dl), mongrel (3.48 g/dl) and Dobermann (3.9 g/dl). Similarly the mean value of albumin level was found to be more than the normal range in the Lhasa apso (6.2 g/dl).

Among the local and exotic breeds

The exotic breeds were found to have higher total protein value (6.88 g/dl) and lower albumin value (3.16 g/dl) than that of the local breeds (6.46 g/dl and 3.48 g/dl respectively).

Sexwise

Males were found to have higher total protein value (7.57 g/dl) and lower albumin value (2.99 g/dl) than that of females (6.61 g/dl and 3.2 g/dl respectively).

Agewise

The mean total protein level was found to be within the normal range (5.3 – 7.6 g/dl) in the dogs of age group <1 years (7.15 g/dl) and 1 – 5 years (7.1 g/dl). It was found higher than the normal range in the age groups 5 – 10 years (7.61 g/dl) and >10 years (8.6 g/dl).

Similarly the mean albumin level was found to be lower than the normal range (3.2 – 4.7 g/dl) in the dogs of age group 1 – 5 years (2.86 g/dl) and 5 – 10 years (3.05 g/dl). It was found within the normal range in the age group <1 year (3.2 g/dl) and higher than the normal range in the age groups >10 years (6.2 g/dl).

ALT

The mean ALT level (24.22 IU/l) of the infected dogs was found to lie within the normal range (10-34 IU/l).

Breedwise

The mean ALT level of dobermann (0.1 IU/l) and boxer (0.2 IU/l) was found to lie below the normal range (10-34 IU/l). It was found within the normal range for labarador (16.4 IU/l) Lhasa apso (16.4 IU/l), mongrel (19.72 IU/l), mastiff (20.575 IU/l), dalmation (22.9 IU/l), Japanese spitz (25 IU/l), terrier (25 IU/l) and German shepherd (32.97 IU/l).

Among the local and exotic breeds

The mean ALT level of both local (19.72 IU/l) and exotic breeds (14.51 IU/l) was found within the normal range (10 – 34 IU/l). But the local breeds had a little bit higher ALT level than the exotic breeds.

Sexwise

The mean ALT level of both male (26.64 IU/l) and female (18.32 IU/l) was found within the normal range (10 – 34 IU/l). But the male had a little bit higher ALT level than the female breeds.

Agewise

The mean ALT level for all the age groups of dogs was found to be within the normal range (10 – 34 IU/l). Among them, the mean ALT level of dogs of different age groups > 10 years, 1 – 5 years, 5 – 10 years and <1 year is 16.4 IU/l, 21.85 IU/l, 28.55 IU/l and 33.7 IU/l respectively.

AST

The mean AST level (40.4 IU/l) of the infected dogs was found to lie within the normal range (10-62 IU/l).

Breedwise

The mean AST level of boxer (1.8 IU/l) and dobermann (1.8 IU/l) and was found to lie below the normal range (10-62 IU/l). It was found within the normal range for labarador (14.9 IU/l), Lhasa

apso (27.4 IU/l), mongrel (28.44 IU/l), mastiff (30.775 IU/l), Japanese spitz (34 IU/l), terrier (34 IU/l), dalmation (43.7 IU/l) and German shepherd (59.86 IU/l). The data is presented in figure 30.

Among the local and exotic breeds

The mean AST level of both local (28.44 IU/l) and exotic breeds (22.05 IU/l) was found within the normal range (10 – 62 IU/l). But the local breeds had a little bit higher AST level than the exotic breeds.

Sexwise

The mean AST level of both male (45.62 IU/l) and female (27.71 IU/l) was found within the normal range (10 – 62 IU/l). But the male had a little bit higher AST level than the female breeds.

Agewise

The mean AST level for all the age groups of dogs was found to be within the normal range (10 – 62 IU/l). Among them, the mean AST level of dogs of different age groups > 10 years, 1 – 5 years, 5 – 10 years and <1 year is 27.4 IU/l, 37.09 IU/l, 44.82 IU/l and 60.5 IU/l respectively.

Bilirubin

Both the mean total bilirubin (1.97 mg/dl) and direct bilirubin (1.54 mg/dl) were found higher than the normal range (0.1 – 0.6 mg/dl and 0 – 0.1 mg/dl respectively).

Breedwise

The mean total bilirubin was found to be within the normal range (0.1 – 0.6 mg/dl) for labarador (0.2 mg/dl) and Lhasa apso (0.2 mg/dl). It was found higher than the normal range for mastiff (1.525 mg/dl), mongrel (1.8 mg/dl), German Shepherd (2.1 mg/dl), Japanese spitz (2.7 mg/dl), terrier (2.7 mg/dl), boxer (2.9 mg/dl), dobermann (2.9 mg/dl) and dalmation (3.2 mg/dl).

The mean direct bilirubin was found higher than the normal range (0 – 0.1 mg/dl) for all the breeds. It was found to be 0.2 mg/dl for labarador and Lhasa apso, 1.175 mg/dl for mastiff, 1.26 mg/dl for mongrel, 1.76 mg/dl for German shepherd, 1.8 mg/dl for Japanese spitz and terrier, 2 mg/dl for boxer and dobermann.

Among the local and exotic breeds

The mean total bilirubin and direct bilirubin level of both local and exotic breeds were found higher than the normal range (0.1 – 0.6 and 0 – 0.1 respectively). But the exotic breeds were found to have higher total bilirubin (1.99 mg/dl) and direct bilirubin (1.52 mg/dl) level than the local breeds (1.8 mg/dl and 1.26 mg/dl respectively).

Sexwise

The mean total bilirubin and direct bilirubin level of both female (2.22 mg/dl and 1.67 mg/dl respectively) and male (1.87 mg/dl and 1.49 mg/dl respectively) were found higher than the normal range (0.1 – 0.6 mg/dl and 0 – 0.1 mg/dl respectively). But the female were found to have higher total bilirubin and direct bilirubin level than the male breeds.

Agewise

The mean total bilirubin of the age group >10 years (0.5 mg/dl) was found to lie in the normal range (0.1 – 0.6 mg/dl). It was found to be higher in other age groups, viz. 3.25 mg/dl for < 1 year, 2.079 mg/dl for 1 – 5 years and 1.57 mg/dl for 5 – 10 years.

The mean direct bilirubin was found to be greater than the normal range (0 – 0.1 mg/dl) for all the age groups, viz. 3.1 mg/dl for <1 year, 1.57 mg/dl for 1 – 5 years, 1.21 mg/dl for 5 – 10 years and 0.2 mg/dl for > 10 years.

Kidney function test

Table 4: Statistical representation of kidney function tests

	Blood urea nitrogen (BUN)	Creatinine (Cr)
Mean	39.48	7.57
Median	37.5	3.35
Mode	88.2	33
SD	30.10	10.67
±SE	4.05	1.43

Blood urea nitrogen (BUN)

The mean BUN level (39.48 mg/dl) was found to be higher than the normal range (7 – 32 mg/dl) in the infected dogs.

Breedwise

The mean BUN level was found within the normal range (7 – 32 mg/dl) for Japanese spitz (15 mg/dl), terrier (15 mg/dl), mongrel (20.112 mg/dl) and labarador (27.5 mg/dl). It was found higher in mastiff (32.99 mg/dl), boxer (37 mg/dl), dobermann (38.06 mg/dl), Lhasa apso (49.7 mg/dl), German shepherd (50.68 mg/dl) and dalmation (88.2 mg/dl).

Among the local and exotic breeds

The mean BUN level was found within the normal range (7 – 32 mg/dl) for the local breeds (20.112 mg/dl) and was found higher than the normal range in exotic breeds (41.2 mg/dl).

Sexwise

The mean BUN level was found within the normal range (7 – 32 mg/dl) for the females (30.22 mg/dl) and was found higher than the normal range in male breeds (43.28 mg/dl).

Agewise

All the age groups had higher BUN level than the normal range (7 – 32 mg/dl). Among them dogs of age group <1 year had highest BUN level (50.4 mg/dl) followed by >10 years (49.7 mg/dl), 1 – 5 years (38.4 mg/dl) and 5- 10 years (37.62 mg/dl).

Creatinine

The mean creatinine level (7.57 mg/dl) was found to be higher than the normal range (0.5 – 1.4 mg/dl) in the infected dogs.

Breedwise

The mean creatinine level was found within the normal range (0.5 – 1.4 mg/dl) for labarador (0.7 mg/dl) only. It was found higher in mongrel (1.48 mg/dl), Japanese spitz (2 mg/dl), terrier (2.3 mg/dl), boxer (3.3 mg/dl), mastiff (3.325 mg/dl), dobermann (3.4 mg/dl), Lhasa apso (7.5 mg/dl), German shepherd (11.78 mg/dl) and dalmation (33 mg/dl).

Among the local and exotic breeds

Local breeds had slight higher (1.48 mg/dl) mean creatinine level than the normal range (0.5 – 1.4 mg/dl). Whereas, exotic breeds had high (7.6 mg/dl) mean creatinine level.

Sexwise

Both the male (8.32 mg/dl) and female (5.76 mg/dl) breeds had high creatinine level than the normal range (0.5 – 1.4 mg/dl). Comparatively, males had high creatinine level than the females.

Agewise

All the age groups of infected dogs had high creatinine level than the normal range (0.5 – 1.4 mg/dl). Among them <1 years had highest creatinine level (17.15 mg/dl) followed by 1-5 years (7.81 mg/dl), >10 years (7.5 mg/dl) and 5 – 10 years (4.25 mg/dl) respectively.

DISCUSSIONS

The sample taken for the study comprised mainly male German shepherd breed. This shows the preferences for male German shepherd breed by the people of Kathmadu valley. The sample size is unequal, which can be one of the draw backs of the study. Similarly adequate sample of Bhaktapur district couldn't be found as very few cases from Bhaktapur came to the study sites in comparison to Lalitpur and Kathmandu districts.

The hematological study revealed statistically significant (at both 0.05% and 0.01%) decrease in the value of PCV, RBC and Hb level which may be due to immune mediated damage to bone marrow stem cells caused by E. canis infection. This data also supports the findings of Reardon & Pierce (1981) that E. canis causes mild-to-moderate nonregenerative anemia.

Hematological findings showed Japanese spitz were found to be most severely affected than other breeds. Interestingly, terriers were found to be least affected, even than the mongrels. This difference may be due to the fact that the different animals might be in the different phases of infection, less number of intermediate hosts (ticks) and anemia rarely occurs in dogs with acute ehrlichiosis unless it is related to blood loss. Pure bred dogs, particularly German Shepherds, in comparison to local breeds, may have depressed cell mediated immunity and acquire severe disease. This study also supports the findings of Frank & Breitschwerdt (1999) and corresponds to the statement E. canis seems more severe in Doberman pinschers and German shepherds breeds of dogs in comparison to mongrel breeds. Regarding the sexes of the dogs, male were found to be more affected (more depressed hematological report) in comparison to the females. This supports the finding of Troy & Forrester (1990) that males are affected more severely than females. (Troy & Forrester 1990). Similarly dogs of <1 years of age group were more severely affected followed by the age group >10 years. This may be due to the low immunity level in the young animals and old animals. Dogs of age group 5-10 years were found to have least depressed hematological parameters (Hb, PCV and TEC).

The differential leukocyte count was found to be different in this study as what is stated in the literature. Statistically significant increase in neutrophil (at both 0.05% and 0.01%) count was observed, whereas the lymphocyte count was found to be within the normal range. Weiser *et al.* (1991) had stated that there occurs mild neutropenia, followed by lymphocytosis in the E. canis infected dogs. There might be other bacterial infections simultaneously with ehrlichiosis in our study population, whereas Weiser *et al.* (1991) had conducted the study in the laboratory. Monocytosis was not found in our study, which may be due to the presence of other simultaneous infections in our study population. The rapid test is not the gold standard test. So, all the animals positive on screening test may not be Ehrlichia positive.

The biochemical study for the liver function tests revealed statistically non-significant (at both 0.05% and 0.01%) change in the value of total protein and albumin level. Similarly, statistically significant (at both 0.05% and 0.01%) increase in the value of total bilirubin and direct bilirubin was found in the study.

The total protein level was found to be within the normal range, which differs to that stated by Troy & Forrester (1990) that mild hyperproteinemia occurs in ehrlichial infection. Whereas the albumin level was found to be slightly decreased but was not statistically significant. This loss of albumin usually occurs due to the loss of albumin through kidney due to renal failure. Whereas, increase in the total protein level is due to the increase in the globulin- part of the protein. Buoloy *et al.* (1994) and Reardon & Pierce (1981) had also reported that there is mild hypoalbuminemia in

ehrlichiosis. The mean ALT and AST level was also found within the normal range, which differs to that stated by Buoloy et al (1994) and Reardon & Pierce (1981) that mild elevation of ALT and ALP occurs in ehrlichial infection. Whereas this study supports the findings of Troy & Forrester (1990) that mild elvation of total and direct bilirubin occurs in ehrlichiosis in dogs.

The mean total protein level of Dalmation, German shepherd, Labarador, Lhasa apso, Japanese spitz and Terrier was found to be greater than the normal range. The mean value of albumin level was found to be lower than the normal range in Boxer, Dobermann, Labarador, Mastiff, Japanese spitz, Terrier and German shepherd. This states that globulin is high in Labarador, German shepherds, Japanese spitz and Terrier breeds suggesting that severe infection occurs in these breeds, which is true to that stated by Troy *et al.* (1980) that pure breeds of dogs like German shepherds and Dobermanns are more severely affected.

The exotic breeds were found to have higher total protein value and lower albumin value than that of the local breeds. This suggests that exotic breeds are more severely affected than that of local breeds. Though, mean ALT and AST level of both local and exotic breeds was found within the normal range, the local breeds had a little bit higher ALT and AST level than the exotic breeds. This difference might be due to the fact that exotic breeds are fed more proper diet with lots of supplements, which make the liver of local breeds more suspetible or more damaged before even the infection occurs. The exotic breeds were found to have higher total and direct bilirubin level than the local breeds, which suggests that local breeds are less severely affected than the exotic breeds.

Males were found to have higher total protein value and lower albumin value in comparison to the females. The male had a little bit higher ALT and AST level than the female breeds. So the males were found to have more severely affected liver function tests value in terms of total protein, albumin, ALT and AST level. Whereas, females were found to have higher total and direct bilirubin level in comparison to the males.

The biochemical study for the kidney function tests revealed statistically non-significant (at 0.01%) increase in the value of BUN. Whereas, the test was significant at 0.05%. Similarly, statistically significant (at both 0.05% and 0.01%) increase in the creatinine value was observed in the study.

The mean BUN and creatinine level was found to be higher than the normal range in the infected dogs. This may be due to the fact that ehrlichiosis causes immune complex glomerulonephritis in the infected dogs. Our result is similar to that of Troy & Forrester (1990).

Dalmation had the highest BUN and creatinine value, followed by German shepherd, Lhasa apso, Dobermann and Mastiff. So, the kidney of Dalmation is more susceptible to ehrlichiosis followed by German shepherd, Lhasa apso, Dobermann and Mastiff.

Exotic breeds had higher BUN and creatinine value than the local breeds. So, the kidney of exotic breeds might be more susceptible to ehrlichiosis than the local breeds.

Male breeds had higher BUN and creatinine value than the females. So, the kidney of male breeds could be more susceptible to ehrlichiosis than the female breeds.

The BUN and creatinine value was found to be highest for dogs of <1 year, followed by >10 years, 1-5 years and 5-10 years respectively for BUN and 1-5 years, >10 years and 5-10 years for creatinine. Hence the kidneys of the young animals < 1 year is more affected than others in ehrlichiosis.

CONCLUSION

The hematological study revealed statistically significant (at both 0.05% and 0.01%) decrease in the value of PCV, RBC and Hb level. The mean PCV, RBC and Hb count was found to be 26.10±1.24 %, 4.48±0.19 X 106 / cu. mm. and 8.79±0.38 g/dl respectively. Hematologically, Japanese spitz were found to be most severely affected and Terriers were found to be least affected. Males, exotic breeds

and dogs <1 years of age group were found to be more severely affected. Statistically significant increase in neutrophil (at both 0.05% and 0.01%) count was observed, whereas the lymphocyte count was found to be within the normal range.

The mean total protein, albumin, AST, ALT, total bilirubin and direct bilirubin level was found to be 7.29±0.24 g/dl, 3.05±0.14 g/dl, 40.41±4.15 IU/l, 24.22±2.42 IU/l, 1.97±0.16 mg/dl and 1.54±0.15 mg/dl respectively. The biochemical study for the liver function tests revealed statistically non-significant (at both 0.05% and 0.01%) change in the value of total protein and albumin level. Similarly, statistically significant (at both 0.05% and 0.01%) increase in the value of total bilirubin and direct bilirubin was found in the study.

The liver function of Labarador, German shepherds, Japanese spitz and Terrier breeds were more severely affected than other breeds. Exotic breeds and males were more severely affected in terms of liver function.

The biochemical study for the kidney function tests revealed statistically non-significant (at 0.01%) increase in the value of BUNwhereas, the test was significant at 0.05%. Similarly, statistically significant (at both 0.05% and 0.01%) increase in the creatinine value was observed in the study.

The kidney of Dalmation was found to be more susceptible to ehrlichiosis followed by German shepherd, Lhasa apso, Dobermann and Mastiff. Kidneys of exotic breeds, males and dogs < 1 year of age were found to be more severely affected.

SUGGESTIONS

Based on this research following recommendations can be made:

- Blood tests should be followed as a routine test in disease diagnosis.
- For prevention of the disease, vector (tick) control is an important aspect. Therefore dog owners should always be careful regarding tick infestation and should deal with it seriously.
- Tick control program should be adopted.
- There have been very few studies regarding occurrence and pattern of ehrlichiosis in dogs. Therefore there is still a broad space for further studies.

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POULTRY HEALTH AND PRODUCTION

HIGHLY PATHOGENIC AVIAN INFLUENZA OUTBREAKS IN NEPAL

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ABSTRACT

Nepal was free from Highly Pathogenic Avian Influenza (HPAI) until 2008. In total eleven outbreaks of HPAI subtype H5N1 virus in domestic poultry were reported in Nepal from 2009 to 2011 at 9 districts of country. Among 11, first two outbreaks of HPAI were reported in Jhapa in 2009, eight outbreaks were in Kailali, Banke, Dang, Rupendehi, Kaski, Nabalparasi, and Chitwan in 2010 and one outbreak was in Bhaktapur of Kathmandu valley in November 2011. Seven outbreaks were limited only in backyard chicken and ducks in 7 districts however 2 outbreaks were also reported in commercial poultry in Chitwan and Kaski districts. Preliminary diagnosis of HPAI virus was carried out at Central Veterinary Laboratory in Kathmandu, whereas molecular characterization and phylogenic analysis was performed at Veterinary Laboratory Agency in UK. All these outbreaks occurred from October to March in winter season of the year resulting into total deaths of 1,366 birds and further outbreaks were successfully controlled by sacrificing 55,905 birds followed by quarantine and bio-security. Three clades of H5N1 virus were identified in Nepal during past 3 years such as HPAIV (H5N1) Clade 2.2 in 2009, HPAIV (H5N1) clade 2.2 and, 2.3.2 in 2010 and HPAIV (H5N1) clade 2.3.2.1 were identified in 2011. Incidence of bird flu in human was not clinically observed and confirmed in exposed person during these outbreaks.

INTRODUCTION

Avian influenza is caused by type A virus of family Orthomyxoviridae. Wild ducks are the main reservoir of influenza A viruses that can be transmitted to domestic poultry and mammals, including humans (Kim *et al.*, 2010). They had considered the duck as the "Trojan Horses" of H5N1 influenza. All 16 HA and 9 NA subtypes have been isolated from aquatic birds. The viruses cause asymptomatic or low pathogenic infection in these natural hosts (Webster, Bean, Gorman, Chambers, & Kawaoka, 1992).

HPAI H5N1 can cause high mortality in poultry and direct economic losses. In 11 countries of South Asia and South East Asia, there were 2,286 outbreaks in poultry, 60 outbreaks in wild birds and 64 human deaths in between November 2010 to October 2011 (USAID, 2012).

As per the report of Central Veterinary Laboratory (CVL), Kathmandu, Nepal was free from this disease up to 2008. In January 2009, the first outbreak occurred in some places of Jhapa district. In subsequent years plenty of outbreaks occurred in the different districts of Nepal. No human death has been recorded so far in Nepal (CVL, 2009, 2010, 2011).

Occurrence

Duck that migrate annually were likely to spread influenza viruses along the migration routes,

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primarily by exposing the resident and domestic duck population at the numerous stopovers sites (Olsen *et al.*, 2006; Wallensten *et al.*, 2007 and Arzel, Elmberg, & Guillemain, 2006). Henning *et al.* (2010) had reported that Scavenging Ducks were the major source of Transmission of Highly Pathogenic Avian Influenza in Java, Indonesia.

In 2009, only clade 2.2 was detected in Nepal and the clade was similar to that found in India, Bangladesh, Bhutan etc. (FAO, 2010). Indriani *et al.* (2010) collected poultry samples from 27 poultry related sites of Indonesia and tested using real time RT –PCR and found 39 (47%) live- bird markets contaminated with avian Influenza virus A (H5N1) in < 1 of the sites sampled.

Reid *et al.* (2010) had reported that clade 2.3.2 of HPAI had affected domestic poultry and wild birds in Romania and Bulgaria. Virus of that clade was identified in Mongolia, Eastern Russia, Bulgaria, India, and Bangladesh and also in some other countries of South East Asia (FAO, 2010).

Li *et al.* (2011) reported that HPAIV clade 2.3.2.had been repeatedly detected in wild birds in Hong Kong, Japan, Russia and Mongolia and they suggested that this clade might have established in migratory birds.

Nagarajan *et al.* (2012) had reported Avian influenza virus of clade 2.3.2 in domestic poultry in India.

Newman *et al.* (2012) had explained the eco- virological approach for assessing the role of wild birds in the spread of avian influenza H5N1 along the central Asian flyway.

Vaccination

Vaccination against HPAI H5N1 was not found ineffective in Egypt (Peyre *et al.*, 2009). Iwami, Suzuki, & Takeuchi, (2009) had concluded that presence of the emergence of a vaccine-resistant strain, the vaccination program cannot simply control the spread of the disease. The control of this infectious disease through vaccination becomes more difficult.

Hinrichs, Otte, & Rushton (2011) had described the technical, epidemiological and financial implications of large-scale national vaccination campaigns to control HPAI H5N1. They had conclude that the high and recurrent costs, technical difficulties and epidemiological drawbacks of large-scale, open-ended, blanket vaccination programmes in national efforts to control HPAI call for careful targeting of vaccination in national control strategies which 'intelligently' combine available disease control measures.

METHODOLOGY

Clinical Sample collections from field, PM Examination of the cases, tracheal/cloacal swabs/tissues or fresh faecal samples were examined.

Sample collection

When unusual mortality reported clinical samples were collected from outbreak districts, buffer zones, during surveillance. Samples were also directly collected from districts, regional labs, National Avian lab, Central Veterinary Hospital and direct collection from CVL staff or from farmers (From 2007 to 2011). Only samples tested at CVL are included in this article. In 2008, majority of samples were from Jhapa, Morang, Sunasari and Surkhet districts. In 2009, tracheal swabs/ tissues , cloacal swabs and fresh faecal samples were collected from 12 districts (Jhapa , Morang , Kaski , Kathmandu , Nawalparasi , Banke , Siraha, Mahottari, Makawanpur, Sindhuli, Surkhet, Sankhuwasabha), whereas in 2010 tracheal swabs/tissues,cloacal swabs and fresh faecal samples were collected from 11 Districts (Jhapa, Morang, Kaski, Kathmandu, Nawalparasi, Banke, Tanahun, Rupendehi, Chitawan, Kailali, Dang). In 2011, similar types of samples were collected from Bhaktapur and other districts. In 2009 six hot spots were identified in Jhapa district, whereas in 2010 during the outbreak, eight hot spots of H5N1 were identified in Kaski district. H5N1Viruses

were also detected in various districts in the same year. Dead chickens, ducks, wild birds were treated as cases and hence were included in the test cases.

Sample size

A total of 14,980 tracheal swabs/tissues, cloacal swabs and fresh faecal samples were collected and tested from July 2007 to Dec. 2011 [July 2007 to Dec 2008 a total of 2,233; In 2009, a total of 4221 + 404 dead birds; in 2010, a total of 2720 swabs + 482 dead birds samples of poultry (backyard chicken, ducks, pigeon, commercial birds), wild birds and migratory birds were tested. In 2011, a total of 4,920 swab samples including samples from 976 dead poultry (backyard chicken, ducks, pigeon, and commercial birds), wild birds were presented in virology unit at CVL].

Post-mortem Findings

Post mortem of 1564 poultry suspected for HPAI were done from 2009 to 2011 at CVL. Haemorrhage in proventriculus, gizzard mucosa, intestinal tract, trachea, heart, brain and peritoneal fat; conjunctivitis, hemorrhage in brain tissue, edema and cyanosis of wattles and combs, haemorrhage in shank region, subcutaneous haemorrhage in skin of head and neck regions and congestion in the body muscle were the postmortem lesions. But there were no clear cut signs in all PM cases. There was also no consistency in the field symptoms.

Testing

Screening for Flu A

Samples submitted to Virological Unit were tested for Flu A using Rapid Test Kits (Anigen / Synbiotic kits) as recommended by manufacturer. Samples showing any degree of positive reaction and ten percent Flu A negative samples were submitted to the Molecular Biology Unit for further investigation.

Detection by RT- PCR and real time RT-PCR

OIE Ref. Lab AAHL, Geelong, Protocol for RT-PCR and OIE Ref. Lab.FLI Germany, Protocol/ techniques for rt RT-PCR (Real Time PCR) were used for the detection of virus in the tissue and / or swab samples obtained from the Virological Unit at CVL, Kathmandu. Viral RNA was extracted from the samples using QIA amp Viral RNA Mini Kit (Qiagen Viral RNeasy® Mini Kit) as recommended by the manufacturer. For detection of influenza type A virus, One step RT-PCR and real time RT-PCR were used. For HA subtype (H5 and H9) One step RT-PCR was carried out, whereas HA subtype H5 and NA subtype N1were carried out by using one step rt RT- PCR .

Further confirmation of H5N1, isolation, sequencing and characterization of HPAI virus (H5N1) were done in VLA / AHVLA. UK.

RESULTS

Flu A - Rapid Test Result

From July 2007 to Dec 2008, a total of 2,233 tracheal and /or cloacal swabs/ tissues were tested at Central Veterinary Laboratory (CVL, 2009) using rapid test kits, out of which all samples were found Negative for Flu A.

In 2009, a total of 4,625 pooled samples were tested using rapid test kits of which 53 were found positive for Flu A. Similarly, in 2010, out of 3,202 pooled samples tested 76 samples were found positive for the same .During 2011, a total of 4,920 samples were tested of which 38 were found positive for Flu A (CVL, 2010 and 2011).

PCR testing Result

At Central Veterinary Laboratory (CVL) Kathmandu, during 2009 and 2011, a total of 186 and 112 samples (tissue/ swabs) were tested by RT-PCR technique for H5 of which 32 samples were found positive for HPAI H5, whereas in 2010, a total of 138 sample (tissue/ swabs) were tested by RT-PCR and Real Time PCR for H5 & H5N1 of which 62 were found positive for HPAI H5 & H5N1. On the basis of the test result it seemed that average rate of positive samples was found 21.56 % (94/436) for H5 / H5N1 in PCR. Besides, most of the Rapid Test positive cases turned positive in PCR Test and from 10% rapid test negative samples tested, 5% were also found positive in PCR (CVL, 2009, 2010 and 2011).

Reconfirmation of Virus from OIE Reference Lab

In 2009, 2010 and 2011, a total 95, 143 and 20 tissues/ swab samples were sent to OIE Ref Lab, Veterinary Laboratory Agency (VLA), Weybridge, U.K for further confirmation of H5N1 out of which 14, 30 and 5 samples were found positive for HPAI (H5N1) respectively.

Virus sequences and clades

According to the VLA, Weybridge U.K., reports of the clades and sequences of Nepal Virus were found as below:

1. In 2009, HPAI H5N1 Virus Clade found was 2.2 with the sequence: PQGERRRKKRGLF (HPAI H5N1) a cleavage site motif, which was closely related to clades from India and Bangladesh.
2. In 2010, HPAI H5N1 Virus Clade found were 2.2 (sequence: PQGERRRKKRGLF (HPAI H5N1) and 2.3.2 with the sequence: PQRERRRKRGLF (HPAI H5N1) a cleavage site motif, which closely related to clade from Mongolia, Rumania, Bulgaria.
3. In 2011, HPAI H5N1 Virus Clade found was 2.3.2.1 with the sequence: PQRERRRKRGLF (HPAI H5N1) a cleavage site motif, which was also found closely related to the clade from Mongolia, Rumania and Bulgaria.

DISCUSSION

Available information shows that during 2006-07, dominant virus clade in South East Asia was 2.3.4. Nepal was free from HPAI up to 2008. In 2009, Nepal experienced HPAI H5N1 of clade 2.2. The first introduction of clade 2.3.2 HPAIV in Nepal occurred in February 2010 and was the first event in South Asia. Again clade 2.3.2.1 HPAI H5N1 was detected in November 2011 in Manohara of Bhaktapur District.

Nagarajan *et al.* (2012) had reported Avian Influenza virus of clade 2.3.2 in domestic poultry in India. Li *et al.* (2011) reported that HPAIV clade 2.3.2.had been repeatedly detected in wild birds in Hong Kong, Japan, Russia and Mongolia and they suggested that this clade might have established in migratory birds. Nepal is temporary stay place (November to March of year) of Migratory Birds of Ciconiformes, Anseriformes, Falconiformes, Galliformes and Charadriiformes family and they come to Nepal from Siberian Plateau and North Africa (Falcon) or Gulf countries. These birds stay on the different wet-lands of Nepal. Some birds migrate even down to Indian Territory. This indicates that there is possibility of entering of HPAIV clade 2.3.2 in Nepal through migratory birds.

Indriani *et al*. (2010) collected poultry samples from 27 poultry related sites of Indonesia and tested using real time RT –PCR and found 39 (47% live- bird markets contaminated with avian Influenza virus A (H5N1) in < 1 of the sites sampled. In Nepal there are no organized poultry markets. Poultry is also marketed on the road- sides in the metropolitan cities. Such live –bird markets might be other source of HPAIV H5N1. In Nepal various types of wild birds are found and many of them mix- up with the domestic poultry. Besides, poultry and various types of birds also enter

or are entered from the porous borders. But it is still unknown that whether these viruses came to Nepal through trade or wild native birds or migratory birds.

It is also unknown till the date that whether the clade 2.3.2.1 of HPAIV H5N1 is replacing clade 2.2 or co-circulating with it. Continued co- circulation of different sub-clades of the HPAIV H5N1 may be easily adapted in the domesticated poultry in Nepal and other countries of South Asia and may increase the probability of evolution of pandemic H5N1 strains. This is also explained by other researchers.

In 11 countries of South Asia and South East Asia, there were 2,286 outbreaks in poultry, 60 outbreaks in wild birds and 64 human deaths in between Nov 2010 to Oct 2011 (USAID, 2012). These facts indicate that there is urgent necessity of further investigation on whether these viruses came to Nepal through migratory birds or poultry trade or from the wild native birds.

Kim *et al.* (2010) had reported the puzzling inefficiency of H5N1 influenza vaccine in Egyptian poultry immunized with commercial H5. Vaccination against HPAI H5N1 was not found ineffective in Egypt (Peyre *et al.*, 2009). This may be due to mismatching of the field virus with vaccine.

CONCLUSIONS

Now it is very important to monitor whether the clade 2.3.2.1 of HPAIV H5N1 is replacing clade 2.2 or co-circulating with it. Continued co- circulation of different sub-clades of the HPAIV H5N1 may be easily adapted in the domesticated poultry in Nepal and other countries of South Asia and may increase the probability of evolution of pandemic H5N1 strains.

Vaccination against this disease seems to be questionable or confusing. There is need of special scientific discussion before going to take policy decision for vaccination against H5N1 Viruses.

RECOMMENDATIONS

On the basis of above facts following recommendations can be made:

- Detection, characterization and phylogenic analysis of H5N1 in wild birds, wet land birds, ducks/ gooses and migratory birds found in Nepal is also urgently necessary.
- To monitor whether the clade 2.3.2.1 of HPAIV (H5N1) is replacing clade 2.2 or co-circulating with it. Monitoring of the different clades of HPAIV (H5N1) with virus sequence is suggestive.
- Close collaboration with wildlife people for the collection of tracheal and swabs from of the wild and migratory birds for the study of clades of HPAIV is quite suggestive. More regional approach to control the AI is suggestive, especially for twinning and lab diagnosis.
- Sharing of information/ technology among the regional and reference laboratory may be more useful to study the nature and sub-clades of the virus.
- Vaccination against this disease seems to be questionable or confusing. There is need of special scientific discussion before going to take policy decision for vaccination against H5N1 Viruses. If policy is framed for vaccination, vaccine production only from Nepal field- strains may be suggestive.

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SEROPREVALENCE OF AVIAN INFLUENZA SUBTYPE H9N2 IN LIVE BIRD MARKET OF KATHMANDU VALLEY

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ABSTRACT

A community Cross-sectional study was conducted in Live Bird Market of Kathmandu Valley to find out the seroprevalence of Avian Influenza subtype H9N2 from August –December 2011. Altogether 92 blood samples and 40 cloacal swab samples were taken from seven different live bird markets of Kathmandu Valley using purposive random sampling method and carried in icebox maintaining 4°C temperature. All the samples were analyzed in virology laboratory, Animal Health Research Division, NARC, Kathmandu. The serum were separated from blood samples and processed for Haemagglutination Inhibition (HI) test. Of the 92 serum samples 24 samples showed HI positive (at >3rd well) revealing 26.086% seroprevalence of LPAI H9N2. The seroprevalences of seven different places were found to be viz: Lagankhel (38.88%), Tankeshwor (41.17%), Maharajgunj (0%), Khasibazar (40%), Sundhara (36.36%), Balkhu (33.33%) and Koteshwor (0%) with their mean antibody titres in log2 form were found to be 5, 5, 0, 5, 4, 4, 0 respectively. The geometric mean titers (GMT) of positive sampled groups were Lagankhel (16.865), Tankeshwor (28.857), Khasibazar (32), Sundhara (13.92) and Balkhu (16). Of the 40 cloacal swab samples 2 samples from each LBM was processed for AI Virus antigen rapid test kit showed all the samples to be negative for the AIV antigen. Low-pathogenic strains (H9) of avian influenza are prevalent in Nepal. Live Bird markets (LBM) are recognized as a reservoir of avian influenza viruses (AIV) and a possible source of infection for domestic poultry. These markets may also facilitate avian influenza virus undergoing reassortment and such reassortment may finally leads to the emergence of high pathogenic avian influenza (HPAI) H5N1 which is devastating for both poultry and humans.

INTRODUCTION

Avian influenza (AI) is an acute contagious respiratory viral disease caused by influenza virus. Among respiratory viruses these influenza viruses have unique features with the segmented RNA genome and highly antigenic diversity. Influenza viruses have been classified in three serotypes

A, B and C. Type A is responsible for highly pathogenic avian influenza (HPAI) and human pandemics worldwide associated with severe morbidity and mortality. Genetically and antigenically, AIV exist as multiple subtypes based on the two glycoproteins (HA and NA) on the virion surface. To date, 16 HA (HA1-16) and 9 NA (NA1-9) subtypes have been identified in aquatic birds (Fouchier *et al.*, 2005). Influenza viruses infecting poultry can be divided in two groups. One is very virulent viruses, anciently called fowl plague and now termed as "Highly pathogenic avian influenza" (HPAI) in which mortality may reach as high as 100 percent. The other is "Low pathogenic avian influenza" (LPAI) which cause disease in milder form, however sometimes may cause high mortality if concurrent infection with other secondary pathogens. LPAI H9N2 causes unapparent

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diseases with mild respiratory signs, egg production losses and sometimes with slightly elevated mortality (Capua and Alexander, 2006).

In the last decade, H9N2 viruses had caused disease outbreaks in chicken in many parts of the world including Germany, Italy, Ireland, Pakistan, Saudi Arabia, South Korea, UAE, India, Israel, Jordan, China, South Africa and USA (Alexander, 2007; Nagarajan *et al*., 2009). Although high pathogenic H5N1 avian influenza has been a major health threat to humans in Southeast Asia since 2003 (Li *et al*., 2003 and Guan *et al*., 2004), *the human* infections with H9N2 have also highlighted the potential of H9N2 influenza virus to become the next pandemic strain.

A key lesson, initially from Hong Kong but confirmed in other parts of Asia and Egypt is that poorly managed live bird markets and traders' yards can play a major role in the persistence and transmission of the avian influenza virus especially if poultry remain in the market over 24 hours, providing opportunities for transmission within market stalls (FAO, 2011).

It has been frequently reported that secondary pathogens such as *E coli*, *Staphylococcus aureus*, *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale* and infectious bronchitis virus played a significant role in aggravating the clinical condition of the birds earlier infected with AIVs, H9N2 (Nilli & Asasi 2002, Bano *et al*., 2003).

MATERIALS AND METHODS

This study was carried out from August 2011 to January 2012 during the internship period at Virology Unit of Animal Health Research Division (AHRD), Khumaltar, Kathmandu. Tests were carried out as per given on the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of OIE, 2008.

Site of study

The study has been carried in different live bird markets (LBM) of Kathmandu valley i.e.Tankeshwor, Balkhu, Sundhara, Khasi bazar, Maharajgunj, Koteshwor and Lagankhel.

Sample and Sample Preparation

92 blood samples and 40 cloacal swab samples were taken from mentioned live bird markets of Kathmandu valley using simple random sampling (Lotterey) method, carrried in ice containing thermocool at 4°C; analysed in virology lab of Animal Health Research Division, Khumaltar.

Reagents Preparation

One ltr of Phosphate Buffer Solution (PBS) solution, 200ml of Alsever's solution and 1% chicken RBC suspension was prepared according to the OIE Manual of diagnostic Tests and Vaccines for Terrestrial Animals, 2008.

Serum Preparation

A total 92 blood samples in vacutainer without EDTA were let to stand in slanting position for 30 minutes without disturbance and then subjected to centrifugation at 1500 rpm for 15 minutes.

Serum was separated at the upper layer which then was collected in serum collection vial and preserved in deep freeze (-50°C) in case there will be any delay in examination.

Rapid Test

Initial screening test with cloacal swabs was performed using rapid test kit for avian influenza virus (Manufactured by Bionote, Inc. 2-9, Seogu-dong, Hwaseong-si, Gyeonggi-do, Korea). Positive sample gave two red/pink color bands in the strip and only one band indicates negative result.

Haemagglutination Inhibition Test (HI)

This quantitative test of serum samples were processed for Haemagglutination Inhibition (HI) test under OIE Manual of diagnostic Tests and Vaccines for Terrestrial Animals, 2008 and the standard Antigen and Antisera was brought from Charles River (USA) for the test of HA and HI.

- The HI titre was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 25µl RBCs and 25µl PBS only) should be considered to show inhibition.
- Positive test with appearance of distinct button or tear shaped and Negative test with agglutination.

RESULTS

Of the 92 serum samples, 24 samples showed HI positive (at >3rd well) revealing 26.086% seroprevalence of LPAI H9N2. The seroprevalences of seven different places were found to be Lagankhel (38.88%), Tankeshwor (41.17%), Maharajgunj (0%), Khasibazar (40%),Sundhara (36.36%), Balkhu (33.33%) and Koteshwor (0%). The mean antibody titres in $\log_2$ form were found to be 5, 5, 0, 5, 4, 4, 0 respectively. The geometric mean titers (GMT) of positive sampled groups were Lagankhel (16.865), Tankeshwor (28.857), Khasibazar (32), Sundhara (13.92) and Balkhu (16).

Of the 40 cloacal swab samples, 2 samples from each LBM was processed for AIV antigen rapid kit test which showed all the samples to be negative for the AIV antigen.

The results were statistically analyzed by Chi-Square test using Mcrosoft Office Excel 2007. No significant variation ($p<0.05$) was observed among different sites in case of prevalence rate. Since calculated Chi-square value ie.11.7488 is less than tabulated Chi-square value ie. 12.59 at 6 degree of freedom so the test is non-significant.

DISCUSSION

The absence of clinical signs of influenza in live bird market chickens, in spite of considerable antibody titers in some birds, could be due to persistent exposure and acquired resistance of these birds to influenza virus in the environment, and therefore these birds would be naturally immunized against this virus. All these reveal the past exposure of birds with H9N2 virus and developed antibody against it but there is no current infection. Since it is known that H9N2 influenza virus in 1999 has acquired its six internal genes from H5N1-like source that was responsible for the initial H5N1 "bird flu" outbreak in HongKong in 1997 (Guan *et al.*, 2000) such circulation of LPAI virus in poultry population for a long time may lead to the mutation creating pandemic novel strain. It has been frequently reported that secondary pathogens such as *E coli*, *Staphylococcus aureus*, *Mycoplasma gallisepticum*, *Ornithobacterium rhinotracheale* and infectious bronchitis virus played a significant role in aggravating the clinical condition of the birds earlier infected with AIVs, H9N2 (Nilli & Asasi, 2002; Bano *et al.*, 2003), recently in chitwan, Piple VDC, Nepal, 2068/8/19 it was reported that 1800 broiler died from a flock of 3000 this may be due to the concurrent infection of the flock with other bacterial or viral infections because H9N2 alone do not cause such mortality.

In Nepal, antibodies to H9N2 influenza A were detected in chickens in Kathmandu and Chitwan in two serum samples (pant *et al.*, 2005). Similarly another study in Nepal for serum sample, 2 samples showed hemagglutination inhibiting activity (9.09%) against H9 (Bhandari, 2008). Though study in subtle amount in Nepal there have been the evidences of H9N2 circulating in the poultry. Our study showed the higher prevalence rate (26.086%) among those studied in Nepal, the reason behind this may be we did the study on Live Bird Market local hens and ducks. Ducks are being the reservoir of the avian influenza virus and the structure of the LBM leading to the potential risk to LPAI H9.

In an study in backyerd chickens around caspian sea in Iran overall antibody titer and seroprevalence of H9N2 avian influenza virus recorded were 6.52 and 72.98%, respectively (Hadipour, M.M., 2009). Similarly Al-Natour *et al.*, (2005) reported that the seroprevalence of avian influenza was 71% among broiler-breeder flocks in Jordan, which is much greater than the prevalence rate of our study. The reason behind this may be due to the greater sample size taken in the study and another strong reason could be due to the migratory wild water birds around the sea. Wild water birds are considered the main reservoir of all subtypes of Avian Influenza Viruses (AIV). Low Pathogenic AIV (LPAIV) are widely distributed in wild avian species around the world. They have been most frequently identified in water birds of the orders Anseriformes (including ducks, geese and swans) and Charadriiformes (particularly gulls and terns). These viruses replicate in epithelial cells of the respiratory and intestinal tracts of birds and are excreted in high concentrations in their faeces (Alexander, 2000).

According to *Hadipour*, *M.M.* (2010) antibodies against H9N2 avian influenza virus were measured using hemagglutination-inhibition (HI) test in sera from 300 individuals in five different population in Fars province, including poultry-farm workers, slaughter-house workers, veterinarians, patients with clinical signs of respiratory disease, and clinically normal individuals, who were not or rarely in contact with poultry. Mean antibody titers of Lagankhel (7.3), Tankeshwor (6.8), Khasibazar (6.1), Sundhara (4.5), and Balkhu (2.9) and seroprevalences of Lagankhel (38.88%), Tankeshwor (41.17%), Maharajgunj (0%), Khasibazar (40%), Sundhara (36.36%), Balkhu (33.33%) and Koteshwor (0%) were determined. This study reveals the zoonotic importance of LPAI H9N2 and shows that poultry farm workers, slaughter house workers and veterinarians are in the greater risk.

In addition to HP H5N1, the H9N2 subtype has been implicated in contributing to the influenza outbreak in 1997 in Hong Kong. Extensive surveillance efforts to discover the source of the Hong Kong pandemic revealed that influenza of subtype H9N2 was being isolated from chickens at the same Live Bird Markets that had HP H5N1-positive chickens (Shortridge, 1997). Although the overall prevalence of the H9N2 subtype was low (4.4%), one market exhibited an unusually high prevalence (36.6%) which is slightly higher than our study. During this outbreak, most chickens appeared healthy (Shortridge, 1997). This observation and later experiments suggest that chickens that were previously infected by a H9N2-subtype virus may have been partially protected from the pathogenicity induced by a HP H5N1-subtype infection, which could have allowed the HP H5N1-subtype virus to 'silently' attain a prevalence where transmission to humans was probable (Khalenkov, 2009; Seo, 2001).

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MODELING THE IMPACT OF INTERVENTIONS ON THE DYNAMICS IN VILLAGE POULTRY SYSTEMS

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ABSTRACT

Backyard poultry is a significant contributor to the livelihoods of marginalized, small holders and landless, especially women where it not only enhances food security but is also enmeshed in the social, religious and cultural milieu of the society. Local Poultry in Nepal are mainly reared by poor farmers and their husbandry is integrated within the whole farming system. The majority of these people have a tradition of poultry keeping, especially chickens. In village conditions, nearly all farmers rear a few chickens and the birds that are managed under a back yard system utilizing their free time at home. The chickens and eggs produced are consumed by the members of family any surplus sold locally. Even though eggs and meat produced from local poultry maintained under back yard system have higher price, consumers have higher preference for them. With this scenario, surprisingly, village farmers seem to be totally unaware of the poultry health and management systems. It suffers huge losses due to various reasons like predation, diseases and theft. Among the diseases, Newcastle Disease (ND) is the most feared and it virtually wipes out village flocks. ND also known as Ranikhet Disease (RD) is endemic all over the country. There are many technical possibilities to improve free-range and backyard poultry keeping. Rural households, however, are not adopting these technologies widely. This paper presents a model approach for ex ante evaluation of interventions in village poultry systems at Khudunabari and Arjundhara VDC,Jhapa. The dynamic deterministic model considers mortality, egg production, reproduction, off take, and their interrelationships. In the base situation, the model reflects the behavior of are relatively stable village poultry flock. The model was used to explore how interventions influence the dynamics of village poultry flock. Over the simulated period of one year, ND(Newcastle Disease) vaccination, Free range in certain closed area, Night time housing, supplementary feeding, and control of broodiness each has a positive effect on bird offtake, egg production, egg offtake, and flock size. The impact of interventions is also related to the use of the available resources along with herbal medicine, ND vaccinations were economically most effective. Different management using local resources and medicine help to reduce input cost drastically. When applied with situation specific input data, the model can be used in first stages of research and development approaches to support decisions on priorities of projects in village poultry production and conservation.

INTRODUCTION

Poultry production as commercial enterprises in Nepal started since last three and half decades and is now it became one of the main national agricultural industries. There is a growing awareness of the nutritive value of eggs and meat among the people. Poultry products (eggs and meat) are a good source of food with high biological value. Therefore, poultry farming is becoming an important business enterprise in both rural and urban areas of the country (Bhurtel and Shah, 2000). Poultry alone contributes 4% of the agricultural GDP of the country with an output of 10 billion rupees from their sector (Dhakal, 2005). The contribution of poultry sector to the agriculture GDP is 38.15

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% and contribution to livestock AGDP is 27.66% (MOAC, 2006).

The increasing trend of poultry population in Nepal is given below:

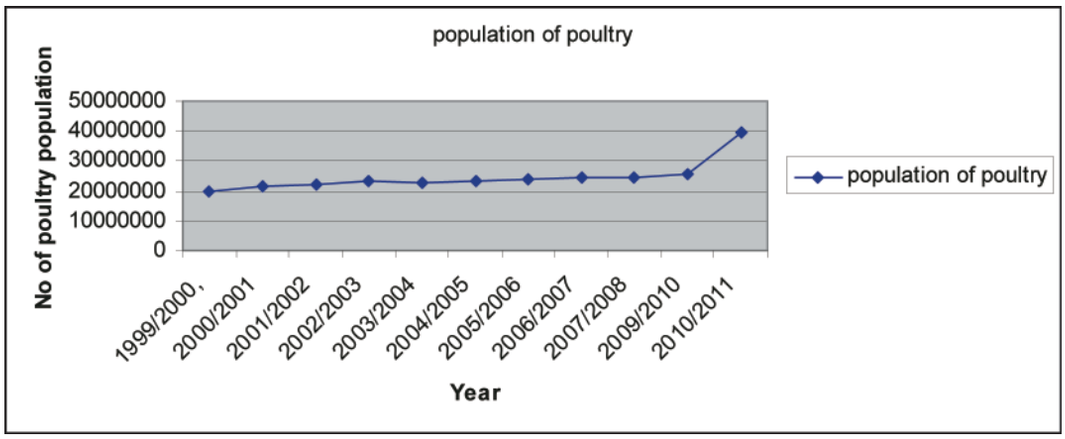

Fig: Trend of increase population of poultry in Nepal (MOAC, 2011).

The annual growth rate during 1988-1998 was 4.2%, which were above all the livestock enterprises (FAO, 1999). Now poultry industry is self sufficient to replace import of feeds, chicks, eggs, and meat products and has great role in national income (Bhattarai, 2005).The average annual growth rate of poultry population was more than 10% despite existing political situation and bird flu media havocs(Bhattarai,2011).

Nepal used to have 3.3 million laying birds, producing an estimated 265 million eggs, an average of 80 eggs/hen/year (DFAMS, 1988) and with the recent data, there are about 25.7 million poultry birds which contribute 634.6 million eggs to the human diets (MOAC, 2010).

Backyard poultry (BYP) are small family flock which may or may not be kept for profits that are reared by scavenging condition. Backyard poultry play significant role to livelihoods of marginalized, small holders and landless, especially women where it not only enhances food security but is also enmeshed in the social, religious and cultural milieu of the society. In village condition Majority of the Farmers have backyard poultry specially chickens that form a part of the family consumption, a means of celebration, sacrifice to the God and a source of income by selling surplus locally when required. They rear a few chickens and the birds are managed under a back yard system without spending much time and money although eggs and meat of back yards have high priced and consumer's preference also high.

Although commercial farming is growing in the urban area of the country, the percentage of Indigenous poultry (Backyards) still exceeds in the rural areas. There are three indigenous breed of chicken are Sakini, Ghanti khuile and Puwankh ulte and out of these, Sakini is the most commonly found. Fifty percent of the total poultry population of the country comprises the indigenous stock (N. Gorkhali and S.P. Neopane, 2008).

In this scenario, village farmers are totally unaware of the poultry health and management systems It suffers huge losses due to various reasons like predation, diseases and theft. Among the Avian diseases New Castle disease (ND) is one of the major causes of high poultry mortality. Although vaccines are available, it is mostly used for commercial poultry farms. Backyard poultry keepers are unaware of the effects of the disease and preventive measures. Those who are aware of the vaccines and are using it are not getting maximum benefit because of the fact that cold chain has not been properly maintained. It can be seen that vaccine sellers and administers are not aware of the importance of maintaining cold chain or were unable to do so because of the lack of appliances needed. There are many technical possibilities to improve free-range and backyard poultry keeping. Rural households, however, are not adopting these technologies widely.

MATERIALS AND METHODS

Location of the study: Arjundhara and Khudunabari VDC of Jhapa that is the easternmost part of Nepal and lies in the fertile Terai plains is around 20 km western from the Indian border. It is also the site for first Bird flu outbreak in Nepal. These villages are home to many ethnic people such as the Danuwar,Limbu, Rai as well as upper castes like Brahmin and Chhetri. Wage labor is the main occupation followed by crop and livestock production.

Selection of household and organizing it in to group: 1904 poultry keeping households (930 from HPN working areas and 974 from HPN non-working areas) were selected. Non Heifer areas were organized in to the group .Total no of local poultry in selected household in two VDC during initial period was 11,994 so no of poultry per household was 6.3.

Productivity of local poultry in selected households: 101 Questionnaire were used to take base data and Flock card and per hen card were distributed to record each data. Initial period no of egg in one clutch was 15-20 and over a year 2-3 clutch so total egg production in one year becomes 30-60. Mortality rate of chicken was 90 %(July –August) In average income per bird/year was Rs. 3600.

Model used and Intervention: Selected households were trained on social mobilization and empowerment through Heifer cornerstone and backyard poultry management trainings. Selected birds were de-wormed using Piperazine liquid and Vaccinated using ND Lasota F1 strain in every 3 months through Community Animal health worker (CAHWs). Birds were housed in night time, Day time free range in certain closed areas. Supplementary feeding using home grown crop and by product, fodder and grasses, 24 hour clean water with add of turmeric powder were provided. Crossbreeding was introduced and control of broodiness was maintained by early separation of hens from their chicks (12-14 days in winter and 3-4 days in summers). Herbal medicine such as zinger, Neem, Tulsi,Turmeric, garlic and other herbal medicine were used preventive and measure. Follow up of project site for 1 year.

Project implementer: Heifer Project Nepal funded by Global Alliance for Livestock Veterinary Medicines (GALVmed)

RESULT AND DISCUSSION

After one year same questionnaire were used to compare the data before and after intervention. Flock cards and per hen cards were analyzed to know clutch per birds, egg production, mortality rate income along with the bird off take, egg off take, and flock size

Table: Productivity and income data

Parameters	Before project intervention	After project intervention
No. of clutch in 1 year	2 - 3	3 - 6
No. of egg laying in each clutch	15 - 20	20-25
Total egg production/ year	30 - 60	80 - 150
Mortality rate	90%	15 -20%
Income per year per bird (Rs)	3,600	7,000-28,000

Number of clutch per year before intervention 2-3 followed similar result found by Brutel R(1995) has increased by double after intervention that may be due to control of broodiness. Chicken resumed egg lying between 11-15 days after weaning the chicks. Similar response was found in performance of local chicken in mill hill and plains in earlier studies Brutel R (1995). Number of eggs lay per clutch 15-20(average of 17) in tandem with case study on indigenous chicken in Tharu community of Chitwan by Thapa Magar DB and MR Kolachhpati(2008) increased significantly i.e. 20-25 after intervention. This may be due to supplementary feedings and cross breeding. Mortality rate is decreased by dramatically due to regular de-worming, vaccination against ND, use of herbal medicine and proper care. The income per year climbed surprisingly by more than seven times

than before intervention due to result of all intervention.

Off take of bird and egg for household consumption were increased drastically. Control of broodiness had a positive effect. Semi intensive farming showed the greatest increase in flock size, followed by ND vaccination, feed supplementation cross breeding and control of broodiness. As a result farmers started to rear large scale, these areas became learning center for backyard poultry. News was Coverage media kantipur daily, NTV, local TV, F.M and newspaper. This model was adopted by non project families and different stakeholders. Overall social Harmony in community was increased. Effect of de-worming and vaccination also reflected for other species of animals. The impact of an intervention involves not only the biological response, but also the resource base of the farming system. The increases in flock size, as a result of the interventions, require additional feed. The study showed that village poultry farmers prefer low input interventions, in particular those that can reduce the loss of chickens Vaccination against ND can increase survival rates dramatically; in our modeling, Constraints of vaccinations are their availability of 100 dose every time and that they have to be repeated at regular intervals. Community activities and training of farmers were needed for successful vaccination programmers. Semi intensive housing showed the most positive response in flock output parameters along with protect from predator.

CONCLUSION AND RECOMMENDATION

Backyard keepers recognized poultry farming as one of the main income generating activities that help them to means of livelihood along with income diversification .All stakeholders are seem very enthusiastic about this model and have already been attempts for replication to more VDC's and districts. Model is a relatively cheap and simple method to improve our understanding of flock dynamics. Modeling can give more direction to the type of village poultry data to be collected in order to study the behavior of poultry systems. The model can help to identify the most likely interventions in village production systems. In this way, the model can be used as part of research and development approaches to understand possibilities of interventions in village poultry systems. ND is endemic all over the country so vaccination is mandatory to control the disease. Proper education on production, management and health aspect is needed to conserve local poultry. Sustainable technologies should be introduced in the backyard raising system .As regular outbreak of bird flu like highly pathogenic poultry diseases in backyard could helpful to control by managing flock with this model. This helps us to conservation and utilization of indigenous poultry.

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ROLE OF COCCIDIAL INFECTION IN THE OCCURRENCE OF NECROTIC ENTERITIS IN COMMERCIAL BROILERS IN AND AROUND KATHMANDU VALLEY

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ABSTRACT

The research was initiated by purposively selecting Kathmandu Valley and it's around. The study was conducted during August to December 2010 with a view of studying role of coccidian in occurrence of necrotic enteritis in commercial Broiler. 100 Intestinal scrapings with necrotic enteritis collected from postmortem examination. These birds were scored for Coccidiosis lesion & evaluated for gross NE lesion score. Out of 100 Samples, sixty nine (69%) found to be positive. As regard to necrotic enteritis, occurrence of NE was found significantly (at 1% and 5%) higher in coccidial infection than in others. Among 69 positive samples, mixed infection were found to be more higher (29%), followed by E. tenella (23%), E. necatrix (9%) and E. acervulina (8%) which was found in intestine of ceaca, mid small SI and upper SI respectively. 35 cases, score 3 (total of 40) were found positive likewise 27 cases, score 2 (total of 35), 4 cases, score 1 (total of 10), 3 cases, score 0 (total of 5) and 1 case, score 4 (total of 5) for coccidial infection respectively. Total of 100 positive cases of NE, 69% of NE predisposed by coccidia i.e. coccidiosis was primary trigger for the occurrence of NE, there after collibacillosis (19%), others (9%) and IBD (3%) respectively. Out of 100 cases of positive NE ,30 Of 23 samples was found to be positive for coccidia in rain/ summer, Likewise 70 of 46 samples was found to be positive for coccidia in dry/ winter season which was 76.66% & 65.71% respectively in rain/ summer & dry/ winter season. There is no significant difference of prevalence of the coccidial infection in between the seasons at 1% and 5%.

Key Words: Necrotic Enteritis, Predisposing factor, Coccidiosis, Lesion Score.

INTRODUCTION

The Agriculture Perceptive Plan (APP 1995-2015) has considered the livestock sector growth crucial to meet its AGDP growth and poverty alleviation objectives. APP has targeted to accelerate the growth rate of the livestock contribution for 31 to 45 percent by the end of APP plan period. The Tenth Five Year Plan has targeted to achieve 303,000 MT meats from the base year productions if 203,300 MT, annual increment of 20,000 MT of meat. As the number of people rearing the livestock is in the decreasing trend and commercial livestock raising is also not in satisfactory level, the only alternative to meet the above meat production can be fast growing broiler industry.

The contribution of agriculture to national GDP is 38.34% of which livestock contributes about one-third of total agricultural GDP. Poultry industry shares 4% of the total GDP and 8% of the agricultural GDP (ABPSD, 2005).

1 NEST, Pokhara
2 Valley Poultry Pvt. Ltd. Balaju

Thus, we can say doubtlessly that poultry industry is of immense importance in the national economy. However, this growing poultry industry is also facing a lot of challenges. Among them, disease outbreak is one of the major problems. Intensive poultry production with fast growing strains and high stocking densities are usually susceptible to infectious agent due to varied reasons and one of the reasons is reduced immune potential (Van der Zijpp, 1983).

Poultry industry is one of the most booming industries of our country. Although Nepal is an agricultural country and economy of our country is basically dependent upon agriculture. It is still at the subsistence level. The additional system of livestock raising is still dominating our agricultural system and commercialization is at the preliminary stage. In contrast to other livestock, poultry husbandry is expanding rapidly and has now transformed into the poultry industry. If any sector of livestock in Nepal is on the road of commercialization, it is none other than poultry sector.

Necrotic Enteritis (NE) is become a top concern in the poultry industry of Nepal, particularly among producers growing broilers without the use of in-feed antibiotic growth promoters. The disease also costs the global poultry industry millions of dollars annually. Clinical NE results in severe intestinal necrosis and increased morbidity and mortality. Therefore, Necrotic enteritis is a serious problem for broiler producers. Experts believe control can be achieved by eliminating triggers of the disease, together with the help of novel preventive approaches that include a new toxoid vaccine (Delmar, 2010).

Poultry farming is the most popular and widely accepted means of livelihood in and around the Kathmandu valley. Kathmandu, the major poultry pockets of the country. Its contribution to the national economy is remarkable. However, the diseases like necrotic enteritis results in heavy loss of economy in broilers production. These pathogens cost poultry producers millions of dollars in lost revenue. The subclinical form of *Clostridium perfringens* associated necrotic enteritis (NE) causes a reduction in performance and overall health of poultry. Recent estimations suggest that the subclinical form of NE cost as much as 5 cents per bird or approximately $450 million/year in the United States (Van der Sluis, 2000). Till date, less work has been done but disease is still prevalent around the world and is a global issue due to huge loss.

MATERIALS AND METHODS

Sample collection

A total of 100 intestinal scrapings with necrotic enteritis were collected by random sampling methods from cases presented for postmortem examination in National Veterinary Polyclinic, Balaju. These birds were scored for Coccidiosis lesion, evaluated for gross NE lesion score using the following scale (Prescott *et al.*, 1978)

Score	Description
0	Normal, no evidence of gross lesions
1	Thin, friable small intestine
2	Focal necrosis and/or ulceration
3	Patchy necrosis
4	Severe extensive necrosis (typically seen in birds which have died from NE)

Differential Characters of important Species of *Eimeria* in fowl:

	E tenella	E acervulina	E necartix
Region	Ceaca	Upper small intestine	Mid small intestine
Intestinal lesion	Haemorrhage, white spot.		Haemorrhage, thicked wall white spots.
Blood in Feaces	++	-	+
Degree of Pathogenicity	++++	++	++++

Source: Urquhart, 1996.

- Negative/not pathogenic + may be present ++ Mild pathogenic ++++ highly Pathogenic

Laboratory Examination:

Microscopic examination of faecal samples by smear method and it was examined under microscope (10 X and 40 X) (Soulsby, 1978).

RESULTS AND DISCUSSION

Attributes of the respondents

Respondent number

A total of 100 samples were examined for the study From Kathmandu and its periphery. The number of samples were highest from Kathmandu (41%), followed by Nuwakot (20%), Lalitpur (17%), Dhading (13) Bhaktapur (6%) and Kavre (3%).

Bird age

Majority of the samples were from the age of 20-30 days (49%), followed by 30-40 days (37%) and only 14% of the sample was that of age of 40 & above.

RESULT

Out of 100 Samples, sixty nine (69%) found to be positive for coccidiosis.

Total coccidial occurrence in necrotic enteritis (NE):

As regard to necrotic enteritis, occurrence of NE was found significantly (at 1% and 5%) higher in coccidial infection than in others.

Distribution among the Coccidial Species

Among 69 positive samples, mixed infection were found to be more higher (29%), followed by *E. tenella* (23%), *E. Necartix* (9%) and *E. acervulina* (8%) which was found in intestine of ceaca, mid small SI and upper SI respectively. The data is presented in Figure 1.

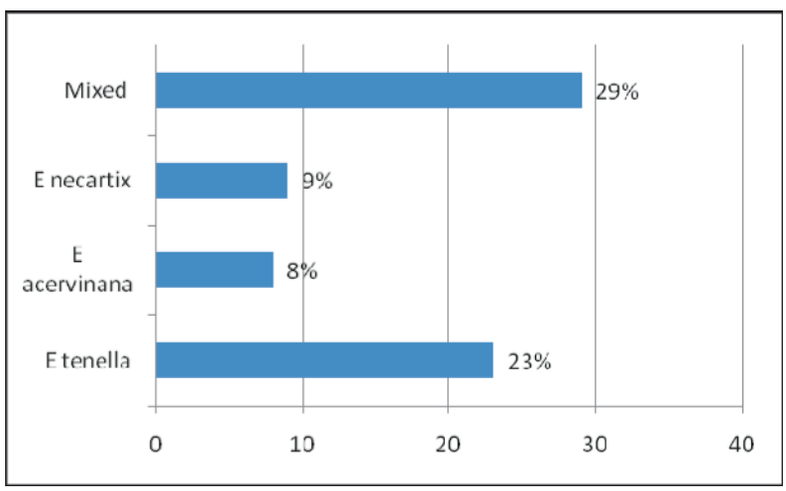

Fig. 1: Distributions among the coccidial species

Agewise distribution of coccidial infection

Out of 49 sample of 20-30 days, 39 sample were positive (56.52%), similarly 37 sample of 30-40 days 26 sample were positive(37.68%),and 14 sample of 40 days and above 4 sample were positive (5.79%) for coccidial infection respectively. The data is presented in Figure 2.

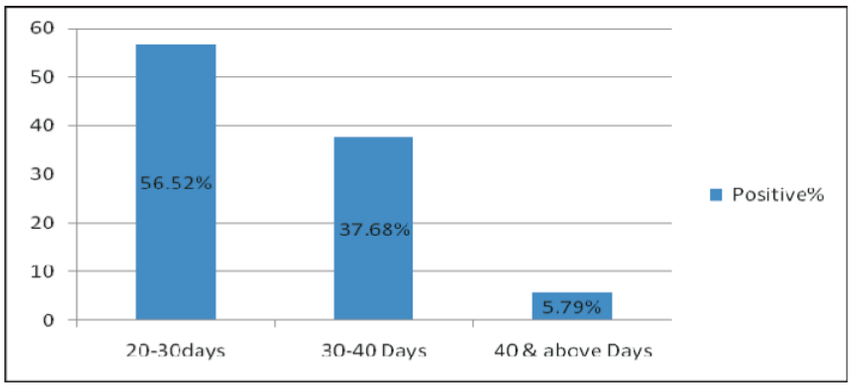

Fig. 2: Agewise distribution of coccidial infection

Lesion score

Out of total of 40 cases, 35 cases, score 3 were found positive likewise 27 cases, score 2 (total of 35), 4 cases, score 1 (total of 10), 3 cases, score 0 (total of 5) and 1 case, score 4 (total of 5) for coccidial infection respectively, which is not similar to Collier *et al.*, (2003) greater in score 2. The data is presented in Figure 3.

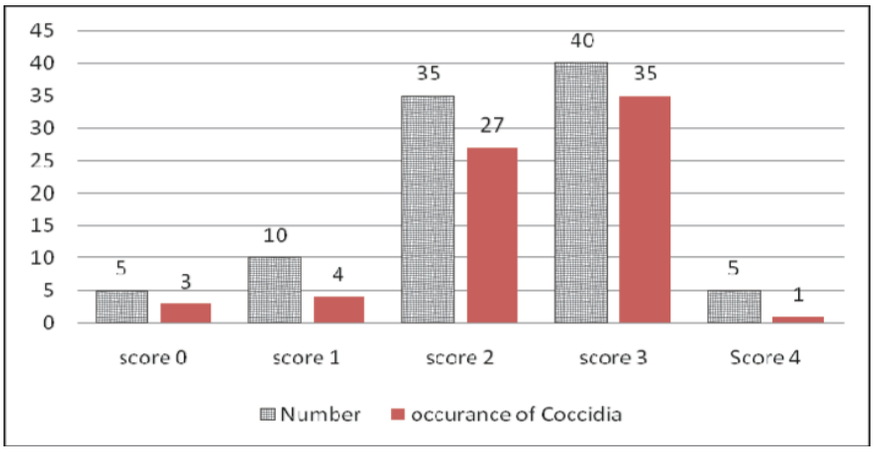

Fig. 3: Lesion Score

Occurrence of NE among the predisposing factor

Total of 100 positive cases of NE, 69% of NE predisposed by coccidia i.e. coccidiosis was primary trigger for the occurrence of NE, there after collibacillosis (19%), others (9%) and IBD (3%) respectively. The data is presented in Figure 4.

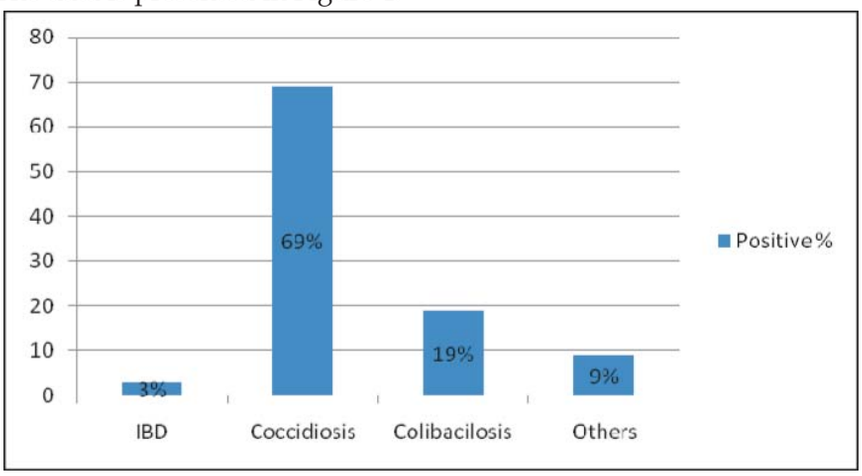

Fig. 4: Occurrence NE among the Predisposing Factor

Distribution of coccidial infection among season & climate

Out of 100 cases of positive NE ,23 out of 30 samples was found to positive for coccidia in rain/ summer, Likewise 46 out of 70 samples was found to positive for coccidia in dry/ winter season which was 76.66% & 65.71% respectively in rain/ summer & dry/ winter season. The data is presented in Figure 8. There is no significant difference of prevalence of the coccidial infection in between the seasons at 1% and 5%.

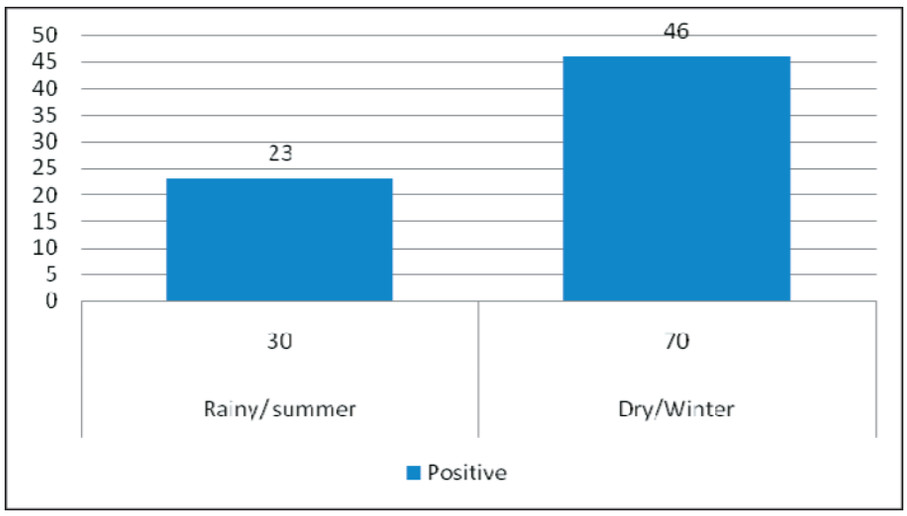

Fig. 5: Distribution of coccidial infection among season and climate

Distribution of coccidial infection in feed & feeding pattern

Total 100 samples 80 farmers fed the mash feed for their birds only 20 farmers used pellet feed. Out of 20 samples 7 cases were positive for coccidiosis and out of 80 samples 62 cases were found to be positive for coccidiosis which was 35% & 77.5% respectively in feeding of pellet and mash feed respectively. The data is presented in Figure 6.

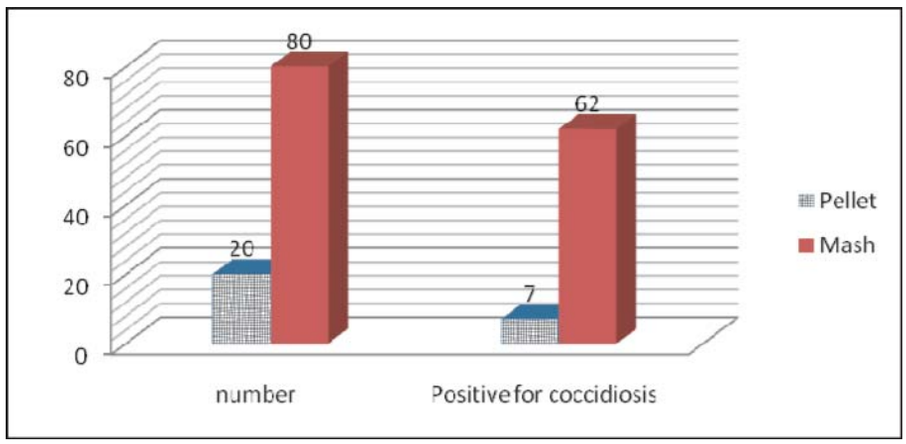

Fig. 6 Distribution of coccidial infection in feed and feeding pattern

Treatment pattern

30 follow up cases, only 18 cases response the treatment, 33.33% response Coccidiostat and Amoxicillin followed by Coccidiostast & Acidifier (27.77%), Coccidiostast & Antibiotic growth promoter (AGP i.e BMD) (22.22) and others (16.66%). The data is presented in Figure 7.

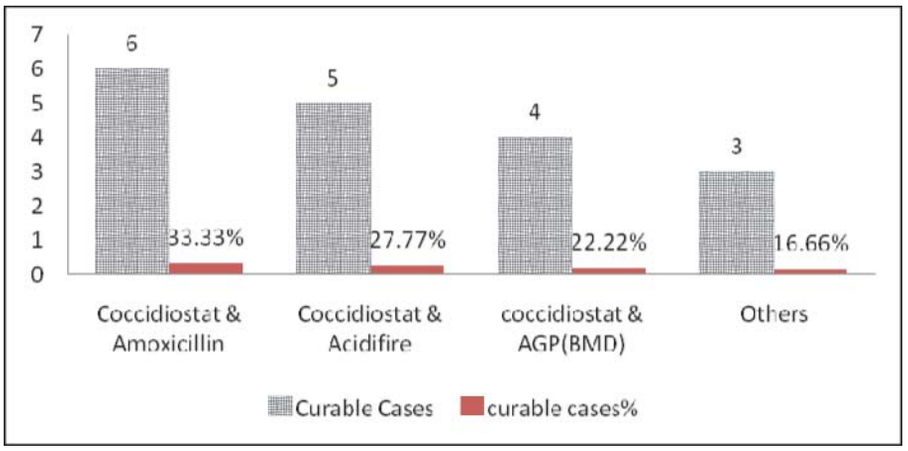

Fig. 7: Treatment pattern

DISCUSSION

Out of 100 Samples, sixty nine (69%) found to be positive for coccidial infection. Among 69 positive samples, mixed infection were found to be higher (29%), followed by *E. tenella* (23%), *E. necartix* (9%) and *E. acervulina* (8%) respectively. But doesn't resemble with (Al-Sheikhly & Al-Saieg (1980) and Ficken & Wages (1997) highest (53%) *E. acervulina* followed by *E. necatrix.*

49 sample of 20-30 days 39 sample were positive (56.52%), similarly 37 sample of 30-40 days 26 sample were positive (37.68%), and 14 sample of 40 days & above 4 sample were positive (5.79%) for coccidial infection respectively which is more or less similar to Islam *et al.* (2008) (52.41%) cases were positive for cocccidia in age of birds ranging from 26 days followed 31 Days (31.76%).

35 cases, score 3 (total of 40) were found positive likewise 27 cases, score 2 (total of 35), 4 cases, score 1 (total of 10), 3 cases, score 0 (total of 5) and 1 case, score 4 (total of 5) for coccidial infection respectively, which is not similar to Collier *et al.*, (2003) greater in score 2. This may be due to the difference that this study only focused in the clinical cases.

Total of 100 positive cases of NE, 69% of NE predisposed by Coccidia i.e. coccidiosis was primary trigger for the occurrence of NE, there after Collibacillosis (19%), Others (9%) and IBD(3%) respectively. Which is more or less similar to Islam *et al.* (2008) (44.44%) cases were positive for cocccidia but not similar to mixed infection (Coccidiosis & IBD) (33.33%) and IBD (22.22%).

Out of 100 samples, 80 farmers fed the mash feed for their birds only 20 farmers used pellet feed. Out of 20 Samples 7 cases were positive for coccidiosis & out of 80 samples 62 cases were found to be positive for coccidiosis which was 35% & 77.5% respectively in feeding of pellet and mash feed respectively.

Out of 30 follow up cases, only 18 cases response the treatment, 33.33% response Coccidiostat & Amoxicillin followed by Coccidiostast & Acidifier (27.77%), Coccidiostast & Antibiotic growth promoter (AGP i.e. BMD) (22.22%) and others (16.66%)which does not resemble the study by Islam *et al.* (2008) where flock's response was better in administration of oxytetracyclin, doxycycline, sulphadiazine-trimethoprim combination, tylosin along with carbon tetracycline respectively.

CONCLUSION

The disease possess serious problem for broiler producers so, the implementation of several approaches, such as improvement of management, feed formulation and limiting exposure to infectious agents through biosecurity, supportive therapy, cleaning and disinfection are essential. In addition, early recognition in managing the enteric disorders may reduce the loss.

Use of Coccidiostat is essential. An important predisposing event for the development of NE is clinical or subclinical is coccidiosis. Risk with any anticoccidial program allows coccidial oocyst cycling. Coccidial cycling causes damage to the intestinal cells, leading to an imbalance in gut bacteria and mucus production. Mucus is a food source for *C. perfringens.*

Use of growth promotants, such as bacitracin, virginiamycin is necessary.

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PREVALENCE OF SALMONELLA IN RETAIL CHICKEN MEAT OF KATHMANDU METROPOLITAN CITY

P. Khanal[1] and S. R. Aryal[2]

ABSTRACT

Contaminated chicken meat has been identified as one of the principal food borne sources of salmonella. A study was conducted from August 2011 to January 2012 to determine the prevalence of salmonella from necks and wings of retail chicken carcass. Fifty retail meat shops were selected randomly and altogether 100 meat samples (50 necks and 50 wings; each neck and wing from same carcass) were collected aseptically from the respective meat shop for microbiological assessment. Isolation of salmonella was done by ISO protocol 6579:2002. Biochemical test and serological test by poly "O" and "H" antisera was done. Ten samples (out of 24 isolates) of neck were positive for poly "O" antisera and 6 samples were positive for "H" antisera. Eight samples (out of 19 isolates) of wing were positive for poly "O" antisera and 4 samples were positive for "H" antisera. Out of the 50 samples tested from neck, salmonella were detected from 10 (20%) and out of 50 samples tested for wing, 8 (16%) samples were detected positive for salmonella. This result shows that there is higher contamination of retail chicken meat. The higher prevalence in neck may be due to contamination of cutting knives and accumulation of drippings of water from the carcass. Hence the utensils, premises of slaughtering should be kept clean and meat should be inspected regularly by concerned authorities.

INTRODUCTION

Meat is an ideal culture medium for the growth of various microorganisms because it is rich in nitrogenous food of various degree of complexity, plentiful supplied with minerals and accessory growth factors, high in moisture (76.78-77.94%), protein (17.49-18.42%), lipid (0.47-0.82%), phospholipids (147.34mg%-206.1mg%), total cholesterol (28.66mg%-34.32mg%) and some fermentable carbohydrate (glycogen). It has favorable pH (5.7-7.2), for most microorganisms and is classified as low-acid food, when classified on the basis of acidity (Frazier and Westhoff, 2003). From human consumption side, meat is a delicious, rich in protein with high nutritional value and consumed by the majority of the people. More people are consuming food of animal origin as compared to vegetable. Due to its taste and good aroma, more people used meat based on their own species affinity and purchasing ability. Besides them Nepalese people, have their hundreds of cultural occasion to slaughter birds and different animal species. Salmonellosis is consistently among the leading source of food-borne disease throughout the world. Salmonella is one of the most common causes of food poisoning is present at varying frequencies on all types of raw chicken meat and its products (Rose *et al.*,2002).

1 Institute of Agriculture and Animal Science, Rampur, Chitwan
2 AHRD, Nepal Agriculture Research Council

MATERIALS AND METHODS

The study was carried out during August 2011 to January 2012. Hundred (50 neck and 50 wing; each wing and neck from same carcass) samples were collected from different retail poultry meat shop of Kathmandu district by simple random sampling method and experiments were carried out in bacteriology and public health laboratory of Animal health research division (AHRD), NARC.

Isolation and Identification

25 gm of meat sample was weighed with forceps, and was put into an Erlenmeyer flask and 225 ml buffered peptone water was added to obtain 1 part sample plus 9 part buffer. It was then mixed and incubated at 37oC overnight. Then 1 ml of the pre enrichment was transferred with sterile pipette to 10ml tetrathionate broth and 0.1 ml of the pre-enrichment was transferred with a sterile pipette to 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth. Tetrathionate broth was incubated at 370C for 18-24 hours and RVS broth was incubated at 420C for 18 -24 hours One loop full from the inoculated and incubated tetrathionate broth and RVS broth was spreaded on XLD and on BGA agar plates and incubated at 37oC overnight (ISO Protocol: 6579: 2002). Pink colonies from BGA and black colonies from XLD were taken and pure culture was performed in Nutrient Agar plates and incubated at 370C for 24 hours. For identification of salmonella spp. Further biochemical test and sugar fermentation were performed.

Morphological and Biochemical test

For the morphological test Grams staining was done. Oxidase test, Citrate utilization test, TSI test, MR-VP test, MIO test, Sugar fermentation test was done for identification.

Serological Confirmation

Two loopfull of normal saline (0.85% Sodium chloride) was placed on a clean glass slide. A small part of a suspected Salmonella colony from an overnight culture plate was taken and mixed thoroughly with both drops of normal saline on the slide to obtain a smooth suspension. One loopful of "O" antisera was added to one bacterial suspension drop on the slide and on the other one loopful of normal saline was added. Antiserum with the bacterial suspension was mixed with the sterile loop. The slide was tilted back and forth for one minute and agglutination was observed under normal lighting conditions .Similarly, test with "H" antisera were performed to detect the flagellar antigen. Agglutinating granules were observed for positive test (PRO-LAB Diagnostics).

Statistical Analysis

All the data available from laboratory findings were subjected to statistical analysis. Descriptive statistics was used to describe the result of prevalence analysis. Prevalence was estimated as the number of samples detected positive to Salmonella isolation from the total sample analyzed.

RESULT AND DISCUSSIONS

A total of 100 (50 neck and 50 wing) fresh chicken meat samples from retail poultry meat of Kathmandu district were processed for isolation of Salmonella. Out of the 50 samples tested from neck, Salmonella were detected from 10 (20%) and out of 50 samples tested for wing 8 (16%) samples were detected positive which is shown in the table 1. The overall prevalence of Salmonella was 18%.

Table 1: Prevalence of Salmonella in chicken meat of Kathmandu district

S.N.	Sites	Total samples	Positive for Salmonella	Prevalence of Salmonella
1.	Neck	50	10	20.0%
2.	Wing	50	8	16.0%

In present study the prevalence rate of salmonella in Kathmandu district was observed 18%. However Joshi *et al.*, (2005) reported 14.5% salmonella in chicken meat. Likewise Maharjan (2006) reported 11.9%, Manandhar *et al.*, (2006) reported 14.5%, Majagaya *et al.*, (2008) reported 11.8%. Likewise Shrestha (2005) reported 13.5% prevalence of Salmonella in chicken meat in a study performed at Central Veterinary Laboratory. The slightly higher percent prevalence of salmonella in chicken meat in present study than the previous studies shows that the salmonella contamination in chicken meat is increasing. This may be due to the technique of hand evisceration which is predominantly practiced in the meat shops under this study.

Acharya *et al.*, (2007) revealed that the prevalence of salmonella was 32.76% which is higher than the present study. Coretz *et al.*, (2006) detected 18% (52/288) salmonella spp. from the samples of feces , feather etc. in Brazil. Nierop *et al.*, (2005) reported 19 positive samples out of 99 fresh and frozen chicken carcasses collected from retail meat shops of South Africa. Cason *et al.*, (1997) found 20% of Salmonella spp. incidence in chicken carcasses in United States. These findings are similar to that of the present study.

Whyte *et al.*, (2002) detected 23% (45/198) salmonella from 198 neck samples by salmonella- specific polymerase chain reaction. The prevalence of Salmonella sp. in chicken breast muscle from various grading of slaughtering facilities such as non-sophisticated, moderate facility, sophisticated and processing plant were 65.71%, 48.57%, 48.57% and 22.86%, respectively and that of thigh muscle were 71.43%, 51.43%, 48.57% and 25.71% respectively. The result of the study revealed that contamination of meat with salmonella decreased with increase in sophistication of slaughter facility and that thigh muscles were highly prone for contamination compared to the breast muscle irrespective of the processing condition (Ruban, 2011). The prevalence of salmonella in this study is lower than that reported by Ruban. The contrast in prevalence of Salmonella may be due to several factors such as differences in origin, time-period and season of the collecting samples. Seasonal variations in the prevalence pattern of salmonella were identified with; a higher prevalence during monsoon months (19.68%) followed by post-monsoon (17.61%) and pre-monsoon (Maripandi *et al.*, 2010). It may be also due to factors like difference in sampling procedures, contamination level of animals, husbandry technologies, slaughterhouse sanitation, level of processing, and method of identification of bacteria and cross contamination of the products.

In this study, the prevalence rate was found just higher in poultry as compared to foreign countries as the prevalence rate was 8.57%, 3%, and1.4% in Turkey, United States, and Denmark respectively (Zhao *et al.*, 2001; Skov *et al.*, 2007). This may be due to sanitation in slaughter house and different methodology for confirmation of salmonella i.e. PCR.

CONCLUSION

It can be concluded that there is higher contamination of retail chicken meat. So there is greater chance of human salmonellosis and other bacterial infection through meat. To minimize the public health risk factors, the slaughtering places must be provided with clean and regular water supply, washable concrete base for slaughtering and proper effluent disposal. Training course should be developed and provided for butchers and meat sellers for proper slaughtering and better meat marketing techniques. Meat in retail shop should be protected from insects, flies, dust and dirt. Premises should be clean and carcass kept for sale must be hanged and properly covered. Regular monitoring of slaughterhouse and effective meat inspection act should be introduced and implemented.

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ZOONOSES, FOOD SAFETY, BIOTECHNOLOGY AND MISCELLANEOUS

SCREENING OF BOVINE TUBERCULOSIS IN DOTS IMPLEMENTED AREA OF KAVREPALANCHOWK DISTRICT OF NEPAL

T.N.Gaire[1] and D.D. Joshi[2]

ABSTRACT

Tuberculosis is chronic insidious contagious disease of man and animals caused by certain pathogenic organism of the genus Mycobacterium. Bovine Tuberculosis results from infection by Mycobacterium bovis Gram positive, an acid-fast bacterium belonging to Mycobacterium Tuberculosis Complex (M. tuberculosis, M. bovis, M. africanum, M. canetti and M. microti).Mycobacterium bovis is significant zoonoses that can spreads through aerosols and by ingestion of raw milk. Pathogenecity of Mycobacterium is attributed to its ability to escape killing by macrophages and induced delayed type of hypersensitivity. Research was conducted to find out the cases of bovine tuberculosis in 50 animals reared by 25 tuberculosis (TB) infected persons that are getting the Directly Observation Treatment Shorts coarse (DOTS) at Kavre District during the period of August to December 2009, for this DOTS centre was visited to find out the Tuberculosis infected person having the animals and immunological test was adopted in their animals for the test of bovine tuberculosis. The intradermal cervical tuberculin test was followed to identify the bovine tuberculosis by injecting 0.1 ml of tuberculin antigen from Canadian Food Agency and measuring the increase in thickness after 48 and 72 hours. Results were taken to be negative if the change in thickness was less than 2.0 mm. The thickness remain the same in 45 animals (90%) and 5 animals (10%) were positive for tuberculin having the change in skin thickness. Similarly among the 25 tuberculosis infected household, 13(52%) had an extra pulmonary type of TB and 12(48%) had pulmonary type of TB. Animals were also higher infected (60%) in household where their owner had extra pulmonary type of TB. Overall prevalence of TB in human where their animal was infected with BTB in study area was 20%. Likewise total 25 TB infected household, 40% had the habit of drinking raw milk, similarly relationship between raw milk ingestion by TB infected person and number of infected animal was markedly higher (50%). Out of 25 TB infected household maximum (44%) were old person and higher animals were (14.28%) associated with infections in same households. The farmers' knowledge on zoonotic aspect of bovine tuberculosis was generally very low (7 %).

INTRODUCTION

Bovine TB (BTB) is important source of human infection (Radostits *et al.*, 2000; Pollock *et al.*, 2005). Developed countries have reduced BTB among the farm animals considerably. However, this disease is important as a major cause of disability and death in many parts of the world. The ease and frequency of the spread of BTB from animals to humans in an uncontrolled environment makes it one of the important zoonoses. Human infection is primarily through digestive tract and secondly by inhalation (Acha and Szyfres, 1980) especially in children (Radostits *et al.*, 2000). BTB is prevalent among cattle and buffaloes in Nepal (Joshi *et al.*, 1999; NZFHRC, 2002; Pun *et al.*, 2004).

The Office International des Epizootics (OIE) classifies BTB as a list B disease, a disease which

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poses great public health significance. It is of significance to the international trade of animals and animals products. As Nepal has already entered into World Trade Organization (WTO), attention must be paid to the meat and milk quality. This could only be possible when diseases like TB are screened and treated or culled accordingly. Therefore the meat and milk should meet the standards established internationally as per the sanitary and phytosanitary (SPS) agreement. The quality management systems like International Organization for Standardization (ISO) and Hazard Analysis and Critical Control Point (HACCP) are still not followed in Nepal.

MATERIALS AND METHODS

Study Area

The site selected for this study was Kavre district of Nepal. Kavre district is located in eastern part of Central Development Region. It is one of the hilly district, 85% of the district is covered by hills out of total 140,486 hector area with altitude ranging from 350-3018 meter from sea level. The district has approximate population of 3, 83,056 with 78.35% depending on agriculture for their livelihood similarly buffalo and cattle population in Kavre district is 1, 29,624 and 1, 29,614. The total milk and meat production are 48,129(MT) and 4,543(MT) respectively (MOAC, 2006).

Sampling of Animals

The secondary data of tuberculosis infected persons rearing the cattle and buffalo was obtained from major DOTS centre and District Public Health Office of Kavre district. Total 50 animals (Cattle and buffaloes of both sexes > 6 months of age) were selected randomly; on the basis of pocket area of the district on proportional basis. Diseased, young calves below 6 months age, animals in advanced pregnancy and those within 6 weeks post partum was be excluded so as to avoid false negative reaction. Possible sampling bias was introduced when the owner himself decided which animals fulfilling the inclusion criteria were tested.

Single intradermal tuberculin test (SITT)

About 12–15 cm apart, on the cervical area of the skin, was clipped, washed with soap and disinfected with 70% ethanol. The initial skin thickness was measured by Vernier Callipers followed by an intradermal injection of 0.1 ml of bovine purified protein derivatives (PPD) with a tuberculin syringe and needle. The site of injection was marked with permanent marker to ease the location of injection in subsequent readings. Subsequent readings of the skin thickness were recorded in 48 and 72 hours post injection as suggested by Radostits *et al.* (2000). The data was compiled and the visible, palpable or measurable change in skin thickness was noted. The animals reacting to the tuberculin with the visible oedematous change as described by Radostits *et al.* (2000) or enlargement in skin thickness by 4 mm or more in 72 hours were declared positive reactors. Mild reactors was indicated by skin thickness increment of between 2 and 4 mm similarly increases in skin thickness less than 2 mm were declared as negative reactor.

RESULTS

A tuberculin screening was conducted among 45 cattle and 5 buffaloes from the farmers getting the DOTS for identifying bovine tuberculosis (BTB), which may have transmitted from animal to humans and vice versa. Four cattle (8.89) and one buffalo (20%) were found positive to tuberculin test. Prevalence between sexes was greatly different in both cattle and buffaloes in which female were more infected. This might be due to large no. of female animals in the study area. Among the animal factors under study, age over 5 years was found to be infected. Similarly, poor nutritional status and management factors such as inadequate ventilation, moisture in floor, common pasture, larger herd size, small floor space and common feeding trough were largely the cause for the infection of tuberculosis in cattle. The study is a screening test of TB in the animals reared by DOTS

taking farmer with a limited sample size from a small geographical area of Nepal. However, it is expected that the results obtained is sufficient and useful for the prediction of prevalence of BTB, which might have transmitted from humans to animals and vice versa.

Table 1: Tuberculin reaction among cattle and buffaloes

Species	Sex	Reaction				Percentage positive
		Positive (4 mm)	Mild (2-3.9 mm)	Negative (<2mm)	Total	
Cattle	Female	2	2	38	42	8.89%
	Male	-	-	3	3	
	Total	2	2	41	45	
Buffaloes	Female	1	-	4	5	20%
	Male	-	-	-		
	Total	1	-	4	5	
Grand total		3	2	45	50	10%

Owners' Knowledge on zoonotic bovine tuberculosis

At the inception of the study, diverse responses on this question were expected. However, none of the farmers who are taking DOTS appeared to be conscious about zoonotic aspects of this disease. The farmers' knowledge of the clinical signs of TB, either in humans or in cattle, was generally very low especially zoonotic aspect (2 %) that the disease could be transmitted from cattle/buffaloes to humans.

Table 2: Age wise distribution of TB and their relationship with ingestion of raw milk

	TB patient(farmer)			Total
	Child	Adult	Old	
Number / Households	5	9	11	25
Habit of raw milk ingestion	3 (60%)	2 (22.22%)	5 (45.45%)	10 (40%)
Animal reared	15	14	21	50
Animal affected(BTB)	1 (6.67%)	1 (7.14%)	3 (14.28%)	5 (10%)

DISCUSSION

It is apparent from the findings of this study that the owners or the farmers do not have knowledge about BTB and its zoonotic importance. These findings may be projected as the situation of the entire country, where neither the formal education nor any other informal media has ever attempted to educate the people about BTB and its zoonotic prevalence.

The higher prevalence in some of the reports may be due to better sensitivity of exotic tuberculin than the relatively indigenous tuberculin used in this study. This may also be due to the change in population with the change in time and various herd factors (Collins, 2003). Transmission of BTB among animals was favourable by (very close contact throughout the year in poor ventilated houses, communal grazing / plugging / threshing / watering, overcrowded pastures). Animals that were not regularly de-wormed were nearly twice at risk for being a reactor. However, this result suggests that high parasitic loads may decrease the animal resistance and make it more susceptible to BTB.

Prevalence among buffaloes in this study was 20 percent. An earlier report in buffaloes by Joshi *et al.* (1974) with 5.13 percent prevalence is much less than our study. It may be due to the fewer no. of sample size for buffalo and the animals being reared by the infected persons.

Status of Zoonoses BTB in study area.

The zoonotic aspect of BTB in our study; people that are consumed the raw milk was mostly affected with TB which is also suggested by (Pande *et al.*, 1995). Similarly out of 36 milk sample from Kaski and Kathmandu in tuberculin positive animals 13 were identified as M. bovis (Jha *et al.*, 2007).A large amount of insufficiently pasteurized milk and dairy product are consumed daily in Nepal, and it has been reported that dairy product are contaminated by various pathogens by pathogens carrying animal thus, it is possible that M. bovis infection may be transmitted to such people in study area and also other part of Nepalese people via dairy product. There is no research done about prevalence of M. bovis in human TB, and this is first study in Nepal to find out the prevalence of BTB in tuberculosis infected farmer.

Similarly higher prevalence of TB in cattle and buffalo in our study was due the animal owned by tuberculous patients which is also reported by (Regassa ,2005).During our study 12 person infected from pulmonary type of TB and 13 person were extra pulmonary type of TB where there animal was infected from BTB, which suggested that those occupational groups working with M. bovis infected cattle or buffalo, on the farm or in the slaughterhouse, are more likely to develop pulmonary disease than alimentary disease (O'Reilly and Daborn, 1995).

It appears that the epidemiology of BTB depending on livestock systems (extensive, intensive), breeds (local, exotic, cross-breed) and also ecological and geographic factors. Further research is needed to better understand BTB transmission in humans from animals by conformation of M.bovis via cultural and biochemistry test as well as PCR based to address the potential of control options.

CONCLUSION

The evidence of BTB in tuberculosis infected person from this study clearly demonstrates that this chronic infection is a lurking threat for economic as well as public health in Nepal and no control or preventive measures have thus been taken for tuberculin positive animal population in the absence of appropriate policy for disease control. The increase of TB in such areas calls for stronger inter sectoral collaboration between the medical and veterinary professions to assess and evaluate the scale of the problem, mostly when zoonotic TB could represent a significant risk, for example, in rural communities and in the workplace. Formulation of legislative measures and government policies such as implementation of meat act 2055 for the control of the diseases mostly focusing on the diseases of public health interest like control of the disease among herds or BCG vaccination.

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STUDY ON STATUS OF PORCINE TRICHINELLOSIS IN KATHMANDU VALLEY DIAGNOSED BY PEPSIN DIGESTION METHOD

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ABSTRACT

A study on porcine trichinellosis was conducted in three districts of Kathmandu valley from August 2011 to October 2011. Ten slaughter slabs were randomly selected (3 from Kathmandu, 4 from Lalitpur and 3 from Bhaktapur) and altogether 210 meat samples were collected for parasitological assessment. For Trichinella detection magnetic stirrer method with sedimentation (protocol for pepsin digestion technique) was practiced. The sediment was observed under microscope for the presence of larva. All the samples showed negative result for Trichinella. From this study it can be concluded that there is a zero prevalence of porcine trichinellosis in Kathmandu valley. However, its validity and accuracy could be challenged through increased sample sizes. Furthermore, continuous surveillance and monitoring have to be implemented before concluding that the Kathmandu valley is free from infection.

INTRODUCTION

Trichinella spp. is one of the most important parasites that plays crucial role in the public health concerns. This parasite is one of the most widespread parasites and can infect more than one hundred and fifty mammalian species including human. So that it brings the concentration of all the veterinary as well as medical researchers to start pivotal step for the prevention and control of trichinellosis. Trichinellosis is a parasitic disease with a world-wide distribution. It remains a serious public threat in both developed and developing countries (Murrel and Pozio, 2000; Liu and Boireau, 2002).

It has been observed in general that most of the pig farmers (73%) reared pigs in the scavenging system in Nepal. Most of the families of the pig rearing communities do not have toilets. They use open field for defecating, which contaminates soil and nearby water streams. This contamination contributes greatly to the parasitic infestation of both pigs and humans. Most of the pigs are kept inside the house at night and are fed on kitchen wastes and excreta. Only 27% farmers reared pigs under intensive system but these sties were also found to be very unsanitary. As for the consumption of pork, 68.19% of the respondents consumed cooked pork, 4.34% of them consumed boiled pork while 8.34% of the people consumed raw pork. No modern slaughterhouse has been constructed and no meat inspection is practiced so far in Kathmandu valley. These are the major factors associated with the zoonotic risk of trichinellosis.

Trichinellosis has been recognized over the past decades in many parts of the world in new hosts and with new epidemiological contexts (Pozio, 2000a; Pozio *et al.*, 2002). *Trichinella spp.* are most commonly transmitted to human through the consumption of raw or undercooked meat of infected

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animals, most commonly domestic pigs. The practice of low heat barbecuing is popular among Nepalese. The certain ethnic groups in eastern region are mainly consuming raw pork in their regular dishes (Joshi *et al.*, 2005). The Scavenging system of pig raising is popular in Nepalese farming .Sporadic suspicions of trichinellosis in humans have been reported by different medical hospitals in Nepal but not yet confirmed. There is no proper lab facility and manpower for the diagnosis of trichinellosis in human hospital.

MATERIALS AND METHODS

Site of study

The research was focused on pig slaughter slabs of different places of Kathmandu Valley and experiment was carried out at parasitology laboratory of Animal Health Research Division, NARC.

Duration of the study

August, 2011 to January, 2012.

Animal species

Domesticated pigs that are of different breeds were taken as sampling unit. In Lalitpur, samples were of Pakhribas black while in Bhaktapur, samples were from Yorkshire and Landrace. But in Kathmandu, samples were of all above mentioned breeds.

Sample size

Ten slaughter slabs were randomly selected (3 from Kathmandu, 4 from Lalitpur and 3 from Bhaktapur). Total of two hundred ten meat samples (70 from Kathmandu, 90 from Lalitpur and remaining 50 from Bhaktapur), used to sell for direct consumption in pig meat slaughter slabs, and were collected for laboratory examination.

Collection and transportation of Samples

The samples were collected directly from the pig meat slaughter slabs of study area irrespective of sex and age. Meat samples were of diaphragmatic pillar. After collection of meat samples, these were stored in defreeze at -200C. From each pig, at least 50 gram of meat was taken.

METHOD

Magnetic stirrer method with sedimentation (protocol for pepsin digestion technique according to the EU-regulation (EG) no. 2075/2005) was practiced which also the most common method world-wide.

Stepwise procedure

Ten samples of 10 g meat were blended in a chopping board minced in a mixture. The minced meat was placed in a 3 litre beaker with a large magnet and 10 g pepsin (1:10,000) was added. Then 2 litre of water (46-480C) with 16 ml 25% HCl was poured into the beaker. The beaker was placed on a heated magnetic stirrer (44-460C). After that, the solution was stirred at 200-300 rotations per minute for 30 minute. The digested fluid was poured into a separation funnel (Baermann apparatus) via 170-200 micron sieve. The fluid was left for sedimentation for 30 min. The 40 ml was left for 10 min to sediment. 30 ml of the supernatant was sucked off. Sediment was tapped into a measuring glass. The remaining 10 ml was examined for the presence of larva with a maximum of 30 min. If a sample was left to sediment for more than 30 min, the final sediment should be re-suspended and sedimented twice to make the final sample clearer.

The following mentioned formula was used based on win Episcope 2.0 to estimate the maximum number of possible positive animals in the Kathmandu valley of Nepal.

$$D = [1- (1 – CL) 1/n] * [N – (n-1)/2]$$

Where, D = Maximum number of *Trichinella* positive animals

CL = Confidence limit as a fraction

n = Samples size that showed negative

N = Total number of pigs in Kathmandu valley

RESULTS

Trichinella investigation through pepsin digestion

The meat samples from a total of 210 pigs were analyzed through the pepsin digestion technique. Trichinella larvae were not found in any of the analyzed sample.

Possible maximum prevalence of Trichinella

It was found that from the all tested meat samples of pig that there was no positive result for Trichinella by the Pepsin digestion method. The possible prevalence of Trichenella positive pigs in Kathmandu valley was found as follows.

$$D = [1-(1 – 0.95) 1/210] * [19491 – (210-1)/2]$$

$$= 275 \text{ Pigs}$$

Possible maximum prevalence = (275 / 19491)* 100 = 1.41% at 95% confidence interval and with sampling fraction of 1.07%.

DISCUSSION

According to Ribicich *et al.*(2009), pigs raised outdoors were more likely to be infected than pigs raised in total or partial confinement ($p< or =0.05$) and pigs fed waste products containing meat were 12.5 times more likely to be infected than pigs not fed waste containing meat ($p<0.01$). In Nepal, many pigs are raised under outdoor management of farming and scavenging system. So, there may be the chance of *Trichinella* infection in swine population. Most of the pigs (53%) are rearing without de-worming. These entire factors are responsible for the incidence of trichinellosis. The questionnaire survey in Central development region during 2006/07 shows that no rodent control (70%), left over feeding practice (65%), garbage dumping and direct outlet of farm waste in the vicinity (82.5%) and the uncooked meat being used as feed (100%) practice existed in farms, which were predetermined risk factors for the *Trichinella* infection (Karna, 2007).

Joshi *et al.*, 2005 has carried out parasitological examination on 425 muscle samples by HCl-pepsin digestion method first time in local pigs of Nepal and found no single Trichinella larva. Again he did same examination on 298 muscles, taken from 8 district of Nepal but same result occurred.

Lea Martinetti observed 40 meat samples from Teku during June-July, 2010 and found zero prevalence from pepsin digestion.

Karna (2007) observed 551 meat samples to investigate *Trichinella* larvae in five major pig producing districts of the central development region of Nepal from November 2006 to end of April 2007 and the Pepsin digestion did not detect *Trichinella* larvae in meat sample.

Similarly, in this research, not a single larva from the 210 samples of Kathmandu valley was detected by pepsin digestion method. Pepsin digestion method is the confirmative diagnostic tool for the diagnosis of the disease.

CONCLUSIONS

This study suggests that Trichinella in this region has zero prevalence from pepsin digestion technique. However, its validity and accuracy could be challenged through increased sample sizes. Furthermore, continuous surveillance and monitoring have to be implemented before concluding that the Kathmandu valley is free from infection. Scavenging system of pig raising should be eliminated in order to minimize the risk factor for parasitic zoonosis. Consumer education regarding public health awareness is found to be essential for the prevention of disease because we cannot able to control such disease due to high cost involvement.

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STUDY ON THE LEVEL OF AFLATOXIN M1 CONTAMINATION IN RAW AND PROCESSED MILK MARKETED IN KATHMANDU VALLEY

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ABSTRACT

Aflatoxin M1 (AFM1) is the principal hydroxylated AFB1 metabolite mainly present in milk, which is getting a worldwide concern due to its serious public health impact. In this study the levels of Aflatoxin M1 (AFM1) in Raw and Pasteurized milk marketed in Kathmandu valley was determined. A total of 32 milk samples (Raw 16, Pasteurized 16) obtained from different areas of Kathmandu valley were analysed for the occurrence and concentration range of AFM1 by Thin Layer Chromatography. The milk samples were analyzed according to the official AOAC methods, which included extraction of toxin using chloroform, clearing by silica gel column chromatography, qualitative analysis by thin layer chromatography and quantification by visual comparison of the spots. AFM1 was found in 14 (43.75%) of milk samples examined. The levels of AFM1 in 7 (21.87%) samples were higher than the maximum tolerance limit (0.05 µg/l) accepted by some European countries while none of the samples exceeded the prescribed limit of US regulations. The mean concentration of AFM1 was higher in Raw milk (0.030 ± 0.042 µg/l) compared to pasteurized (0.022 ± 0.039 but the difference was not statistically significant (P>0.05). This finding reflects that milk marketed in Kathmandu valley contains Aflatoxin M1 residue and risks on public health exist from consumption of milk with Aflatoxin M1 contamination. Thus, milk and milk products should be screened and controlled periodically for AFM1 contamination. Also, dairy cow feeds should be stored in such a way that they do not become contaminated with mycotoxins.

INTRODUCTION

Aflatoxins are a group of closely related heterocyclic compounds produced predominantly by two filamentous fungi, *Aspergillus flavus* and *Aspergillus parasiticus*. Recent studies have shown that some *A. nominus* and *A. tamarii* strains also produce aflatoxin, of which *A. nominus* is phenotypically similar to *A. flavus* (Kurtzman et al, 1987; Goto *et al.*, 1997). Presently, 18 different types of aflatoxins have been identified, with aflatoxins AFB1, AFB2, AFG1, AFG2, AFM1, and AFM2 being the most common (Beuchat, 1978). Aflatoxins M_1 and M_2 are the hydroxylated metabolites of aflatoxins B_1 and B_2 and can be found in milk or milk products obtained from livestock that have ingested aflatoxin contaminated feed.

Aflatoxin M2 is rarer than M1 and not as toxic so it receives little interest. Aflatoxin M1 has also been isolated on highly contaminated corn samples where it occurs 1000 times lower concentration than aflatoxin B1 (Shotwell *et al.*, 1976. As with all aflatoxin it is a highly oxygenated heterocyclic compound. Aflatoxin M1 is chemically stable; it is not destroyed under domestic conditions such as microwave or oven heating however the stability of aflatoxin M1 during pasteurization is in

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debate. Bakirci, (2001) and Henry *et al.*,(1997) reported that pasteurization has no effect whereas Deveci and Sezgin (2006) suggested that pasteurization causes a 16% decrease, hypothesising that the decrease is due to heat treatment causing casein decomposition.

The WHO International Agency for Research on Cancer (IARC) has classified both aflatoxin B1 and aflatoxin M1 as carcinogenic agents to humans (IARC, 2002). Aflatoxin M1 manifests its toxic effects by linking its adverse effects with the nucleic acid in toxic ways leading to hepatotoxicity and carcinogenicity (Wong *et al.*, 2000).

Aflatoxicosis is the name given to the disease caused by the harmful effects of aflatoxin. There are two courses of the disease: acute and chronic. Acute aflatoxicosis results in deaths from hepatic necrosis and liver failure. Chronic aflatoxicosis in humans and animals are related with cancer, immune suppression, heptocellular carcinoma, Reyes syndrome, cirrhosis and kwashiorkor (Stora *et al.*, 1983; Bennett and Klich, 2003).

EU countries have the lowest allowable concentrations of AFM1 in milk, which is 0.05 µg/l (Commission Regulation (EC) N. 466/2001), while other countries have legislation for this mycotoxins ten times higher, which made allowable concentrations of 0.5 µg/l.

Behind the veil of opaque whiteness, every quart of milk may hide a potential peril to the public health. To the unaided scenes, unwholesome or dangerous milk may present exactly the same appearance as the purest and safest supply obtained. Today all over the globe the health conscious consumers are looking towards the products not only clean and pure but for the possible contamination by the residues which impart possible health hazards in long run. For this reason, many countries have regulations to control the levels of aflatoxin B1 in feeds and to purpose maximum permissible levels of AFM1 in milk to reduce this risk. As milk is the main nutrient for infants and children and who are considered to be more susceptible to adverse effects of mycotoxins, the presence of aflatoxin M1 in milk is a concern.

MATERIALS AND METHODS

Samples

Thirty two samples of raw and pasteurized milk were bought from different dairy collection centres and supermarkets of different areas around Kathmandu valley. Samples were collected and analysed during August to November 2011. All samples were analyzed before their expiry date. Sixteen raw milk samples were purchased from different local small dairy collection centers from various regions of Kathmandu valley. The collection centres collects milk daily directly from the farmers and sells to the consumers without any processing.

Sixteen samples of commercial pasteurized milk were purchased from supermarkets and local shops from the study area. Samples were from different commonly consumed brands. Packet milk from respective milk industries were analyzed before their expiry date.

Methods

All the milk samples were analyzed by Thin Layer Chromatography (TLC) technique for the presence of aflatoxin M1 according to the official methods given by Association of Analytical chemists (AOAC, 2000) with some modifications.

The basic procedure involved- Extraction of aflatoxin from milk samples, using chloroform; clearing or cleaning up bycolumn chromatography, silica gel. Qualitative estimation of Aflatoxin is by thin layer chromatography. Quantitative estimation of aflatoxin M1 is in uv cabinet by visual comparison technique. Confirmatory test is by H2SO4 Spray test.

All 32 samples were taken in the period from September to December 2011. Method which was used to determine Aflatoxin M1 combines cleanup process with silica gel columns and TLC

determination (AOAC, 17th Edition 2000).

Extraction and cleanup

50 ml milk, 10 ml of saturated salt solution (40 gm NaCl / 100 ml water), and 120 ml chloroform at 300C in a 250 ml separating funnel was shaken and allowed to separate for 2 minutes. lower CHCl3 layer was Drained into 125 ml Erlenmeyer flask .Centrifuge if layers do not separate.(15 minutes at 2000 rpm).10 gm anhydrous Sodium Sulphate was added to CHCl3 with stirring. The final filtrate was collected in a graduated cylinder, final volume of which was recorded and saved for column chromatography.The column was half filled with CHCl3. 2 gm silica was made gel slurry with CHCl3 and put into the column followed by adding 2 gm Sod sulphate above silica gel. Sample extract was now added and entire solution was drained through column by gravity. This was followed by washing column with 25 ml toluene – acetic acid (9 + 1) to remove coloured compounds and with 25 ml of hexane – ether – acetonitrile (5 + 3 + 2) to remove fat. Elution of Aflatoxin M1 was done with 40 ml CHCl3 – acetone (4 + 1). The final volume was evaporated to dryness and the purified extract was stored in freeze or used immediately for further testing.

Thin Layer chromatography

The sample residue was dissolved in 100 µl of benzene – acetonitrile (9 + 1), mixed well in vortex mixture. At the same time the Pre-coated TLC plate (TLC silica Gel 60, Merks, Dimensions 20 x 20 cm2) were activated in hot air oven (110oC) for 1 hr. 40 µl of sample solution was spotted in one side and 4, 8, 12, 16 and 20 µl M1 standard (0.25 µl / ml) in the same line to the other side of the plate. The plate was developed in developing chamber containing chloroform - acetone – isopropanol (87 + 10 + 3). The solvent system was let to rise for about 12 cm in the plate. After drying for some time the plate was viewed in uv cabinet (366 nm λ), Checked for the spots of the sample in same rf value as that of standard. Comparison was done between the intensity of spots of the standard spot to that of sample, visualized and noted the matching spot and the volume of standard spotted which matched to that of sample. The collected information was placed to the working formula and the level of aflatoxin M1 was calculates according to the formula.

Calculation

Aflatoxin M1 in µg/kg or µg/l is given by the formula

$$\frac{Vst \times Cst \times Vet}{Vm \times M \times Vf/120}$$

Where, Vst is the Volume in µl of the AFM1 standard used which matches the nearest spot intensity to the florescence intensity of the sample. Cst is the Mass concentration in µ/ml of the AFM1 standard. Vext denotes the volume in µl in which sample extract was dissolved used in the test. Vm represents volume in µl of the sample of the sample extract used for the test. M is the volume of milk in ml used for the test. Vf is the volume in ml of the filtrate obtained in extraction steps. 120 comes from the volume of chloroform, in ml, used for extraction.

Confirmatory test (Blaney *et al.* 1985)

The developed TLC plate was sprayed with 25% Sulphuric Acid by the help of sprayer. The colour of the spot fluorescence given by the toxin, changed from bluish to yellowish blue which confirms the presence of Aflatoxin M1 in the spot.

Statistical analysis

Data were analyzed by SPSS software (Version 16.0.0, Macrovision Corporation, USA). Overall prevalence was calculated using MS-Excel. Results were expressed as mean ± standard deviation (SD) and also as minimum and maximum concentration of AFM1. Differences in AFM1

concentration between different types of milk were examined using one-way analysis of variance (ANOVA). Fisher Exact's test was applied to compare the means among different categories of level of AFM1 between raw and pasteurized milk samples. The differences between values were considered significant at $P \leq 0.05$.

RESULT AND DISCUSSION

Table 1: Number and percent of negative and positive samples for each kind of milk.

Types of milk	No.Of samples	No of Positive samples	No of Negative samples
Raw Milk	16	8 (50%)	8 (50%)
Pasteurized milk	16	6 (37.5%)	10 (62.5%)
Total	32	14(43.75%)	18 (56.25)

Table 1 summarizes the number of samples analyzed and the number of samples found to contain detectable levels of AFM1 contamination in Kathmandu Valley. From a total 32 samples, 14 (43.75%) contained AFM1. The number of positive samples for raw and pasteurized milk was 8 (50%) and 6 (35.75%) respectively. Above table shows more positive samples for raw milk than that of pasteurized milk.

Being only the first study of AFM1 in milk marketed in Nepal, there are no any previous works to compare the contamination level of this study however lot of studies have been carried out in the Asian countries which can be works to compare with.

The contamination percentage form the present study is lower than various studies by different researchers in turkey, 64.9%, 84%, and 72.5% respectively. (Aseem *et al.*, 2011, Davoudi *et al.*, 2011, Fallah *et al.* 2010).

Similar studies in other Asian countries like India, Indonesia, South Korea yielded comparatively higher percentage of contamination, 57.5% by Nuryono *et al.*, (2009) in Indonesia, 96.3% by Lee *et al.*, 2009 in South Korea, 72% by Choudhary *et al.*, 2007 in India, 87.3% by Shipra *et al.*, 2004 in India.

The results revealed by this study is on the lower side than the numerous results of numerous studies abroad but it is hard to conclude the presence lower risk of AFM1 exposure in our country. This is the first study of its kind and lots more is to be revealed in the future. The comparatively smaller contamination percentage might have resulted due to the fewer sample size and less sensitive analytical method(TLC) compared to the HPLC, ELISA etc which is considered more sensitive analytical method.

Table 2: Level of Aflatoxin M1 in Raw and Pasteurized milk

Type of milk	Range of AFM1	Mean ±SD	P-value
Raw Milk	0.026-0.138	0.030±0.042	
Pasteurized milk	0.025-0.127	0.022± 0.039	0.594
Total	0.025-0.138	0.026± 0.040	

Table 2 summarises the level of Aflatoxin M1 in raw and pasteurized milk samples. In total the level of AFM1 was found in concentrations ranging from 0.025 to 0.138 µg/ltr. (Mean=0.026 ± 0.040 µg/ltr.). The mean value of raw milk is 0.030 ± 0.042 which is larger than the mean value of pasteurized milk. However, this difference was found to be insignificant by the one way analysis of variance ($P > 0.05$).

Table 3: Different level of AFM1 contamination in raw and pasteurized milk samples

Type of milk	Frequency distribution of samples in µg/ltr (%)		
	< 0.025 µg/ltr	0.025 - 0.05 µg/ltr	>0.05 µg/ltr
Raw Milk	8/16 (50%)	4/16 (25%)	4/16 (25%)
Pasteurized Milk	10/16 (62.5%)	3/16 (18.75)	3/16 (18.75%)
Total	18/32 (56.25%)	7/32 (21.87%)	7/32 (21.87%)
P- value	> 0.05	> 0.05	> 0.05

From table 3 it can be inferred that all positive samples were within the tolerance limit (0.5 µg/ltr) determined by USA regulations. However, 7 samples (21.87% of the positive samples) contained concentrations above 0.05µg/ltr which is the tolerance limit adopted by the European Community and Codex Alimentarius Commission for liquid milk and processed milk products (Codex Alimentarius Commission 2001; Creppy 2002).

The lowest concentration detected by the employed method of analysis was 0.025 µg/ltr. A total of 18 samples were detected negative. However, the possibility of these samples containing AFM1 can't be ruled out as the negative samples don't necessarily mean the concentration level 0 µg/ltr. These samples may contain AFM1 which was not detected by the test. 43.75 % of the tested samples contained AFM1 in the detectable level. 7 (21.87%) of the samples contained the level of AFM1 between the range (0.025-0.05 µg/ltr) which can be considered safe. Similarly 7(21.87%) of the tested samples exceeded the tolerance limit by EU (>0.05 µg/ltr). Among the exceeded samples the number of raw samples was high 4 (25%) compared to pasteurized 3(18.75%). However, these findings are proved insignificant statistically by the fisher exact's test as P>0.05. The variation in the concentration of AFM1 between raw and pasteurized sample was proved insignificant by the statistical analysis, which supports the findings given by Bakirci, (2001) and Henry *et al.*,(1997) indicating there is no effect of pasteurization in stability of Aflatoxin M1.Keeping the results in mind it should be noted that pasteurization by no way renders milk completely safe. The threat of mycotoxins contamination which is a concern of serious public health is still prevalent although one may feel the milk is completely safe for consumption.

CONCLUSION

Aflatoxin M1 concentration of milk and milk products is potentially a serious public health problem as all age groups, including infants and children, consume these products worldwide. For this reason, milk and dairy products have to be inspected and controlled continuously for AFM1 contamination. Where concentrations are unacceptably high, careful investigation of feedstuffs for contamination by AFB1 must be made, the reason for this established and the cause eliminated.

With a view of the fact that milk is used by all the age groups including infants and children in Kathmandu valley, even the low amount of Aflatoxin M1 in milk can be a serious public health problem. Since the commission of the European communities stated that even if Aflatoxin M1 is regarded a less dangerous genotoxic carcinogenic substance than Aflatoxin B1, it is necessary to prevent the presence in milk and consequently in milk products, intended for human consumption and for young children in particular (Prandini *et al.*, 2009).

Nepal is a country where the pasture is not always widely available and the cattle and buffalos are fed with great amounts of concentrated feeds. The storage mechanism of the concentrated feed is not well developed and people do not care about the storage methods as a result of which these feed easily grow the fungus producing mycotoxins in them as a result of which there is always the possibility of milk being contaminated with the metabolised mycotoxins, mostly AFM1. The result of this study implies that more emphasis should be given to the routine AFM1 inspection of milk and dairy products as well as storage of animal feeds in Nepal. It is important to maintain control and to apply an ideal recommended limit to minimize the health hazard from Aflatoxin M1 contamination in milk which it can be used by infants and children. About this, governments have

responsibility for making regulations to protect consumers against harm arising from chemical in milk. Government and producer must apply some methods and plans for prevention and control of Aflatoxin M1 in milk and dairy products. About this, application of the Good Agricultural Practices (GAP) and Good Veterinary Practices (GVP) by agriculture and also the Hazard Analysis and Critical Control Point (HACCP) system as a draft code of practice for preharvest and postharvest control of dairy cow's feed and in milk and dairy products processing is effective. (Kamkar et.al., 2011). Precautions must be taken in the storage of feed commodities. Low moisture content, low temperature and low humidity conditions should be maintained during storage because these depress the fungus growth and thus eliminate Aflatoxin contamination. Responsibility for Aflatoxin M1 control in milk and dairy products lies with all participants in the production process, from farmers through to consumers.

Analysis of Aflatoxin at µg/ L or kg level needs high tech. laboratories equipped with highly sophisticated instrumentation. Adequate number of laboratories must be established for proper analysis of aflatoxins in different foods and feed commodities and also for certification purposes, as required by the international trade. There should be continuous surveillance programs in the country to monitor the occurrence of Aflatoxin regularly in milk and milk products. This is a first research of its kind in Nepal and there is always a scope for further research on detection of the mycotoxins in dairy products and commonly consumed food by the public. Furthermore this research will provide a baseline result to carry out research at more depth and broader scale and assign a permissible national limit for Aflatoxin M1 in milk products as imposed by many countries over the globe.

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ASSESSMENT OF PIG FARMER ATTRIBUTES IN KATHMANDU DISTRICT FOCUSING JAPANESE ENCEPHALITIS RISK FACTORS

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ABSTRACT

Japanese encephalitis (JE) is the most important cause of viral encephalitis in the world and its range has been expanding in Asia. In Nepal JE cases have been reported from 54 out of 75 districts. Since pigs can amplify JE virus, pig farming can be a high risk occupation and risk compounds when other risk factors like rice field, ducks, wild birds, mosquito vectors etc. are present nearby human dwellings. This cross sectional study carried out from September to December, 2011 had 3 goals: 1) to document the presence and nature of JE risk factors for pig farmers; (2) document their knowledge and practices for JE control and (3) identify opportunity for future extension of JE education. A standardized survey was conducted to 100 randomly selected pig farmers in Kathmandu district. The analysis involved descriptive data and univariate associations of demographic factors and knowledge and practices. The principle findings support the conclusion that the pig farmers are exposed to multiple JE risks, that they have very low knowledge of JE and its prevention and generally have low uptake of personal protection including vaccination and mosquito avoiding practices. Future education must be targeted to these low-income, illiterate farmers and must include women in the education process. Veterinarians, para-veterinarians and neighbours appear to be the trusted sources of educational material.

INTRODUCTION

Japanese encephalitis is a mosquito borne zoonotic disease caused by an arbovirus of flaviviridae family (Lindenbach *et al.*, 2001). It is the single largest cause of viral encephalitis in the world (Kinchi *et al.*, 2010), with annual case reports ranging from 30,000-50,000 (Solomon, 2006) but, estimations are even higher (Tsai, 2000). In Nepal, JE has been endemic in southern region (60-300 masl) since 1980s. Suddenly, JE cases reported from Kathmandu valley (1300 masl) in 1997 and in subsequent years from other districts of hill (300-2,000 masl). The hilly area, Kathmandu valley, is now endemic for Japanese encephalitis.

Culex tritaeniorhynchus is the predominant mosquito vector of JE (Philip Samuel *et al.*, 2000) that becomes active during dawn and dusk (Pant, 2009; Baik & Joo, 1991) and has average flight range of 1.5 Km (Henrich *et al.*, 2003). Though Culex tritaeniorhynchus breeds in open sunlit temporary and permanent habitats with vegetation, it prefers rice field (Keiser *et al.*, 2005; Richards et.al., 2010; Mani *et al.*, 1991) which also provides foraging sites for wading birds, the primary enzootic hosts for Japanese encephalitis virus (JEV). Thus, JE outbreak, spread or re-emergence is directly associated with irrigated rice agriculture and extension of flooded surface area (Hurk, 2009; Impoinvil *et al.*, 2011; Keiser *et al.*, 2005). In Nepal, ducks have been incriminated as the potential risk factor for JEV transmission (Joshi *et al.*, 2004; Pant, 2006). Since pig develops prolonged viremia after JEV infection pig rearing in presence of other risk factors such as wild birds, ducks, mosquito vectors, rice field and

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standing water sources may be a high risk occupation in Nepal. Risk may compound if preventive measures like vaccination and mosquito avoiding practices are also not carried out properly.

Other countries like Japan, South Korea and Taiwan have successfully controlled JE to great extent by effective immunization for human and pigs, modernization of pig farms, change in agricultural practices and improved living standards (Erlanger *et al.*, 2009; Igarashi, 2002). These are not feasible in Nepal under the current socio-economic conditions of the pig sector and the country. Therefore, other prevention methods of JE must be found. The objectives of this project were to survey randomly selected pig farmers in order (i) to document their exposure to risk factors (ii) characterize their knowledge and practices for JE control and (iii) identify factors that could influence the design of future educational programs to pig farmers.

MATERIALS AND METHOD

This study was conducted from September 2011 to December 2011. A research team at the National Zoonosis and Food Hygiene Research Center (NZFHRC) used published literature to identify known risk factors to include in the questionnaire survey. The questionnaire was pre-tested for clarity and feasibility on a sample of 20 farmers in a region outside of the study area and semi-structured questionnaire was finalized. Within Kathmandu district four sites namely Gothatar, Gokarna, Balaju and Jadibuti-Manahara area were selected, the number of pig farms counted in each area and in total 100 farms (out of total 180) were selected (19, 20, 28 and 33 respectively from Balaju, Gokarna, Gothatar and Jadibuti-Manahara) by simple random sampling (lottery) method. The data were collected, answers of open ended questionnaires were classified and converted to close ended form, answers coded and entered into the SPSS software version 19. Descriptive statistics was used for analysis of data and univariate associations of demographic factors and knowledge and practices were determined by using Chi-squared test.

RESULTS AND DISCUSSION

Pig farmer attributes

There was an equal number of male (n=50) and female (n=50) in our randomly selected study group. Among them, 33% had got primary education, 22% secondary education and 6% some college education. The monthly income of the farmers was less than 10,000 Nepalese rupees per month. Pig farming was the sole source of income for 73% of the farmers. Others had peripherals such as farming, working on daily wages, working as a hotel worker or as a porter for tourists. Only 15% farmers own land and only 16% had got any form of pig raising training.

This study shows equal involvement of male and female in pig farming. The pig farming sub-population in Kathmandu had low level of education, poor income status and no better side occupation. The fact that they are raising pigs on others land makes modernization or improvement of pig farm also impossible at present.

Exposure or closeness to risk factors

All farmers reported being bitten by mosquitoes and seeing their pigs being bitten. All farms were within 20 m from human dwelling. They were living very close to the mosquito breeding places, 95% of them lived within 1 kilometre distance from the rice field while 99% within 1 kilometre from standing water source. All had encountered variety of birds on their farms, all year round including wading birds, waterfowl and other wild birds. One third of them had raised ducks (39%) by themselves and others responded nearest duck farm being within 0.5 kilometres from their house. Ducks were raised under free ranging system, allowing direct contact with pig housing, food and water sources.

Indian research involving rice paddy ecosystem with herons and without herons had revealed greater role of herons for seroconversion rate in children (Mani et.al., 1991). The role of various migratory birds had also been incriminated for expansion of JEV genotype 1 in Asia (Nga et.al,

2004). Besides the migratory birds, role of ducks for JEV transmission had already been incriminated in Nepal by Joshi and Gaidamovisch (1981/82). In Nepal a stronger association was found to exist between JE incidence and percentage of irrigated land, mainly the paddy field (Impoinvil *et al.*, 2011). A case control study in Bali, Indonesia (Liu *et al.*, 2010) had also revealed proximity to rice field and pig ownership by family or neighbourhood to be independently associated with risk of JE. The pig farming sub-population in Kathmandu is also exposed to multiple JE risk factors including pigs, mosquito bites, rice field, wild birds, stagnant water sources, ducks etc. which creates a threat of JE all the time.

Knowledge of Japanese encephalitis

Only 42% had heard about JE and most of them knew it as a disease of human and few knew it causes problem in pigs. Just 20 individuals actually knew it gets transmitted by means of mosquito bite and almost half of them didn't know it is a vaccine preventable disease in pigs and human both. Friends and community was found to be the major source of JE and zoonotic disease information (48%) followed by mass media (38%), trainings (12%) and academic study (2%). There was a significant association between gender of farmer and knowledge about JE ($\chi^2 = 5.911$; $P < 0.05$); males being more aware than female. More exposure of males to the external environment, greater reach of them into mass media and greater male involvement in trainings and academic study may be the reason for males being more aware than female. SEARO-WHO (2002) quoted "health education and training is an important part of JE control". Nepal government ministry of health and USAID also noted the lack of awareness being potential risk factor of JE. Here, we found low level of awareness among the pig farmers which should be considered in future JE intervention programs.

Prevention measures used

The government of Nepal had launched an active vaccination campaign in Kathmandu for public in recent years. The vaccination program was conducted through different health centers free of cost. However, none of the pig farmers were immunized and they even couldn't figure out either of their family members being immunized. Despite the fact that 87% of the pig farmers had vaccinated their pigs for other diseases, none did for JE. Almost all (99%) of pig farmers claimed they knew mosquito bite prevention techniques and used at least one. The percentage of farmers using window screen, repellents, mosquito coils, staying indoor at dawn and dusk, wearing full length cloths, maintaining proper drainage and using mosquito nets were, however, quite less 11,25,69,39,40,38 and 41 respectively. Seventeen percentage of pig farmers reported they practiced mosquito avoidance techniques in the shed. Their techniques involved spraying chemical, maintaining cleanliness, smoking and using repellent. We found no relationship between whether a person knew of JE and whether he or she used at least one known preventive measures against mosquito exposure and bite (= 0.731,P=0.392). This is a very interesting finding which makes us to think twice. It means having knowledge doesn't mean he/she will practice it. So, future JE intervention strategies should take under consideration all the enablers and barriers for turning knowledge into day to day practices.

CONCLUSION

The pig farmers of Kathmandu district are continuously exposed to multiple JE risk factors. However, besides this bitter truth, the best JE control measures like effective vaccination and modernization of pig farms as adopted by many other countries are not possible at present context of Nepal. For us, awareness generation is the best cost effective method of JE prevention and control. This was able to find some interesting trends like low level of education, low level of income and females in need of more awareness. Future JE intervention program should focus on educating all (with special privilege to women); using veterinarians for educating the farmers and making use of mass media considering their high illiteracy. This kind of study should be carried out in other JE endemic districts as well to draw a clear picture of pig farmers attributes on JE and other zoonotic diseases as well.

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CANINE LEPTOSPIROSIS - A CASE STUDY

S.K. Paudel[1]

ABSTRACT

An 8 month old, uncastrated male Great Dane was presented to the Animal Medical Center, Kuala Lumpur with a history of anorexia and weakness for 2 days. Vomiting was observed by the owner on the day of presentation. The abnormalities noted on physical examination were depression, conjunctival congestion, dry muzzle and about 7% dehydration. The rectal temperature was normal. Hematological and biochemical analyses suggested acute renal failure with cholestatic liver disease. A tentative diagnosis of Leptospirosis was made based on the clinical signs and hematological and biochemical abnormalities. Leptospirosis was later confirmed by microscopic agglutination test (MAT), that detected diagnostic levels of antibody titer (1:1600) to serovar pomona. The animal was successfully treated with ampicillin, amoxicillin and doxycycline with supportive treatment.

BACKGROUND INFORMATION

Leptospirosis is a zoonotic disease caused by molecular distinct Leptospira serogroups. These spirochaetal bacteria can persist in dogs and wildlife reservoirs, which shed organisms contaminating the environment (Geisen et al., 2007). Currently, more than 200 serovars and 23 pathogenic serogroups have been identified (Guerra, 2009). Most commonly found pathogenic serovars in dogs include bratislava, canicola, icterohaemorrhagiae, pomona, and grippotyphosa (Ananda et al., 2008) The canine disease presents as an acute infection of kidney and liver and, sometimes, as a septicemia (Mc Donough, 2007). Common clinical signs reported include depression, anorexia, vomiting, diarrhoea, occulonasal discharge, renal pain, muscle pain and icterus. Three clinical syndromes (i.e. acute hemorrhagic, icteric, and uraemic) are recognized. A dog may have one or more of the syndromes, although uremic syndrome appears to be the most common (Harkin et al., 1996). Widespread use of a bivalent vaccine against Leptospira canicola and Leptospira icterohaemorrhagiae have led to a decreased incidence of leptospirosis in dogs. In the past years, however, veterinarians have become increasingly aware that a number of newly identified serovars can cause clinical disease in dogs. The available bivalent vaccine is serovar-specific and does not induce immunity towards these new serovars (Adin et al., 2000)

Achieving as definitive a diagnosis as possible should be of special importance to veterinary practitioners because of the zoonotic potential of the disease and the possibility of the dog serving as a reservoir for other dogs and humans. Unfortunately, achieving a definitive diagnosis is often difficult with the tools in use today. The first difficulty faced is that the clinical signs associated with this disease are often vague and are typically nonspecific. The clinic-pathologic data are often more of a function of the end-organ damage and nonspecific as well. Subtle abnormalities and combinations of abnormal clinic-pathologic data are often the key for a high index of suspicion necessary in these cases. Specific leptospirosis testing in practice today is typically still limited to serology although PCR testing may become a more common modality in the future, especially for

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acute cases. The MAT commonly used today lacks both sensitivity (negative results early in the disease process) and specificity (reacts positively with vaccinal antibodies) when a single test is performed. Thus, a high index of suspicion is required and veterinarians most often have to submit repeated samples to obtain a definitive diagnosis (Goldstein, 2010).

CASE REPORT

An 8 month old, uncastrated male Great Dane was presented to the Animal Medical Center, Kuala Lumpur with a history of anorexia and weakness for 2 days. Vomiting was observed by the owner on the day of presentation. The abnormalities noted on physical examination were depression, conjunctival congestion, dry muzzle and about 7% dehydration. The rectal temperature was 38.5 oC. Vaccination was done against distemper virus, adenovirus, leptospira (serovar canicola and icterohaemorrhagiae), parainfluenza, parvovirus and coronavirus.

Hematological abnormalities included leukocytosis (white blood cells 21.20 K /µL; reference range: 5.05-16.76 K /µL), mild anemia (pack cell volume (PCV) 30%; reference range: 37.3-61.7) and thrombocytopenia (platelets 50 K /µL; reference range: 148-484 K /µL). Serum biochemical abnormalities included increase in alkaline phosphatase ((ALP), 932 u/l reference range: 46-337 u/l), total billirubin (3.4 mg/dl; reference range: 0.0-0.9 mg/dl), serum creatinin(8.2 mg/dl; reference range: 0.5-1.8 mg/dl), blood urea nitrogen(109 mg/dl; reference range: 7-29 mg/dl), phosphorus(12.7 mg/dl; reference range: 2.5-6.8 mg/dl) and electrolyte imbalance with hyperkalemia(6.4 mmol/l; reference range: 3.5-5.8 mmol/l). The parameters indicated an acute renal failure and cholestatic hepatic disease with hyperbilirubinemia. A tentative diagnosis of Leptospirosis was made based upon clinical signs and above parameters. Leptospirosis was later confirmed by microscopic agglutination test (MAT) that detected diagnostic level of antibody titer (1:1600) to serovar pomona.

The Animal was hospitalized and aggressive Intravenous Fluid therapy was started. After rehydration, Mannitol (1g/kg body weight, IV over 15-20 min, q12h) was administered and continued for 5 days. Initial antibiotic therapy Included ampicillin (22 mg/kg body weight, IV,q8h)and was switched to oral amoxicillin (22 mg/kg body weight,q8h for two weeks) when it could tolerate oral medications, after 4 days. Other supportive therapy included ranitidine (2 mg/kg body weight, IV, q12h) and oral liver tonic and vitamin B complex. Supportive therapy was continued for 2 weeks. Following Amoxicillin therapy, the animal was kept on oral doxycycline (5 mg/kg body weight, q12h), which was continued for two weeks. Further hematological and biochemical analyses indicated gradual improvement over next two weeks (Table 1).

Table1: Clinical Laboratory Data Prior to and During Treatment for Canine Leptospirosis

	Time (Days) Since Initial Presentation								Hospital Normals
Parameters	0*	2	3	4	6	9	12	15	
PCV (%)	30	27	--	--	35	--	37	39	37.3-61.7
TWBC Count(K/µL)	21.20	8.80	--	--	10.00	--	8.40	12.40	5.05-16.76
ALP(u/l)	932	727	594	492	386	384	210	200	46-337
Total Bilirubin (mg/dl)	3.4	5.6	7.7	7.0	9.4	8.1	3.1	0.9	0.0-0.9
Serum Creatinine(mg/dl)	8.2	11.0	12.9	8.5	2.4	2.1	1.4	1.4	0.5-1.8
BUN (mg/dl)	109	116	125	111	53	32	25	18	7-29

*= Initial Presentation; PCV = Packed Cell Volume; TWBC= Total White Blood Cells; ALP = Alkaline phosphatase; BUN = Blood Urea Nitrogen.

DISCUSSION

According to Greene et. al., (1998), the kidney and liver are the major organs affected by the leptospiremia. This may result in azotemia and elevated liver-enzymes. In our study as well biochemical analysis revealed severe azotemia and high alkaline phosphatase with hyperbilirubinaemia that suggested acute renal failure and cholestatic hepatic disease. Leukocytosis was the next supportive finding in this study.

Detection of serum antibodies using MAT is the most commonly used diagnostic method for leptospirosis in dogs (Geisen et al., 2007). A high single MAT titre in combination with clinical signs was diagnostic in this study. The problem in interpretation of antibody test results is that vaccines can induce very high titres to other serogroups, which can be even higher than the titre against the vaccinal serogroups (Barr et al., 2005). A high MAT titre to a non-vaccinal serogroup and no (or only low) titres against vaccinal serogroups accompanied by clinical signs of leptospirosis must be considered highly suggestive of active infection (Baldwin, et al., 1987). Another diagnostic criterion is a four-fold rise in convalescent serum MAT titres (Birnbaum et al., 1998).

Successful therapy is dependent on good supportive care and appropriate antibiotics. Therapy with procaine penicillin G and dihydrostreptomycin has been recommended, but the need for intramuscular administration is a definite draw back. Therapy with intravenous ampicillin (22mg/kg body weight, IV,q8h) and continuing with oral amoxicillin (22mg/kg body weight,q8h) when tolerated, for two weeks, followed by two weeks of oral doxycycline (5mg/kg body weight, q12h) appeared to be the best therapy for leptospirosis (Harkin, et al., 1996). In this present case, the animal showed great improvement after the same line of treatment.

CONCLUSION

Leptospirosis should be considered in any dog presented with acute renal failure, acute cholestatic hepatic disease, or both. MAT can be used for the diagnosis of leptospirosis infection in dogs. A high single MAT titer in combination with azotemia and increased liver specific enzyme was diagnostic for this study. The infected animal can be successfully treated with ampicillin, amoxicillin and doxycycline with supportive care. As the human population continues to grow and housing developments encroach areas previously inhabited by wildlife, there may be a re-emergence of diseases like leptospirosis, that have natural reservoirof infection. Veterinarians have a responsibility to identify those infections with a zoonotic potential.

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OPTIMISATION OF AN IMMUNOHISTOCHEMISTRY FOR THE DETECTION OF PORCINE CIRCOVIRUS TYPE 2.

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ABSTRACT

Porcine circovirus type 2 (PCV2), a small non-enveloped single stranded DNA virus is the necessary causative agent of postweaning multisystem wasting syndrome (PMWS). Currently, methods to detect PCV2 include PCR, virus isolation, in situ hybridization and immunohistochemistry (IHC). IHC can be used routinely in diagnostic laboratories and provide histopathological lesions and detection of PCV2 in the same section so that virus and tissue lesions can be linked. IHC is however, a complex method that requires individual laboratory development and optimization. A series of experiments to optimize dilution and incubation parameters of a monoclonal antibody against PCV2 as well as optimization of epitope retrieval method were done in the Pathology and Infectious Disease Lab in Royal Veterinary College, Hawkstead, UK from 1st May, 2010- 30 August 2010. Formalin fixed-paraffin embedded tissue blocks of pigs consisting lymph nodes (Mesenteric, inguinal and tracheal) were used for the experiments. The NovolinkTM polymer kit was used to detect bound antibody. The optimum assay performance conditions for PCV2 detection were a dilution of 1:200 of the primary anti-PCV2 monoclonal antibody, incubation of tissue sections for 2 hours with this monoclonal antibody at room temperature and protease induced antigen retrieval (PIER). While further work needs to be done to develop and optimize a successful IHC protocol, this work provides initial data that can be used for this purpose.

INTRODUCTION

There are two known porcine circoviruses: Porcine circovirus type1 (PCV1) and Porcine circovirus type 2 (PCV2). They are small, non-enveloped single stranded virus containing circular DNA genome (Finsterbusch & Mankertz, 2009). They are categorised into the circoviridae family and the genus circovirus together with a number of avian viruses with similar molecular characteristics. PCV1 was first described as a contaminant of the porcine kidney cell line PK-15 in 1974 (Tischer, Gelderblom, Vettermann, & Koch, 1982; Tischer, Rasch, & Tochtermann, 1974) and was considered non pathogenic (Tischer, Mields, Wolff, Vagt, & Griem, 1986). Another variant of PCV was isolated later from pigs affected by postweaning multisystemic wasting syndrome (PMWS) (Allan et al., 1998) initially diagnosed and reported in Canada in 1991 (Clark, 1997). This variant was termed PCV2 as it was phylogenetically and serologically distinct from PCV1 (Meehan et al., 1998).

PCV2 virus can be transmitted horizontally by the direct contact between the infected and healthy pigs (Bolin, Stoffregen, Nayar, & Hamel, 2001)and vertically by infected sows and boars (Park et al., 2005). PCV2 has been accepted to be the necessary etiological agent of post weaning multisystemic wasting syndrome (PMWS), an emerging and multifactorial disease in swine (Bolin, et al., 2001; P. A. Harms et al., 2001). However, PCV2 alone is not sufficient to cause the full course of disease (Tomas, Fernandes, Valero, & Segales, 2008).

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PCV2 infection is ubiquitous in domestic pigs. PMWS, which has spread worldwide, is a disease of major concern in swine producing areas of the world because of its abundant damaging effects on growth performances. The disease is characterized by progressive weight loss, respiratory signs, diarrhoea and paleness of the skin (Allan & Ellis, 2000; Allan et al., 1999; Chae, 2004). PMWS is often accompanied by other viral and bacterial pathogens such as porcine reproductive respiratory syndrome virus (PRRSV), swine influenza virus, porcine parvovirus (PPV), Haemophilus parasuis, Actinobacillus pleuropneumoniae, streptococcus suis and Mycoplasma hyopneumoniae (J. Kim, chunj, H.-K., Jung, T., Cho, W.-S., Choi, C., Chae, C., , 2002; Pallares, 2002). PCV2 infection is divided into two main categories: 1) clinical form which has been described above. 2) Subclinical form which is characterized by absence of clinical signs, no or mild lesions with low counts of PCV2 (Hansen, Pors, Bille-Hansen, Kjerulff, & Nielsen, 2010). PCV2 can be present in healthy pigs and the trigger factors for the development of PMWS includes several factors like susceptibility and immune status of the pig (McKeown et al., 2005), the timing of PCV2 infection (Rose et al., 2009), and co-infections (Rose et al., 2003).

Some studies have shown that PMWS is manifested in pigs aged 7-15 weeks but most cases have been recorded at 6-8 weeks of life (Chae, 2004; Harding , Clark, Strokappe, P.I., & Ellis, 1998). The clinical signs are variable and unspecific. Morbidity and mortality associated with PMWS depend on the stage of outbreak and management within affected units (Allan & Ellis, 2000). The overall mortality can reach 10% in an acute outbreak whereas in endemically infected herds, morbidity and mortality are less (Allan & Ellis, 2000).

Histopathologically, PMWS is characterized by the presence of granulomatous inflammation in lymph nodes, liver, spleen, tonsil, thymus and peyer's patches and occurs consistently in superficial inguinal lymph nodes. There is lymphocyte depletion and replacement with histiocytes, with or without the presence of multinucleated giant cells. There is also a presence of intracytoplasmic inclusion bodies which are large, multiple grape-like structures often seen in the cytoplasm of histiocytic cells and multinucleated giant cells (Chae, 2004). Histological changes in liver include moderate inflammatory cell infiltration of portal areas, some hepatocellular vacuolation and swelling and sinusoidal collapse (Allan & Ellis, 2000; Chae, 2004). Histological features of PMWS in lung include multifocal lymphohistiocytic interstitial pneumonia and in kidney it includes lymphocytic infiltration in the renal interstitium. In intestine, intestinal villous atrophy from mild to severe with variable sloughing or regeneration of glandular and cryptal epithelial cells are exhibited (Allan & Ellis, 2000).

Mere presence of PCV2 and its detection does not indicate PMWS but PCV2 infection. The diagnosis of PMWS needs three criteria: i) the presence of compatible clinical signs, ii) the presence of moderate to severe characteristic microscopic lymphoid lesions (lymphoid depletion and lymphohistiocytic to granulomatous inflammation, sometimes multinucleated giant cells and basophilic inclusion bodies in histiocytes), and iii) the presence of moderate to high amounts of PCV2 within these lesions (Sorden, 2000).

PMWS can be controlled to a certain extent by improving good husbandry practices. The main focus for controlling PMWS is controlling other co-infections that can potentiate PCV2 infection. Management strategies such as decreasing stocking density , all-in/all-out production by facility, age segregation and good sanitation are important aspects of control (P.A. Harms, 2002). There is no effective treatment for PMWS, however vaccination of gilts, sows and piglets help to reduce the occurrence of PMWS by increasing PCV2 antibody titres in serum and colostrums and protects the piglets from developing PMWS (Opriessnig et al., 2010). Piglets can be vaccinated after 3 weeks of age as the maternal antibodies wane.

Techniques such as immunohistochemistry (IHC), in situ hybridization, polymerase chain reaction (PCR) and virus isolation (VI) can be used for the diagnosis of PCV2 antigen. In situ hybridization and immunohistochemistry provide histopathological lesions and detection of PCV2 in the same section so that virus and tissue lesions can be linked. These tests define the localization of the PCV2

antigen in tissues. Therefore these two methods are considered to be useful in diagnosis of PMWS. In comparison to in situ hybridization, immunohistochemistry has been proved more sensitive in the detection of PCV2 antigen (McNeilly et al., 1999). The probes used in that study were based on PCV1 whole genomic sequence. On another study PCV2 specific digoxigenin labelled DNA probe was used and found that in situ hybridization to be more sensitive than immunohistochemistry (D. Kim et al., 2009). However, in situ hybridization need greater technical complexity and expense compared with immunohistochemistry and so creates difficulty for using routinely in diagnostic laboratories.

Immunohistochemistry is based on the ability of primary antibody to bind to the antigen of interest in the tissue specimen and the subsequent detection of this reaction (Haines & Chelack, 1991). Formalin fixation is the standard method for tissue preservation in veterinary and human medicine (McNeilly, et al., 1999). Despite preserving tissues formalin fixation alters protein biochemistry such that the epitope of interest is masked making them undetected by primary antibodies for binding. Masking of epitope can be caused by forming cross links between amino acids and the epitope, cross links with unrelated peptides at or near an epitope altering the conformation of an epitope or altering the electrostatic charge of the antigen. The successful detection of antigens requires the good antigen retrieval method that reverses the changes produced during fixation. Antigen retrieval depends on the multiple variables such as the target antigen, the antibody used, the type of tissue, and the method of fixation. Generally, two techniques of antigen retrieval: Heat induced epitope retrieval (HIER) and Protease induced epitope retrieval (PIER) is popularly used for reversing the chemical modification caused by formalin fixation so that antibodies can bind with the antigen.

The effect of PIER depends on the concentration and type of enzyme, incubation parameters (time, temperature, and pH), and the duration of fixation (Battifora & Kopinski, 1986). PIER has not only low success rate for restoring immunoreactivity but it also potentiates for destroying both tissue morphology and antigen of interest (Ordonez, Manning, & Brooks, 1988). Different antibodies require different antigen retrieval (AR) method for the detection of antigen of interest.

Heat induced epitope retrieval can be carried out by applying different heating devices such as pressure cooker, microwave oven, vegetable steamer or autoclave in a buffer. HIER acts by hydrolyzing methylene cross links (Van Hecke, 2002). However, other unknown mechanisms are also involved in enhancing immunostaining of tissues fixed in ethanol, which does not produce cross links (Hayat, 2002). The effectiveness of HIER depends upon time, temperature, buffer composition and pH. Time and temperature are inversely proportional. The pH of the retrieval solution is very important (Shi, Cote, & Taylor, 2001). It is because different antibodies bind at different pH. Some antibodies bind at neutral pH, some at high pH and some at low ph. Commonly used buffers are 0.1M citrate buffer (pH 6), 0.1M EDTA (pH 8), 0.5M Tris buffer (pH10), and 0.05M glycine/Hcl buffer.

Success of epitope retrieval depends on the type of antibody used for the detection of antigen. The aim of this study was to develop immunohistochemical techniques for the detection of PCV2 anitgen in lymph node tissues that is known to contain antigen. The success of IHC depends on the antigen retrieval method used for restoring antigen of interest. HIER has been replacing enzymatic digestion method of epitope retrieval as it is easy to use. In addition, it increases the intensity of staining and the number of cells stained, demonstrate antigens that are not normally demonstrable in formalin fixed paraffin embedded (FFPE) tissues and produces consistent, reliable, high quality immunochemical staining of formaldehyde sensitive antigens.

Heat induced epitope retrieval has been successfully used in diagnosing infectious diseases in human and veterinary medicine. However, the studies related to PCV2 detection by immunohistochemistry was based on PIER (Chianini, Majo, Segales, Dominguez, & Domingo, 2003; Grierson et al., 2004; Hamberg, Ringler, & Krakowka, 2007; Hansen, et al., 2010; D. Kim, et al., 2009; McNeilly, et al., 1999; Opriessnig et al., 2004; Van Hecke, 2002). The objective of this study is to optimize immunohistochemistry for the detection of PCV2 antigen using anti-PCV2 NC monoclonal antibody bought from Jeno Biotech Inc, Korea.

MATERIALS AND METHODS

Tissue samples

Formalin fixed-paraffin embedded tissue blocks of pigs consisting lymph nodes (Mesenteric, inguinal and tracheal) and had been detected positive for PCV2 when tested by ELISA and PCR were available as positive control for the optimization of IHC protocol. The tissue blocks were fixed in 10% formalin for 2 weeks and embedded in the paraffin. The tissue blocks were re-embedded before cutting into 6µm tissue sections using a microtome and mounted on SuperFrost R plus slides (VWR international). Another positive tissue control available was the tissue sections that had been positive to IHC and had been obtained from Sandra Schoeinger (Lecturer Veterinary Pathology), RVC, UK. Negative control tissue that is not known to contain PCV2 antigen of interest was collected from pigs that is healthy and did not show any signs of PMWS from abattoir.

Haematoxylin and Eosin staining

For microscopical examination, tissue sections were stained with haematoxylin and eosin stain. Tissue sections were deparaffinised in clearene bath I (Neoclear Merck KGaA, Germany) for 20 min and then on clearene bath II for 1 min and rehydrated in graded alcohol bath and then washed in distilled water. Then slides were stained in Ehrlich's haematoxylin for 10 minutes followed by washing in distilled water. Slides were dipped in 1% acid quickly for differentiation followed by washing in tap water. Then the slides were dipped in Eosin for 5 minute and then washed in running tap water. The slides were then dehydrated in graded alcohol baths: 50% alcohol bath I for 30 sec, 70% alcohol bath for 30 sec, 90% alcohol bath for 1 min, 100% alcohol bath for 1 min, and again 100% alcohol bath for 1 min. Then it is cleared in xylene bath I for 5 min and xylene bath II for 10 min and then mounted and coveslipped.

Immunohistochemistry

A series of immunihistochemical test was performed for the standardization of primary antibody, optimisation of primary antibody incubation time and optimisation of antigen retrieval. Positive controls tissue sections that is known to contain antigen by IHC and negative controls tissue sections that is not known to contain antigen and collected from healthy pig were applied for the experiments. Negative control tissue sections were used for confirming the specificity of the test and for assessing the degree of non specific background staining.

The tissue sections were incubated in an oven at 60°c for 35 minutes before dewaxing in xylene bath I and xylene bath II and then rehydrated in graded alcohol series: 90% alcohol bath I and 100% alcohol bath II followed by washing in tap water bath.

Tissue sections were treated with two different antigen retrieval heat induced epitope retrieval and protease induced epitope retrieval. Heat induced antigen retrieval was carried out by heating tissue sections on a citrate buffer (pH 6.1) in a microwave oven. Citrate buffer was heated in a microwave oven for 3 minutes and slides were added in boiled citrate buffer and heated at 10 dp (dielectric permeability) for 5 minutes, topped up citrate buffer if neccessary and reheated again at 10 (dielectric permeability) for 5 minutes followed by cooling for 10 minutes.

Protease induced epitope retrieval was carried out by incubating slides with 0.05% (0.025g/50ml) protease xiv (sigma, Aldrich, St.Louis, MO, USA) for 15 min at room temperature. The protease digest solution was pre warmed for 10 min at 37°c before incubation. Then the slides were washed in wash buffer.

IHC was performed using NovolinkTM polymer kit; Ref no RE7140-K, LOT – 6002217 (Leica Biosystems Newcastle ltd, UK). The slides were first treated with peroxidase block for 5 minutes and then with protein block for the same duration before incubation with 1:200 diluted anti PCV2 monoclonal antibody for 2 hours at room temperature

For standardization of primary antibody (anti PCV2 NC monoclonal antibody 12C.48), tissue sections were incubated with serial dilution of primary antibody 1:100, 1:200, 1:400, 1:800, 1:1600 and 1:3200 for two hours at room temperature respectively. The antibody diluents used was Dako antibody diluent with background reducing components (Dako North America, United States).

For optimization of primary antibody incubation time, tissue sections were incubated overnight with primary antibody at 4 c and for 2 hours at room temperature. After incubation with primary antibody slides of tissue sections were incubated with protein block for 30 minutes followed by incubation with Novolink polymer for 30 minutes. Before application of reagent in every step the slides were washed in Dako wash buffer for 5 minutes twice. For the detection of antigen antibody reactions, DAB solution was used and counterstained with haematoxylin. The sections were then dehydrated in graded alcohol bath series: 90% alcohol bath I, 100% alcohol bath II and 100% alcohol bath III and cleared in xylene bath I and xylene bath II. Finally slides were cover slipped using histomount and observed under microscope.

RESULT

Histopathological features of lymph node tissue

Hematoxylin and eosin staining of pig lymphocytes (fig 1.b) exhibited the presence of scattered multinucleated giant cells as well as residual follicles demonstrating central depletion. Other features demonstrating PCV2 infection included medullary oedema and lymphatic dilation. Moderate to marked lymphoid depletion with loss of corticomedullary demarcation were also observed. Additionally, infiltration of a large number macrophages accompanied by small numbers of lymphocytes and plasma cells was detected. Normal uninfected tissue did not show any pathological features (fig 1)

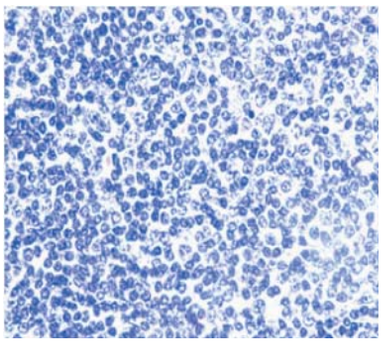


Fig (1.a): Photomicrograph of pig lymph node (control positive) showing multinucleated giant cells and macrophages, HE 40X.

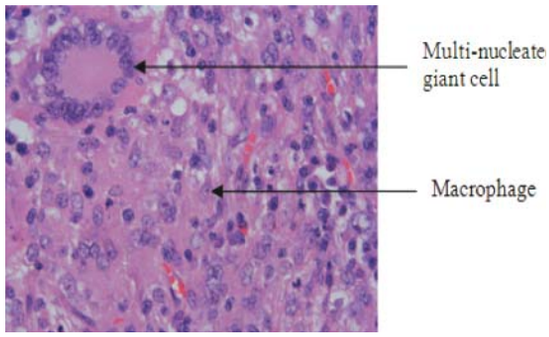


Fig (1.b): Lymph node of pig (control negative) showing lymphocytes and follicles, HE 40X.

Immunohistochemistry:

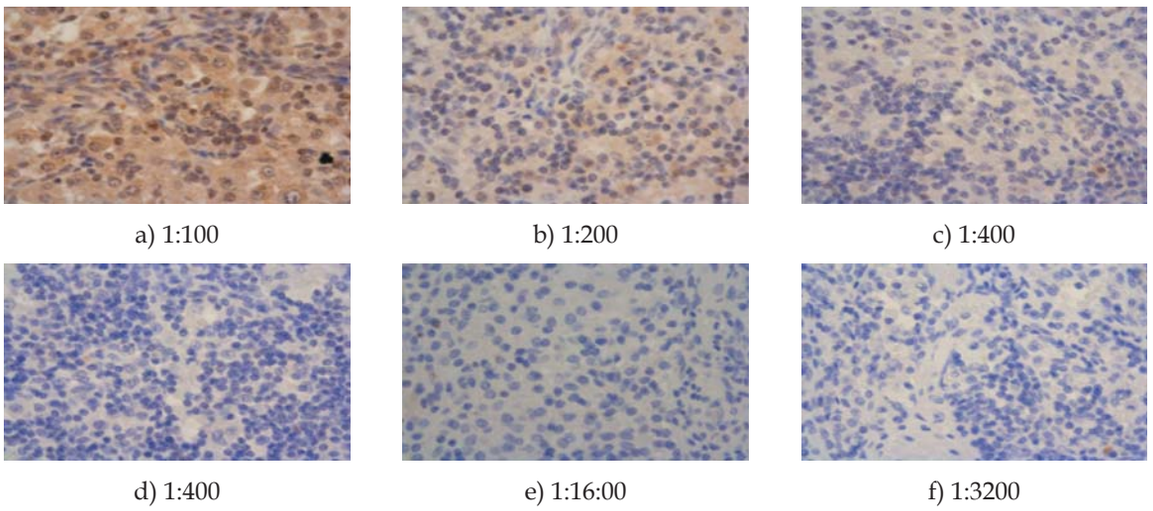
Standardization of Primary antibody:

On examining the slides that had been treated with different dilutions of primary antibody for testing optimal dilutions of antibody, high non specific background staining was observed at 1:100 dilution and low at 1:3200 dilutions (fig 2: a-f). The results obtained were shown in table 1

Table 1: Standardization of primary antibody

Antibody dilution	1:100	1:200	1:400	1:800	1:1600	1:3200
Background stain	High	Medium	Low	Low	Low	Low

From the result 1:200 was standardized as the optimal dilution of primary antibody for detecting PCV2 antigen by immunohistochemistry.



a) 1:100 b) 1:200 c) 1:400

d) 1:400 e) 1:16:00 f) 1:3200

Fig. 2 (a-f). Photomicrographs of sections showing results of different dilutions of primary antibody to determine antibody dilution that results in the maximum staining of positive antigens while maintaining minimum background staining. Primary antibody dilutions are shown for each micrograph. (Mag x40)

Optimization of primary antibody incubation time

In an experiment for standardization of primary antibody incubation time, tissue sections were incubated with primary antibody for 2 hours at room temperature and another for overnight at 4°C. Heat induced antigen retrieval was carried out on tissue sections using steamer and microwave to compare the intensity of antigen retrieval from heating devices. The outline of experiment and the results were shown in the table 2 and table 3.

Table 2: Tissue sections incubated with primary antibody for two hours at room temperature and overnight at 4˚c using microwave for antigen retrieval

HIER	Primary antibody incubation for two hours at room temperature	Overnight primary antibody incubation at 4˚c
Device	Microwave	Microwave
Result	Medium background staining	High Background staining

Table 3: Tissue sections incubated with primary antibody for two hours at room temperature and overnight at 4˚c using steamer for antigen retrieval

HIER	Primary antibody incubation for two hours at room temperature	Overnight primary antibody incubation at 4˚c
Device	Steamer	Steamer
Results	Medium background staining	High background staining

The results from this experiment showed that the slides incubated overnight showed high background staining (fig 3a). The intensity of non specific background staining was very high compared to the slides incubated for 2 hours at room temperature (fig 3b). The non specific background staining observed was staining of blood serum, plasma cells and some lymphocyte cells. Steamer and microwave heating devices was found to have no any effect on antigen retrieval. However, from this experiment two hours primary antibody incubation time was found optimum for antigen antibody binding in immunohistochemstry for PCV2 detection.

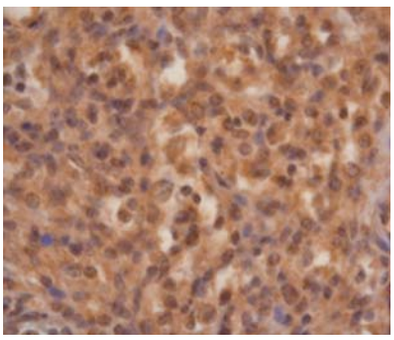


Fig. 3(a): Photomicrograph showing the high background staining after overnight incubation (4°C) of tissue sections with the primary antibody against PCV-2.

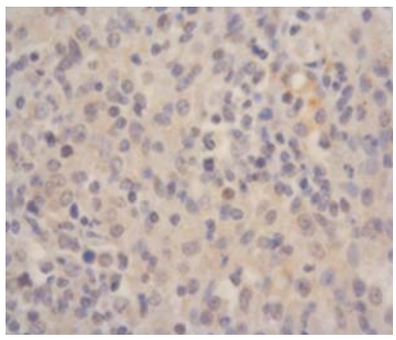


Fig. 3(b): Photomicrograph showing the medium background staining after a 2 hour incubation (room temperature) of tissue sections with the primary antibody against PCV-2 for 2 hours. (Mag x40).

Immunohistochemistry for the optimisation of epitope retrieval

In this experiment, slides were treated with two different kinds of epitope retrieval: HIER and PIER. On examining the slides that had been treated by heating on microwave on citrate buffer (pH6.1), no any specific staining was observed (fig 4c). Some back ground staining of the plasma cells, lymphocytic cells and the blood serum was observed. On the other hand slides treated with protease showed specific staining, along with staining of plasma cells and blood serum (fig 4a). However, the signal of intensity of specific staining was weak along with the presence of non specific staining of plasma cells and blood serum.

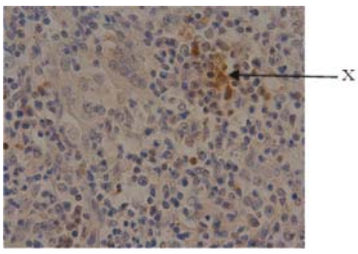


Fig. 4(a): A photomicrograph showing PCV-2 antigen using protease induced antigen retrieval. X refers to specific staining of antigen in macrophages.

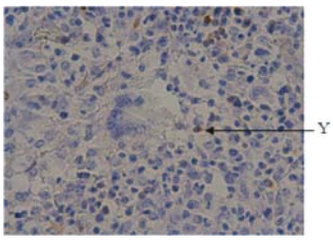


Fig. 4(b): Photomicrograph of a negative control slide in which the primary antibody against PCV-2 was omitted, and the antigen was retrieved using protease.

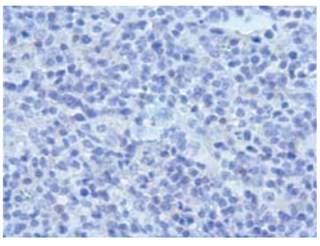


Fig. 4(c): A photomicrograph showing the absence of specific staining after tissue sections were subjected to antigen retrieval using heat.

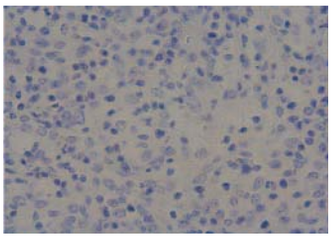


Fig. 4(d): Photomicrograph showing a tissue section in which no primary antibody was added after retrieval of antigen using heat. The magnification of all micrographs is x40.

DISCUSSION

This study attempted to develop immuno-histochemistry for the detection of the presence of PCV2 antigen in the lymph nodes of infected pigs. Before developing this method, the tissues were studied under Haematoxylin and Eosin stain. Lymphnode pathology observed in these sections was consistent with published literature (Chianini, et al., 2003). A commercial monoclonal antibody against PCV2 was used to develop the IHC assay as monoclonal antibodies in comparison to polyclonal antibodies has higher specificity, high homogeneity, ease of characterization and

minimal batch to batch or lot-to- lot variability.

Before starting the experiments, standardization of primary antibody was carried out and though this experiment couldnot detect the specific staining in any of the dilution of antibody, the background staining observed at 1:200 dilution was found optimum. The reason for not obtaining specific detection could be the inability of heat induced antigen retrieval to retrieve antigen or it could be the insufficient time for primary antibody to bind with the antigen.

The results obtained from the optimization of the length of primary antibody incubation time showed 2 hrs of incubation at room temperature as the optimum time. The conditions for incubation of primary antibody depend on the antibody characteristics such as affinity, environmental factors such as temperature, and procedure. Normally, incubation for most routine IHC protocol is 30-90 min at room temperature. In the study by Grierson et al. (2004) monoclonal antibody was applied at a dilution of 1:1000 for one hour at room temperature, while the study conducted by Hansen et.al (2010) applied monoclonal antibody at a dilution of 1:150 dilution for one hour at room temperature.

Then for optimizing antigen retrieval, two antigen retrieval methods were attempted: protease induced antigen retrieval (PIER) and heat induced antigen retrieval (HIER) whilst the mechanism by which either facilitates epitope exposure is not clearly understood, both mechanisms are widely used in antigen retrieval for immunohistochemistry studies.

The results of the present work indicate that PIER would be the most effective method for PCV2 antigen retrieval. In this work, it was possible to obtain an optimum noise to signal ratio using PIER method. Successful immunohistochemistry using PIER depends on proper optimization of both temperature and incubation time because PIER has the potential of destroying tissue morphology and also the antigen of interest. It is therefore necessary to optimize all these conditions in order to have a reliable IHC method based on PIER. This is especially important because enzymes are believed to cleave the bonds non specifically formed by proteins and fixative (McNicol & Richmond, 1998). Attempts to carry out IHC using HIER were unsuccessful since the outcome resulted in non specific staining of plasma cells and blood serum and no any specific staining (fig 4c). Repeated efforts to optimize HIER by changing incubation time with primary antibody as well as several sources of heat showed high non specific staining and so proved unsuccessful.

The use of HIER for antigen detection in immunohistochemistry has been effective for other infectious agents (Paul, 2003). However, in some cases , the use of this method has been shown to reduce or abolish the detection of antigen instead of improving the detection (Paul, 2003). It is however difficult to conclude that this was the case in the present study, since rather than obtaining no staining, tissues subjected to HIER failed to show specific staining, the same tissues clearly resolved and antigen was believed to be visible when subjected to PIER. The signal of intensity of staining was low. Since it could not be compared with known stained slides, it is quite difficult to reach at confirmation. However it can be associated with the microscopic lesions seen in the tisues. Further research to increase the intensity of specific staining should be carried out with minimal nonspecific staining and for the interpretation gold standard slides should be used. In addition further work should be carried out to reduce the non specific staining of the antibody by adding detergents to the antibody diluents and washing buffers or increasing the protein block incubation time

This study has shown that the monoclonal antibody 12C.48 can be used in an IHC test to confirm the presence of a PCV2 infection in pig. Preliminary data from this work suggest that PIER could be developed and further optimized and be adopted as routine confirmation test for PCV2 with minimal background staining.

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EFFECT OF ALOE VERA ON IMMUNOMODULATION, LIVER FUNCTION, BLOOD GLUCOSE AND PERFORMANCE OF BROILER CHICKENS

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ABSTRACTS

A study was conducted to characterize the immunomodulatory, hepatic hypoglycemic and growth performance effect of commercially available Aloe vera juice in commercial broiler chickens following experimental feeding. A total of 225 commercial broiler chicks were divided into 5 treatment groups each replicated three times. (not clear)One group getting no aloe vera served as control. The study of experiment was conducted under completely randomized design (CRD). The changes in the level of immunity acquired in the treatment groups were measured through different parameters: ; effect on liver by measuring liver enzymes and hypoglycemic effect by measuring glucose in the serum. Overall performance was estimated by measuring mortality, weight gain and feed conversion ratio (FCR) every weeks up to 42days. Significantly higher ($p \leq 0.05$) log2 HI titre against ND with high globulin levels and moderately higher level of lymphocyte counts were recorded in all the treatment groups as compared to control group. There were no significant changes in liver enzymes and blood glucose levels in the treated groups as compared to the control group. No microscopic changes were found in the liver of either treatment or control groups except fatty lipidosis. The ratio of bursa, spleen, thymus to body weight was significantly higher for treatment groups as compared to the control. The bursal atrophy was significantly higher in control group after hot IBDV vaccine challenge as compared to treatment groups, but the lesion was not consistent for all the samples. The weight gain was non-significantly higher and FCR was significantly lower for treatment groups as compared to control group with a high level (15.56%) of mortality in the control group as compared to treatment groups. Result thus suggests that feeding aloe vera juice to broiler chicken has an immunopotentiating effect and has beneficial effect on overall performance without any adverse effect on liver and blood glucose levels.

INTRODUCTION

Poor health and set back due to frequent outbreaks of much disease conditions are the major problem of poultry farming in south asian countries which in turn leads to the loss of vitality in proper immune status (Prabhakaran, 2003). Conventional treatments with antibiotics cannot control the disease condition in an individual animal or flocks which have once suffered the immunosuppression resulting huge economic losses and hence there is continuous search of alternative medicine (Angell and Kassirer, 1998). An attempt to overcome this problem has been made by introducing the concept of "Pro-Host Therapy". This approach aims at administering those products which can either boost host immune response against infections or can down regulate an

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explicitly activated cells and organs of the immune system without any toxicity or side effects on the host. The term immunomodulators are used for such class of drugs (Chatterjee, 1994).

Immunostimulation in a drug induced immunosuppressing model and immunosuppression in an experimental hyperactivity model by the same preparation can be said to be true immunomodulation (Zhang et al., 1996). A number of plant products, which are claimed to be having minimal side effects, being investigated for immune response modifying activity. Aloe vera has enjoyed a long history of lay acceptance as an herbal remedy and is perhaps the most popular herbal remedy employed today (Davis et al., 1994). They are rich source of substances which are claimed to induce paraimmunity, the non specific immunomodulation comprised of granulocytes, macrophages, natural killer cells and complement functions (Qui et al. 2000) and in tropical region, they are being used as livertonics, anti-stress, antioxidant, antitoxic, antibacterials, growth promoters and immunodmodulator properties in human as well as in poultry.

MATERIAL AND METHODS

Two hundred and twenty five day-old broiler chicks of the same breeder flock and same hatching lot vaccinated against Marek's disease were divided into five treatment groups with respect to feeding Aloe vera each containing 45 birds which was then divided into three replications each replicate containing 15 birds.

T0 = Basal diet with no Aloe vera as a water treatment.

T1 = Basal diet with Aloe vera @5ml/ 2 litre drinking water per day for 42 days

T2 = Basal diet with Aloe vera @5ml/ 2 litres drinking water per day for 15 days

T3 = Basal diet with Aloe vera @10 ml/ litre drinking water per day for 42 days

T4 = Basal diet with Aloe vera @10 ml/ 2 litre drinking water per day for 15 days

The commercial juice from a reputed Company Patanjali Yogapitha, Haridwar, India was used. The birds were maintained on standard management procedures with commercial pellet feeds. At 5 and 19 days of age, all birds were vaccinated with Newcastle Disease Virus (NDV) and at 14 days of age, birds were challenged with hot strains of infectious bursal disease virus (IBDV) vaccine. Samples were collected at the end of each week i. e. 7th, 14th, 21st, 28th, 35th and 42nd days from randomly selected birds of each replication unit.

Blood was collected by puncturing from the jugular vein while liver, bursa, spleen, thymus were collected after slaughter. Blood samples were allowed to clot and centrifuged for 20 min at 1500 rpm to separate sera and were stored at -200 C for biochemical analysis while the organs were preserved in 10% buffered formalin for histopathological study.

The immunomodulating effect was evaluated by measuring antibody titer (Hemagglutination inhibition) log2 against NDV vaccine, general immune response by lymphocyte count, and globulin at weekly interval upto 42 days of age. The effect on liver was estimated by measuring liver enzymes Serum Glutamic Oxaloacetate Transaminase (SGOT) and Serum Glutamic Pyruvate Transminase (SGPT), and its histopathology. The hypoglycemic effect was estimated by measuring glucose level in the serum at weekly interval. The morphological changes in the bursa, spleen, and thymus were estimated by measuring ratio score of the respective organs to the body weight and bursal atrophy.

Differential Leucocyte count (DLC) was done by counting the percentage of lymphocytes in the thin smear prepared from blood (Coles, 1974); hemagglutination inhibition (HI) antibody titre in the serum against New castle disease was estimated by microtitre plate method (Mahato, 2003) and biochemical estimation of globulin, liver enzymes SGOT and SGPT and glucose were done by kit method as per the manufacturer's protocol (Doumas, 1975). Histopathological study of bursa and spleen were done by standard techniques of dehydration in ascending grade of alcohols and cleared in xylene followed by embedding in paraffin wax and sectioned into 5 mm thick tissue, then stained with hematoxylene and and eosin. Ration of bursa-spleen-thymus: Body weight

ratios (gross Pathology) were calculated by taking their respective weights in the same scale. Data collected on various parameters were subjected to statistical analysis using CRD as per method described by (Steel and Toorie, 1980).

RESULTS AND DISUCUSSIONS

The results obtained during the experiment are presented with the help of the tables. The research was designed to evaluate mainly for the immunomodulatory effect of Aloe vera. There are many parameters through which we can measure immunity. Lymphocytes count serves as the indicator of the cellular immunity while HI titres, globulin levels are the indicators of humoral immunity (Chauhan, 1999); ratio score bursa, spleen and thymus weight to body weight, the broad indicators of nonspecific immunity (Chauhan and Singh, 2001). The hemagglutination inhibition (HI) antibody titre is the most commonly used serological test and exclusive measure for determining immunological status of chicken against viral infections of family paramyxoviridae, mainly Newcastle Disease Virus (Spanoghe et al., 1977; Giambrone, 1981). All of these parameters are important and thus have been studied and discussed hereby. The research also comprises of a study on hepatoprotective effect of aloe as well as its effects on blood glucose levels. The results have been dealt in sequence.

Table 1: Effect of Aloe vera levels on lymphocytes count (%) at different weeks of age of broilers

Treatment (Aloe vera per 2 litre drinking water)	Age in weeks					
	1	2	3	4	5	6
Control (Zero)	73.67± 3.79b	71.33± 2.52b	56.67± 2.08c	63.67± 1.53c	75.00± 2.00ab	74.66± 2.00
T1 (5 ml/ 2l x 42 days)	78.67± 1.53a	78.00± 1.00a	71.67± 2.52a	73.00± 1.00a	74.00± 2.00bc	73.00± 0.58
T2 (5 ml/ 2l x 15 days)	79.00± 2.00a	78.00± 1.00a	64.00± 1.00b	68.00± 3.00b	73.00± 2.00bc	73.66± 0.58
T3 (10 ml/ 2l x 42 days)	78.00± 1.00a	78.67± 0.58a	72.33± 3.79a	73.00± 1.00a	76.66± 1.53a	73.33± 1.00
T4 (10 ml/ 2l x 15 days)	78.33± 1.53a	78.00± 1.00a	62.00± 3.00b	68.33± 2.52b	71.66± 1.15c	74.00± 2.08
Probability	0.0719	0.0003	0.0001	0.0008	0.0122	-
F-value	3.007*	14.621**	18.976**	11.763**	5.638*	0.617ns
CV %	2.83%	1.81%	4.05%	2.87%	1.88%	1.92%
LSD	3.99	2.10	4.81	4.81	3.6	Ns

Means in column with different superscripts are significantly different; *Significant at 5% ($p<0.05$) **Significant at1% ($p<0.01$);

Table 2: Effect of Aloe vera levels on log 2 HI titres at different weeks of age of broilers

Treatment (Aloe vera per 2l drinking water)	Age in weeks					
	1	2	3	4	5	6
Control (Zero)	7.33± 0.58c	6.33± 0.58c	3.67± 0.58b	4.66± 0.58c	3.33± 0.58c	3.33± 0.58b
T1 (5 ml/ 2l x 42 days)	9.67± 0.58ab	9.66± 1.53a	9.00± 1.73a	7.66± 1.53a	7.66± 0.58a	6.33± 0.58a
T2 (5 ml/ 2l x 15 days)	9.67± 0.58ab	9.33± 0.58ab	8.00±0.00a	6.00± 0.00bc	5.33± 0.58b	4.66± 1.53ab
T3 (10 ml/ 2l x 42 days)	10.00± 1.00a	8.00± 0.00b	7.33± 0.58a	7.00± 1.00ab	8.00± 0.00a	6.33± 1.53a
T4 (10 ml/ 2l x 15 days)	8.66± 0.58b	8.00± 0.00b	7.33± 1.15a	6.33± 0.58ab	5.66± 0.58b	4.33± 0.58b
Probability	0.0043	0.0027	0.0007	0.0203	0.0000	0.0236
F-value	7.643**	8.72**	12.23**	4.792**	40.625**	4.559**
CV %	7.53%	9.37%	14.15%	14.12%	8.61%	21.29%
LSD	1.24	1.41	1.82	1.63	0.94	1.94

Means in column with different superscripts are significantly different; *Significant at 5% (p<0.05) **Significant at1% (p<0.01)

Table 3. Effect of Aloe vera feeding on g/dl globulin levels (Mean ± SD) in serum at different weeks of age of broilers

Treatment (Aloe vera per 2litre drinking water)	Age in weeks					
	1	2	3	4	5	6
Control (Zero)	3.14±0.66c	3.09 ±0.59b	2.45±0.35b	2.64±0.56b	2.64±0.95b	2.34±0.72c
T1(5 ml/ 2l x 42 days)	4.04±0.23ab	4.17 ±0.06a	3.91±0.38a	4.28±0.44a	6.14±0.14a	6.94±0.92 a
T2(5 ml/ 2l x 15 days)	3.79±0.43bc	4.12 ±0.41a	3.77±0.31a	4.82±1.24a	5.00±0.88a	4.80±0.93b
T3(10 ml/ 2l x 42 days)	4.64±0.25a	4.22 ±0.06a	3.91±0.26a	4.84±1.11a	4.96±1.07a	4.61±0.72b
T4(10 ml/ 2l x 15 days)	4.30±0.37ab	4.50±0.22a	4.10±0.34a	4.48±0.29a	4.75±1.50a	4.27±1.14b
Probability	0.0127	0.0045	0.0007	0.0429	0.0211	0.0016
F-value	5.573*	7.553**	12.378*	3.685*	4.729*	9.921*
CV %	10.43%	8.47%	9.10%	19.51%	21.51%	19.60%
LSD	0.76	0.62	0.60	1.50	1.840	1.64

Means in column with different superscripts are significantly different; *Significant at 5% (p<0.05) **Significant at1% (p<0.01)

As the table-1, 2, 3 shows, the value for lymphocyte count, HI titres as well as globulin levels were higher for the treatment groups as compared to control group throughout the experiment, with highly significant (p<0.01) for HI titre and significant differences for globulin and lymphocyte count statistical differences as depicted in the tables. The differences among different treatment combinations were not significant. The biochemical assessment of liver enzymes showed only non-significant variations with histopathology of liver showing mild degree of fatty degenerations and lipidosis in the control group as compared to the control group. The blood glucose values were within normal range in all the groups with no reduction in the aloe treated groups. This may be due to effect of Aloe vera on immunomodulatory organs. Since Aloe vera were given from the first day of life in all treatment groups except control, the effect can be ascertained to be due to treatment because there was no difference except the applied treatment. The findings are in agreement with the previous findings. The highest log2 antibody titre values 10 were recorded in day old age; decline with increase in the age of the bird and on 7th day of age, the titre value markedly declined in control group chicken which had not received any immunostimulants (Sedekar et al., 1998 and Mani et al., 2001). They reported that the HI titre increase with Tulsi and vitamin E and selenium in poultry. The higher titre value in aloe treated group reported by Mehla and Moorthy, 2008.

Histopatholgy revealed normal structural pattern with homogenous distribution of hepatocytes, central vein and bile duct in treatment groups in contrast to fatty degenerations, lipidosis and vacualations in the control group. Sections of bursa of fabricius from the control showed loss of the normal corticomedullary architecture of bursa and extensive expansion of the interfollicular connective tissues with loss of lymphocyte depletion and somewhere edema and cellular infiltration by heterophils, macrophages and plasma cells as compared with very thin band of interfollicular connective tissues in the treatement groups.

The effect on the growth was interesting. Initially the weight gain was higher in the control group as compared to aloe medicated group but in the third week, the treated group surpasses the control group; higher rate of weight gain were then recorded in each of medicated group with the highest cumulative weight gain in the T1 group at the end of experiment on 42 days. The difference was more than 200 g but the relativity of difference was statistically nonsignificant as compared with the control group as well as among the treatment groups. FCR was very high for the control group as compared to the treatment groups; i. e. more the feed they consumed, less they produced as

compared to treatment groups. Many reports have been in existence showing diverse effects on the weight gain in poultry birds as well as other including humans. Many reports have been in existence showing diverse effects on the weight gain in poultry birds as well as other including humans. Sinurat et al. (2002) stated supplementation of fresh aloe gel 0.25 g/kg and dry aloe gel 0.25 g/kg and 1.0 g/kg in broiler diet from 1-day old to 5 weeks of age showed no significant effect on carcass yield and internal organs.

The lack of overt clinical signs or less mortality, 15.56%, against IBDV inoculated group of chicks in different regimes of treatment studies are contrary to the findings by Cheetle et al. (1989) who reported 50% mortality in White Leghorn chicks infected with the 52/70 strain of IBDV. Similarly, Broiler flocks often experience mortality rates of 20 to 30% (Spanoghe et al., 1977) although an average rate is more like 15% (Stuart, 1989).

CONCLUSION

In conclusion, the birds fed with juice of Aloe vera showed high immune status for both the generalized cellular and humoral immunity which can act against any opportunistic pathogens as well as specific immunity against New castle disease virus. The immunosuppression after the hot infectious bursal disease virus vaccine challenge was less in the treatment groups as compared to control group with minimal atrophy in the bursa. The effects on liver and blood glucose level were not significant. Thus, it can be concluded that feeding aloe vera juice to commercial broiler chicken has immunopotentiating effect without having any adverse effects on liver and normal blood glucose level.

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EVALUATION OF ANTIBODY TITRES AFTER IMMUNIZATION WITH DIFFERENT NEWCASTLE DISEASE VACCINES AND USES OF IMMUNE-MODULATORS IN HYLINE LAYERS

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ABSTRACT

This was conducted to evaluate the antibody titres after immunization with different Newcastle disease vaccines and uses of immune-modulators in Hyline layers. The first experiment was carried out in 40-week-old layers flock (4000 size), which was divided into four groups of 1000 birds- A, B, C, and D.. Group A was used as control, and group B, C, and D were vaccinated with Indovac-FR (F strain), Himvac-B1R (B1 strain), and Indovac-LaSotaR (LaSota strain), respectively. The second experiment was carried out in 45-week-old layers flock (3200 size), which was divided into four groups of 800 birds- E, F, G, and H. Group E, F, G, H were vaccinated with Indovac-LaSotaR, and Pentavit-CR, E-Care-SeR, Levamisole-200R were given to group F, G, H respectively once a day for 7 days at recommended doses by manufacturers. Blood samples were collected for sera from 30 randomly selected birds from each group at 1 day pre-vaccination (1DPrV), 7th, 14th, 21st, 28th, and 35th days post-vaccination (DPV) to measure antibody titre using haemagglutination inhibition (HI) test. It was found that peak antibody titres (Mean±SD) and GMT occurred at 14th days post-vaccination, then decreasing gradually. Among three commercial vaccines, Himvac-B1R appeared to be slightly superior compared to those of Indovac-FR and Indovac-LaSotaR in respect of HI antibody response. Also, among three commercial immune-modulators, Pentavit-CR appeared to be slightly superior compared to those of E-Care-SeR and Levamisole-200R. The F-values of HI titres among three vaccines were significantly different ($p<0.05$) except at 1DPrV. Similarly, F-values of HI titres among three immune-modulators were significantly different at 7th, 28th and 35thDPV, and not significant ($p>0.05$) at 1DPrV, 14th and 21st DPV

INTRODUCTION

The ND also known as Ranikhet disease stands as a major problem towards the development of poultry industry in Nepal. The widespread distribution of the disease has occurred over the last few decades provide example of the negative impact of such disease on the poultry industry and on society as a wholel. . In a study, it was shown that the most common viral diseases of poultry were Infectious Bursal Disease (IBD) followed by ND, Infectious Bronchitis (IB), Marek's Disease (MD), Fowl Pox and Egg Drop Syndrome 76 (EDS 76) from commercial poultry pockets of Nepal (Dhakal, 2002). It is an endemic and sometimes epizootic disease in chickens and it causes a great loss in domestic and wild birds.

Routine vaccination combined with sacrifice of affected birds has helped to control the disease caused by the Newcastle disease virus (NDV). In order to control the disease, various vaccination

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programs against the disease are employed. Vaccines and vaccination programs vary widely, depending on several local factors (e.g. type of production, level of biosecurity, local pattern of disease, status of maternal immunity, vaccines available, costs and potential losses). Despite the vaccinations against ND, there are reports worldwide of birds dying or still showing clinical infections (Allan et al., 1978 a,b; Aldous and Alexander, 2001). This might be due to the use of either low quality vaccine, failure of maintenance of cold chain or interference of vaccine antigen with the maternal antibody (Rahman et al., 2002) or the inability of the birds to maintain the immunity after the vaccination due to lack of immune-modulating agents during vaccination.

In Nepal, various live vaccines such as F, B1, LaSota, VG/GA, ND-clone are brought by several importer but efficacy of these vaccines in relation to climatic condition, distribution and transportation are not always investigated properly and thoroughly either by the importer or by the user. Sometimes, the farmers are suspicious about the protective nature of those imported NDV vaccines. A number of relevant questions are faced by scientists and field veterinarians of this country as to the immunogenicity, virus titre, stability and such other qualities of those vaccines. Still, there is no practice of using immune-modulators during vaccination though lots of immune-stimulating agents are found in local markets. These farmers required to be encouraged with continuous support of disease preventive measures. As such prophylaxis against ND comes under consideration where the schedule of vaccination demands evaluation on the performance or efficacy of vaccines. In order to address such query, the present study was undertaken to evaluate the antibody titres after immunization with different ND vaccines as Indovac-FR (F), Himvac-B1R (B1) and Indovac-LaSotaR (LaSota), and uses of immune-modulators as Pentavit-CR, E-Care-SeR, and Levamisole-200R in Hyline layers.

MATERIALS AND METHODS

Site of the study

The study was conducted in 40 and 45-week-old Hyline layers flock at Mangalpur VDC-1, and Bharatpur Municipality-13 respectively from March to May 2011. All the laboratory work was done at Veterinary Teaching Hospital, Microbiology laboratory, Department of Microbiology and Parasitology, IAAS, Rampur, Nepal.

Newcastle disease vaccines and immune-modulators

Different lentogenic strain vaccines F (Indovac-FR, Batch No: 1010, Indovac Pvt. Ltd.), B1 (Himvac-B1R, Batch. No: 1A0410, Himvac Pvt. Ltd.) and LaSota (Indovac-LaSotaR, Batch. No: L2909, Indovac Pvt. Ltd.) were collected from local market and used during the experiment. The vaccines were stored and diluted during use according to the instruction of manufacturers. Similarly, three immune-modulators as Pentavit-CR (Livecare Nepal Pvt. Ltd., Nepal), E-Care-SeR (Provimi Animal Nutrition Pvt. Ltd., India) and Levamisole-200R (Intercheme Pvt. Ltd., Holand) were collected from local market and used during the experiment. The doses of these drugs were applied according to the recommendation of manufacturers as 10 ml/100 birds (vitamin A, vitamin D3, vitamin E, vitamin B12 and vitamin C at the dose rate of 666 IU, 333 IU, 2.66 mg 1.11 mcg and 0.55 mg per kg body weight respectively), 5 gm/200 birds (vitamin E at the dose rate of 1.38 mg/kg body weight, selenium at the dose rate of 0.0277 ppm/kg body weight) and 5 gm/200 birds (2.5 mg/kg body weight), respectively.

Experimental designs

The 40-week-old Hyline layers flock (4000 size, vaccinated with Indovac-LaSotaR 60 days before) was divided into four groups (A, B, C, and D) having 1000 birds each. Group A was used as control i.e. not vaccinated; and B, C and D groups were vaccinated with Indovac-FR (F), Himvac-B1R (B1) and Indovac-LaSotaR (LaSota) vaccines, respectively with skimmed milk powder.

Another 45-week-old Hyline layers flock (3200 size, vaccinated with Indovac-LaSotaR 60 days before) was divided into four groups (E, F, G, and H) having 800 birds each. Group E was used as control i.e. vaccinated with Indovac-LaSotaR. Also, groups F, G and H were vaccinated with Indovac-LaSotaR with skimmed milk powder. Pentavit-CR, E-Care-SeR and Levamisole-200R were given as immune-modulators in drinking water once a day for 7 days to groups F, G and H, respectively.

Collection of serum sample

Blood samples were collected for sera from 30 randomly selected birds from each group at 1 day pre-vaccination (DPrV), and 7th, 14th, 21st, 28th and 35th days post-vaccination (DPV) for the determination of haemagglutination inhibition (HI) antibody titre using standard HI test (Allan and Gough, 1974). To obtain serum which was tested for NDV antibodies, the blood was allowed to clot. Serum was obtained by tilting the syringe containing the blood, at an angle of 30^{o} to the horizontal surface. The sera were stored at -20^{o}C in the freezer until used. The suspension of 1% cRBC was stored at 40C and PBS solution at 40C for long period without contamination.

Haemagglutination (HA) test

The test was carried out (Allan and Gough, 1974) by two-fold serial dilutions of the viral suspensions in a U-shaped plate to determine 8 HA units (8HA/ 50µl). Commercially available F, B1, and LaSota strain vaccine was used as antigen for haemagglutination (HA) test.

Haemagglutination inhibition (HI) test

The test was performed (Allan and Gough, 1974) to determine the HI titre of the sera samples (detection and quantification of antibodies against NDV) collected from the birds. The test was conducted by using constant 8HA unit antigen and decreasing serum method (β procedure).

Statistical analysis

The data obtained were analyzed using computerized program MicrosoftR Office Excel (2007) to determine the Mean±SD and GMT of antibody titres, and computerized statistical program (SPSS-16) to determine Analysis of Variance (ANOVA), F-value, Coefficient of Variance (CV), and Least Significant Difference (LSD). For all statistical tests a p-value of <0.05 was taken to be statistically significant.

RESULTS AND DISCUSSION

The Mean±SD of HI titres and Geometric mean titres (GMT) in case of first experiment are presented in Table 1 and 2 respectively.

Table 1: Comparative HI titre in sera of 40-week-old Hyline layers following vaccination with different vaccines

Bird Group	Vaccine	HI titre (Mean ± SD) at different time periods					
		1DPrV	7DPV	14DPV	21DPV	28DPV	35DPV
A	Control (non vaccinated)	13.73±a 8.64	13.60±c 9.13	13.86±c 7.25	13.60±c 8.17	11.73±c 4.05	9.73±c 4.02
B	Indovac-FR	10.13±a 7.70	128.00±ab 71.3	960.00±ab 451.92	580.26±b 211.88	311.46±ab 173.63	130.13±b 56.95
C	Himvac-B1R	9.86±a 7.62	147.20±a 82.59	1075.20±a 364.52	742.40±a 271.84	362.66±a 157.96	168.53±a 70.27
D	Indovac-LaSotaR	10.40±a 8.57	98.13±b 72.74	819.20±b 255.11	503.46±b 264.53	258.13±b 154.48	109.86±b 51.58
F-value		1.485ns	24.158*	68.832*	62.566*	36.729*	50.707*

Bird Group	Vaccine	HI titre (Mean ± SD) at different time periods					
		1DPrV	7DPV	14DPV	21DPV	28DPV	35DPV
CV %		73.86	67.95	44.22	47.24	59.54	49.83
LSD		4.168	33.61	162.20	111.10	71.86	26.65

Values with different superscripts in column differ significantly at p<0.05.

DPrV=Day Pre-vaccination, DPV=Days Post-vaccination, SD=Standard deviation, ns = non significant,

- = significant at p<0.05.

Table 2: Comparative GMT in sera of 40-week-old Hyline layers following vaccination with different vaccines

Bird Group	Vaccine	GMT at different time periods					
		1DPrV	7DPV	14DPV	21DPV	28DPV	35DPV
A	Control (non vaccinated)	11.31a	11.31c	12.40c	11.84c	11.05c	8.97c
B	Indovac-FR	8.00a	111.43ab	851.18ab	548.74b	261.98ab	119.42b
C	Himvac-B1R	7.81a	125.00a	1024.00a	691.37a	322.53a	153.98a
D	Indovac-LaSotaR	8.00a	82.51b	776.04b	445.72b	217.77b	99.27b
F-Value		1.485ns	24.158*	68.832*	62.566*	36.729*	50.707*
CV %		73.86	67.95	44.22	47.24	59.54	49.83
LSD		4.168	33.61	162.20	111.10	71.86	26.65

Values with different superscripts in column differ significantly at p<0.05.

DPrV=Day Pre-vaccination, DPV=Days Post-vaccination, SD=Standard deviation, ns = non significant,

* = significant at p<0.05.

It was observed that the Mean±SD and GMT of groups A, B, C and D were statistically similar (p>0.05) at 1DPrV. The HI titre started to increase after vaccination in groups B, C, and D except A. There was increasing trend in HI titre up to 14DPV, afterward decreasing gradually. There was peak titre at 14DPV. But, the HI titre remained at protective stage up to 35DPV, at which good quantity of serum antibodies remained in blood. Among three vaccines, Himvac-B1R (B1 strain) showed higher HI antibody titres than Indovac-FR (F) and Indovac-LaSotaR (LaSota) respectively at 7th, 14th, 21st, 28th, and 35th DPV. It was observed that the F-value of HI titres among three vaccines was significantly different (p<0.05) at 7th, 14th, 21st, 28th, and 35th DPV except 1DPrV. The HI titres of group B, C and D were statistically differ (p<0.05) from control group A at 7th, 14th, 21st, 28th and 35th DPV. Similarly, the HI titres of group B and C, and C and D were statistically differ (p<0.05) from each other at 21st, 35th DPV, and 7th, 14th, 21st, 28th, 35th DPV respectively. The HI titres of group B and D were statistically similar (p>0.05).

The Mean±SD of HI titres and Geometric mean titres (GMT) in case of second experiment are presented in Table 3 and 4 respectively.

Table 3: Comparative HI titre in sera of 45-week-old Hyline layers following uses of different immune-modulators

Bird Group	Vaccine	HI titre (Mean ± SD) at different time periods					
		1DPrV	7DPV	14DPV	21DPV	28DPV	35DPV
E	Control (Indovac-LaSotaR)	5.93±a 3.46	98.43±b 66.66	708.26±a 313.05	418.00±b 215.06	247.46±b 146.24	93.86±c 35.58
F	Indovac-LaSotaR and Pentavit-CR	5.93±a 3.50	174.93±a 69.16	878.93±a 412.69	554.66±a 233.69	358.40±a 151.82	179.20±a 70.10
G	Indovac-LaSotaR and E-Care-SeR	6.80±a 4.59	128.00±b 58.22	785.06±a 307.54	503.46±ab 207.03	294.40±ab 130.83	146.10±b 67.06
H	Indovac-LaSotaR and Levamisole-200R	5.06±a 2.01	106.66±b 65.81	725.33±a 336.70	460.80±ab 194.84	273.06±b 119.94	115.20±c 50.84
F-value		1.284ns	8.367*	1.497ns	2.256ns	3.555*	12.527*
CV %		58.61	51.28	44.56	44.01	46.98	43.10
LSD		1.783	33.29	173.50	109.00	70.47	29.45

Values with different superscripts in each column differ significantly at p<0.05.

DPrV=Day Pre-vaccination, DPV=Days Post-vaccination, SD=Standard deviation, ns = non significant,

* = significant at p<0.05.

Table 4: Comparative GMT in sera of 45-week-old Hyline layers following uses of different immune-modulators

Bird Group	Vaccine	GMT at different time periods					
		1DPrV	7DPV	14DPV	21DPV	28DPV	35DPV
E	Control (Indovac-LaSotaR)	5.15a	84.44b	630.34a	370.50b	212.79b	86.42c
F	Indovac-LaSotaR and Pentavit-CR	5.15a	161.26a	794.18a	512.00a	322.53a	165.03a
G	Indovac-LaSotaR and E-Care-SeR	5.65a	116.7b	707.53a	466.80ab	268.10ab	130.99b
H	Indovac-LaSotaR and Levamisole-200R	4.70a	92.62b	630.34a	425.59ab	250.15b	103.96c
F-value		1.284ns	8.367*	1.497ns	2.256ns	3.555*	12.527*
CV %		58.61	51.28	44.56	44.01	46.98	43.10
LSD		1.783	33.29	173.50	109.00	70.47	29.45

Values with different superscripts in each column differ significantly at p<0.05.

DPrV=Day Pre-vaccination, DPV=Days Post-vaccination, SD=Standard deviation, ns = non significant,

* = significant at p<0.05.

It was observed that the Mean±SD of HI titres and GMT of groups E, F, G and H were statistically similar (p>0.05) at 1DPrV. The HI titre started to increase after vaccination in all groups. There was increasing trend in HI titre up to 14DPV, afterward decreasing gradually. There was peak titre at 14DPV. But, the HI titre remained at protective stage up to 35DPV, at which good quantity of serum antibodies remained in blood. Among three immune-modulators, Pentavit-CR showed higher HI titre than E-Care-SeR and Levamisole-200R respectively at 7th, 14th, 21st, 28th and 35th DPV. It was observed that the F-value of HI titres among three immune-modulators was significantly different (p<0.05) at 7th, 28th and 35th DPV; But, not significantly different (p>0.05) at 1DPrV, 14th and 21st DPV. The HI titres of group F showed statistically differ (P<0.05) from group E at 7th, 21st, 28th and 35th DPV. But, HI titres of G differ from group E at 35 DPV and no statistical difference between group E and H. There were statistical difference between group F and H at 7th, 28th and 35th DPV, F and G at 7th and 35th DPV, and G and H at 35DPV.

One of the principal objectives of this study was to evaluate the HI antibody titres after immunization with Indovac-FR (F), Himvac-B1R (B1) and Indovac-LaSotaR (LaSota) vaccines, and uses of Pentavit-CR, E-Care-SeR and Levamisole-200R in Hyline layers. There was increasing trend in HI antibody titre (Mean ± SD) and GMT up to 14DPV, which is in agreement with the statement of Chauhan and Roy (2003). Peak level of HI titres (Mean±SD) and GMT was found at 14DPV in group B, C, D, E, F, G and H, which is similar to observation of Rahman et al. (2004) where the author stated that HI antibody titres reached the peak levels between 2 to 3 weeks of vaccination. The peak HI titre was higher in the group C of birds vaccinated with Himvac-B1R (B1) than group B vaccinated with Indovac-FR (F) and group D vaccinated with Indovac-LaSotaR (LaSota) which is similar to the findings of Barua et al. (2008). There was statistical difference of group C from A, B and D. Similarly, birds in group B vaccinated with Indovac-FR (F) showed higher HI titre than group D vaccinated with Indovac-LaSotaR (LaSota), though there was statistical similarity between them. A comparison of those would indicate the superiority of Himvac-B1R to Indovac-FR and Indovac-LaSotaR. Though, previous result of Kamrunnahar et al. (2010) clearly indicated higher value of Mean±SD (production of HI antibody) in Cevac Vitapest- LR (LaSota strain) vaccinated flock than BCRDVR (F strain) and Izovac B1 HitchnerR (B1 strain) vaccinated flock. In contrast to the finding of this study, it has been found that LaSota strain gave better immunity than B1 (Creanga, 1972). Banu et al. (2009) found that LaSota strain produced higher immune response than those of other strains- B1 and Clone 30 strain. This finding is strongly supported to the findings of Almassy et al. (1979) and Mallick et al. (1969) who reported that LaSota strain provided superior antibody production after vaccination compared to B1. Westbury et al. (1984) also observed that LaSota is much more immunogenic than the Hitchner B1. The contrast in results of this study with others' might be due to geographical variation, management variation, and variation in vaccine and field strain to immune response based on different factors. However, Ibrahim et al. (1983) compared F strain of ND vaccine with B1 and LaSota, and found no significant difference among these three strains. It was earlier stated that among the lentogenic strains, LaSota strain is more virulent in the host than B1 or F, and causes more post-vaccination respiratory symptoms (Allan et al., 1978 a, b), whereas B1 was a little more virulent and effective than F (Bran et al., 1961). This statement suggests that B1 is better to use than F and LaSota respectively.

The HI antibody titre (Mean±SD) and GMT of group F treated with Pentavit-CR (mulitvitamins) was comparatively higher than that of group G treated with E-Care-SeR (vitamin E and selenium), and group H treated with Levamisole-200R. A comparison of those would indicate the superiority of Pentavit-CR to E-Care-SeR and Levamisole-200R. The result of group F revealed positive effects of Pentavit-CR (multivitamins) on the immune response (HI antibody titres) when vaccinated with NDV, which has statistically different HI titres from group E. The result of HI test had a close relationship with the finding of Sklan et al. (1994) who observed that the immune response of chicks were higher when fed with additional vitamin A in their normal ration compared to the additional vitamin A deprived group. Vitamins represents a heterogeneous group of fat soluble (A, D, E and K) and water-soluble (vitamin B complex, vitamin C) chemical compounds essential in nutrition. All recognized vitamins with the exception of vitamin C are dietary essentials for poultry. Mineral preparations are important for growth, development and immunity. The result of group G revealed that E-Care-SeR has positive effects on HI antibody titres, but HI titres differ staitstically from control group E only at 35DPV. This finding is in agreement with the findings of Swain et al. (2000) who recorded that vitamins and minerals increased cellular and humoral immunity in broiler. However, Brunner and Muscoplat (1980) indicated that immunomodulators do not exert their effect on normal cells; there were higher titres in group F and G than in group E. The similarity in Mean±SD and GMT of Levamisole-200R treated group H and control group E seemed to indicate that levamisole as used in this study have limited effect (no appreciable effect) on the humoral immunity of ND vaccination, which is similar to the findings of Sanda et al. (2008).

Immune response of birds towards virus (antigen) is divided into four different phase as Recognition phase (0-7 days), Effector phase (7-14 days), Decling phase (14-30 days) and Memory phase (>30 days) (H.B. Basnet, 11 december, 2011, personal communication). In recognition phase,

there is initiation in antibody production against specific antigen, which becomes optimum in effector phase. The antibody titres then after gradually decline in decling phase and remains minimal in memory phase having memory cells mostly. In initiation, decling and memory phase of antibody production, there is sub-optimal antibody titre and the immune-modulators have positive stimulating effects on antibody producing cells. But, in effector phase, there is already presence of high titre and antibody producing cells beyond this is in platue, at where immune-modulators have negative stimulating effects on antibody producing cells. This above statements support occurrence of significant different among three immune-modulators at 7th, 28th and 35th DPV (recognition, decling and memory phases) and non significant different at 1DPrV (no use of immune-modulators), 14th and 21th DPV (effector phase).

In a study, Numan et al. (2005) found HI GMT for layer of 37- 47 weeks age group was to be 149.62, concluded to be satisfactory in level of protection against NDV. This finding is in agreement with finding of the study that the antibody titre started to increase after vaccination and remained peak at 14 DPV, and at protective stage up to 35 DPV.

CONCLUSIONS

As regards to the principal objective of the present study it may be stated that production of antibody titres (Mean±SD and GMT) is higher in flock vaccinated with Himvac-B1R (B1 strain) than Indovac-FR (F) and Indovac-LaSotaR (LaSota). A comparison among three vaccines used indicates the superiority of Himvac-B1R to Indovac-FR and Indovac-LaSotaR in seroimmunologic aspect. Similarly, it may be stated that production of antibody titres is higher in flock treated with Pentavit-CR (multivitamins) than E-Care-SeR (vitamin E and Se) and Levamisole-200R. A comparison among three immune-modulators used indicates the superiority of Pentavit-CR to E-Care-SeR and Levamisole-200R in seroimmunologic aspect. It is better to use immune-modulators during vaccination for 7 days, mostly Pentavit-CR (multivitamins) and E-Care-SeR (vitamin E and Se). However, Levamisole-200R has limited effect on antibody production after immunization with ND vaccines. Post vaccine immune response must be monitored by serological tests to make sure that the vaccination was proper and evoked adequate immunity. The HI test is most widely used test to do seromonitoring after vaccination. The antibody titre started to increase after vaccination and remained peak at 14 DPV, afterward decreasing gradually. But, the titres remained at protective stage up to 35 DPV at which good quantity of serum antibodies remained in blood.

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STUDY ON THE EFFECT OF STINGING NETTLE *(Urtica dioica)* ON PRODUCTIVE PERFORMANCE OF LAYER CHICKEN

M. Dahal[1] and D.R. Khanal[2]

ABSTRACT

The properties of stinging nettle (Urtica dioica) on productive performance in layer chicken have been demonstrated in a pilot study conducted at the Animal Health and Research Division (AHRD), Khumaltar, Lalitpur from September 2010 to February 2011. A total of 50 day old female chicks and 10 male chicks of Hyaline strain were divided into two groups; as control and treatment comprising of 25 female chicks and 5 male chicks in each group. The treatment (T) group received commercial layer feed along with shade dried nettle powder from eight weeks onwards at 1% daily level up to 16 weeks. From 17th weeks onwards treatment group received 7% nettle once a week up to 20 weeks while the control (C) group received only commercial layer feed. In both groups, the amount of commercial feed offered to chicken was based on the standard feeding norms. Daily egg production was recorded to determine weekly laying performance. Egg quality parameters such as egg shell thickness and egg albumen height were measured by using micrometer screw gauze and tripod micrometer, respectively. Blood samples were collected on the 8th week of post vaccination against Newcastle disease (ND) and antibody against ND viruses was measured by haemagglutination inhibition (HI) method. Haugh Unit of the opened eggs was also calculated from both groups. Available data until 20 weeks of trial revealed higher number of egg production in treatment group than in control group by 19.7 %. The albumin height was significantly ($p<0.01$) higher in T (6.54±0.23) mm group than in C (5.76±0.22) mm group. The egg shell thickness was significantly higher in T group (0.40±0.23) mm than C (0.37±0.22) mm group. Treatment group had higher level of antibody titer against ND virus (64-512) on 4HAU as compared to control group (32-128). Haugh unit was relatively higher in T (82.43±2.29) group than in C (75.69±1.44) group. These findings indicated that nettle supplementation increases the egg productivity, improves the egg quality and immune status of laying hens.

INTRODUCTION

Poultry industry is rapidly growing in Nepal. Since last decades more number of peoples are adopting this business. Country is gradually shifting towards intensive farming system from the backyard farming. Poultry industry shares 3% of the total GDP and 8% of the agricultural GDP (APSD, 2009). Therefore it is obvious that poultry industry has importance to contribute nation's economy (VEC, 2007). The demand of eggs and chicken meat has risen steadily over years. This is due to rise in the income level and of people changing food habit. This will further create more market and opportunities for further expansion of this sector (Neupane et al., 2009). According to recent data total no of poultry in the country is approximately 25 million among which the laying hens are about 71, 53,088 which produce 61, 74, 55,000 eggs per year. Though the production seems to be high, total production per household per year is only 150 (VEC, 2007).

The outbreak of different diseases like avian influenza, ND, IBD has marred the poultry industry.

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In 2009 alone, 5,900 and 12,015 birds had died from ND and IBD respectively in the Katmandu (VEC, 2009). Similarly, the endemic diseases have seriously affected the industry that decreased the production. Import of inferior chicks through illegal channel from India in the domestic market has created a low productive performance. This has created imbalance between production and consumption. Herbal preparations are more affordable than chemical drugs. In the recent years, some drug companies are claiming to have developed immunomodulators to be used in commercial poultry farms. Many herbal preparations are presumed to have immunomodulatory effect with no or less side effects. In this regards , rearch was conducted to evaluate the effect of stinning nettle (Urtica dioica) Immunomodulatory property of nettle (locally known as Sisno) have been documented by (Khanal, 2005a, 2005b), Piya (2006), Gautam (2007), Maharjan (2008), Poudel (2009) and Regmi (2010) at Agricultural Research Station, Pakhribas and Animal Health Research Division (AHRD), Khumaltar, respectively.

The goal of present study was nettle fed to layers poultry diet and its effect on immune status against ND and its productivity parameters were investigated.

MATERIALS AND METHODS

This study was carried out at Animal Health Research Division (AHRD), Khumaltar, Lalitpur from September 2010 to March 2011.

Experimental setup

A total of 60 chicks (50 female and 10 male) of Hyaline strain were purchased from Avinash Hatchery, allocated randomly into two groups, each group comprising 25 female and 5 male chickens. The birds were divided into two groups namely;

- Control(C) receiving only a layers diet (Pellet).
- Treatment (T) receiving a pellet layers diet + Nettle (from 8th week onwards at 1% daily level up to 16 weeks. From 17th weeks onwards treatment group received 7% nettle once weekly up to 20 week).

Feeding schedule

The adult birds after 20 weeks were fed commercial pellet layers ration at the rate of 150 gm per bird per day. During the day time no additional light was provided, but at night time one CFL bulb was provided for light in each room. Water sanitized with water guard (chlorine) was given ad libitum. The birds were fed with commercially available feed as starter, grower , developer and layers feed in the age group of 1-4 weeks, 5-10 weeks, 10-16 weeks, and >16 weeks respectively on the standard feeding norms.

Nettle supplementation

Nettle was supplemented at 1% level daily from 8th to 16th week of age. From 17th week onward 7% nettle was supplemented once weekly until the end of trial.

Assesment of egg production

The eggs were collected daily and total number of eggs laid was recorded on weekly basis for 4 weeks only due to lack of time.

Assesment of egg quality

Egg shell thickness

Ten eggs were collected from both groups to measure egg shell thickness by using micrometer screw gauze. Three readings were taken from the single shell of three different parts namely apex, base, and middle part.

Egg albumen height

Ten eggs from each group were broken and content was poured into a petridish and albumen height was measured by using tripod micrometer (spherometer) as described by Haugh (1973).

Calculation of the Haugh Unit

With the available data of albumen height and egg weight, the Haugh unit was calculated to determine the quality of opened egg, using standard formula as given by HU= 100 log [H+ 7.57-1.7 W.37] (Card & Neschiem, pp, 291-295, 11th edition).

Assessment of immune status

Blood samples (0.5-1ml) of five birds from each treatment and control group were randomly collected from wing vein using 22 gauge needles on 8th week of post vaccination with Newcastle Diseases Virus. The syringes with blood were kept in inclined position for 24 hours. Then the syringe was kept in refrigeration for 2 hours. Clear serum separated in the upper portion of syringe was poured in sterile serum containing vials and was stored in the freezer. The serological tests followed were Hemagglutination (HA) and Hemagglutination Inhibition (HI) tests as described by OIE Manual (2004).

Preparation of blood smears and differential leucocytes counts

Thin blood smear was prepared from the blood samples from both treatment and control groups collected on 28 days post vaccination. The smears was stained with Giemsa stain and allowed to dry. After complete drying the slides were observed for differential leukocytes count.

Data Analysis

The experiment values were statistically processed and then the comparisons between means were done, using t-test , included within the MS-Excel (2003) software program.

RESULTS AND DISCUSSIONS

Egg production

The treatment group started to lay eggs one day earlier than the control group. The egg production data was recorded for four weeks. The egg laying performance of control and treatment group is presented in figure 1. The increment in the egg production in the treatment group was 19.71% as compared to control group (255 versus 213).Khanal et,al (2009) has also indicated a positive response of nettle on performance of ready to cull hens that had a significant increase in production after nettle supplementation. Improvement on egg laying performance may be attributed to presence of high amount of calcium, phosphorus, vitamins and non- specific immunomodulators in the nettle that might activate the gene responsible for egg laying (Khanal, et., al 2008). Poudel (2009) and Regmi (2010) also reported that the supplementation of nettle on chickens significantly increased the egg production.

Assessment of Egg quality

Table 1: Showing parameters of egg quality

Parameters	Control	Treatment
Albumin Height (mm)	(5.76±0.22)	(6.54±0.23)
Shell Thickness (mm)	(0.37±0.22)	(0.40±0.23)

Albumin Height

The thickness of albumen height measured by Tripoid micrometer (Spherometer) showed higher albumen height in the egg of nettle supplemented birds than in the eggs of control group. Similar result was also obtained by Poudel (2009) and Regmi (2010) with better response in 10% nettle supplemented groups.

Shell thickness

Measurement of shell thickness of eggs revealed that treatment group had higher thickness (0.40±0.23mm) compared to control group (0.37±0.22mm). Higher shell thickness in nettle supplemented group was attributed to higher calcium content in the stinging nettle. The diet of hens must contain adequate calcium in a form that can be utilized efficiently (Roberts, 2004). Roland et al (1994) have stated that adequate calcium in poultry diet enhances shell quality. Since nettle has considerably higher level of calcium in diet, this may have enhanced the shell thickness due to more deposition of calcium carbonate in eggshell.

Effect on antibody titer of ND in serum:

The result of HI test in sera showed that the HI titer against NDV at 8th weeks post vaccination with Lasota vaccine was higher in the treatment group than the control group. The details are presented in annex 1.

Table 2: HI titer against NDV

Parameters	Control	Treatment
Range of HI Titers against NDV (n=10)	32-218	32-512

The result of HI test in sera showed that the titer against NDV at post vaccination with lasota vaccine was higher in the treatment group than the control group. Similar result was obtained by (Allan & Gough, 1974). The results were similar to that of Piya (2006) and Maharjan (2008) where immune status was analyzed in broilers supplemented with nettle powder. Vijaya (1994) also found similar evidence that immune status in broilers administered with Zeetress (Indian Herbs, Banglore) exhibited higher antibody titers compare to control group.

White blood cells pattern

The treatment group with nettle supplementation had comparatively higher proportion of lymphocytes than non- supplemented group.

Table.3. Differential white blood cells in two different of groups.

	Treatment	Control
Lymphocytes	64 ± 2.38	62 ± 2.68
Heterophils	18.4 ± 4.05	18 ± 2.75
Monocytes	4.8 ± 1.01	3.6 ± 0.74
Eosinophils	6.4 ± 2.06	5 ± 1.58

Similar result was also found by Poudel (2009). Wagner et al. (1989) has also demonstrated an increased lymphocyte proliferation by nettle extract on experimental animals. Immune stimulation causes peripheral lymphocytes with more number of reactive lymphocytes (Khan, 2005). Thus it can be conclude that nettle feeding has stimulating action on immune system.

Colour of the eggs

Colour of the eggs in nettle supplemented groups was comparatively more appealing than the non- supplemented group. White spots were seen at times on the outer surface of the shell in the control group eggs but treatment groups eggs were dark brown in colour.

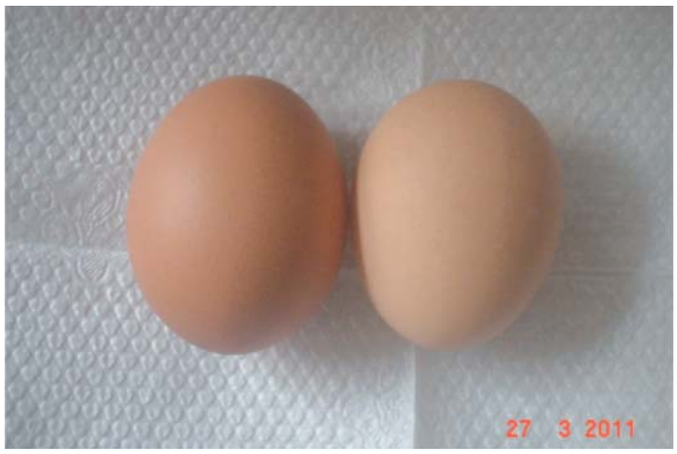

Fig. 1: Comparision of colour between control & treatment group's egg.

Calculation of Haugh Unit

The relation of Haugh unit releaved that (75.69±1.44) on control group whereas (82.43±2.29) on treatment group. Haugh Unit significance quality of opened egg. High HU indicate higher grade of eggs (USAD quality score for 2-ounce eggs).

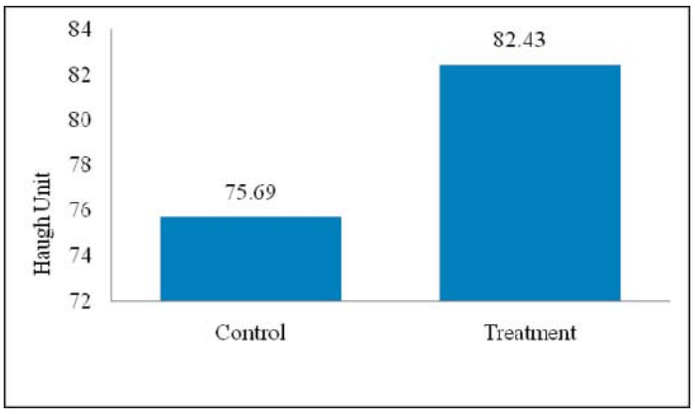

Fig. 2: Showing the Haugh unit of two different groups.

CONCLUSIONS

From the study, it can be concluded that nettle can enhance the antibody titer against New Castle diseases in laying hens. Nettle can also increases the calcium content of egg and has positive influence on egg albumen content. Egg albumen height, shell thickness, colour of the eggs, egg size and weekly egg production intensity all were different between the treatment and the control groups. The main component in nettle with combination of good amount of vitamins and minerals make it a promising plant enhance organic poultry farming and reduce reliance on chemical drugs.. Nettle is beneficial in improving the productivity, eggs quality and immunity in poultry industries.

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SERO-BIOLOGICAL EVALUATION OF THREE DIFFERENT STRAINS OF NEWCASTLE DISEASE VACCINES IN MALE LAYER CHICKEN

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ABSTRACT

The efficacy of F, B1 and LaSota strains of Newcastle disease vaccine in male layer chicks were compared by two methods: i) monitoring the serum antibody level using Hemagglutination Inhibition test after intra-ocular primary vaccination on day 3rd of age followed by intra-ocular secondary vaccination at day 23rd of age, and ii) monitoring clinical signs and mortality for 12 days after challenging the chicks with velogenic strain of Newcastle disease virus on day 48th of age. Serum antibody levels, after 12, 14 and 16 days of primary vaccination, were highly significant (p=0.01) for the groups of chicks vaccinated with B1 strain, while those for the Groups vaccinated with F and LaSota strains were significantly high (P=0.05). After 12, 14 and 16 days of secondary vaccination, highly significant (p=0.01) serum antibody levels were recorded for the groups of chicks vaccinated with B1 or LaSota strains, that were previously vaccinated with B1 strain on day 3rd of age. Serum antibody levels for the groups of chicks vaccinated with F and LaSota strains were significantly high (p=0.05). After both primary and secondary vaccination, significant difference in the serum antibody levels were found for the groups of chicks vaccinated with B1 strain on day 3rd of age than groups of chicks vaccinated with F or LaSota strains. Morbidity and mortality after disease challenge were 100% in the unvaccinated control group, while lowest morbidity was recorded in group vaccinated with B1 strain on day 3rd and day 23rd of age, which was 20%, followed by group that received B1 strain on day 3rd and LaSota strain on day 23rd, in which morbidity was 30%. None of the chicks died in these two groups, while there were variable level of morbidity and mortality in other groups of chicks not vaccinated with B1 strain. Thus for primary vaccination, B1 strain among the three strains seems to be best. For secondary vaccination B1 or LaSota strains can be used to produce maximum serum antibody level. Protection level against the disease challenge with velogenic NDV is associated with higher serum antibody level and unvaccinated chicks cannot resist the disease challenge.

INTRODUCTION

Newcastle disease (ND) is caused by Newcastle disease virus of the avian paramyxovirus type I (APMV-1) serotype of the genus Avulavirus belonging to the family Paramyxoviridae. There are nine serotypes of avian paramyxoviruses designated APMV-I to APMV-9, APMV-I being most pathogenic to the poultry (OIE, 2008). NDV has been shown to be able to infect over 250 (Alexander et al., 1997) species of birds, but the severity of disease produced varies with both host and strain of virus. The less pathogenic strains may induce severe disease when exacerbated by the presence of other organisms or by adverse environmental conditions. The preferred method of diagnosis is virus isolation and subsequent characterization. ND is the highly contagious and destructive disease which attack mostly chicken, turkey, pigeons etc. Water chicks and sea chicks act as carriers of the virus. In Nepal, ND is popularly known as Ranikhet disease, and it is the most notorious

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malady in backyard poultry, claiming 90% of the total mortality with great economic loss, estimated at 74.77 million rupees in 1990 (Mishra 1992). However Paudel, (2011) found that 5% of samples were sero-positive for NDV in Chitwan and Nawalparasi district.

Newcastle disease is one of the most dangerous diseases in poultry industry and till date there is no any method or drug available that can cure this condition. ND is responsible for great economic losses to the farmers every year. Quarantine, vaccination and bio-security are the only tools to combat this problem. Vaccination against ND is practiced in almost all the commercial poultry farms of the country. There are different vaccines available of different strains, companies and countries and are greatly effective too. However frequent outbreaks of ND all over the country have been reported throughout the year. In this scenario, the present study is beneficial from the point that it not only enumerates the different levels of antibody in response to the vaccination with different strains, but also studies the response of chicks after challenge by live ND virus and different pathological and clinical changes in chicks due to ND.

MATERIALS AND METHODOLOGY

Even A total number of 74 day-old Hyline male layer chicks were collected from local market. 10-days old live chicken embryos were purchased for the virus titration process.

The experiment was conducted at 'Livestock Development Farm', Institute of Agriculture and Animal Sciences. Two separate sheds were used during the experiment, the first one was considered as Treatment Unit and second one as Control Unit. In treatment unit, all the chicks were kept from day-old to 48 days. At age of 48 days, 10 chicks were transferred to a control shed and all the remaining chicks in treatment group were challenged with velogenic strain of Newcastle disease virus.

The experimental sheds were fumigated with formalin and potassium permagnet, according to the recommendation of Food and Agriculture Organization (FAO). Within the sheds the different groups of chicks were separated into 4 different compartments on day 3, three groups did contain 20 chicks and one group was with 10 chicks. On day 23, 10 chicks from each group 1, 2 and 3 were taken and were reared in three different compartments and named group 7, 5 and 6, now there are 7 different groups with 10 birds in each group.

On day three, 4 chicks were selected randomly and sacrificed for the collection of blood samples to know the maternal antibody level.

On day 3 the chicks were vaccinated against Newcastle disease Intra-occularly with 'Freeze Dried Culture of Attenuated Newcastle Disease Virus (F Strain)', 'Freeze Dried Live Attenuated Himmvac Newcastle B1 Live Vaccine', 'Freeze-Dried Culture of Lentogenic LaSota Strain' to the groups 2, 3and 4 respectively (Table 1), while group 1 was used as unvaccinated controlled group.

On day 23 again the chicks from compartments 2 and 3 were vaccinated with 'Freeze Dried Culture of Attenuated Newcastle Disease Virus (F Strain)', 'Freeze Dried Live Attenuated Himmvac Newcastle B1 Live Vaccine', respectively and groups 4, 5 and 6 were vaccinated with 'Freeze-Dried Culture of Lentogenic LaSota Strain'.

On day 48 all 10 chicks from group 7 were transferred to control unit (separate shed). Rests of the groups were challenged with indigenous isolate of live Newcastle Disease virus of velogenic strain. Virus was obtained from Central Veterinary Laboratory, Kathmandu. Strain and concentration of the sample was determined by conducting the Embryo Infectious dose (EID50) and Mean Death time.

All the dead chicks were taken to Veterinary Teaching Hospital, IAAS Rampur, for post mortem examination. Tissue samples were collected and were processed and then inoculated in chicken embryo for isolation of the virus and for confirmative diagnosis of the Newcastle disease.

On day 48 all chicks from group 1, 2, 3, 4, 5 and 6 were challenged with concentration of 105EID50/ml velogenic strain of Newcastle disease lives virus orally. Chicks in the 7th group were not challenged and were kept as negative control group (no vaccination, no challenge). Chicks were observed for clinical signs and mortality for 12 days post challenge. All dead chicks were sent for postmortem examination to the Veterinary Teaching Hospital. Tissue samples were stored in 50%buffered glycerol. These tissue samples were ground, mixed with antibiotic solution and were inoculated in 10 day old chicken embryo. HA and HI test was performed for the confirmatory diagnosis of Newcastle disease.

RESULTS

Maternal antibody response

Maternally derived antibody (MDA) was derived on day 3. Blood samples of four chicks were subjected to HI test and the geometric mean of HI titrel was found to be 38.06 HI units, with a standard deviation of ±16.

Serum antibody response after vaccination

The principle goal of this study was to determine the immune response of commonly used three different strains of vaccine in male layer chicken.

It was observed that on day 12, after primary vaccination, the serum antibody titres (HI titres) were 2.7±0.80, 5.57±0.79, 6.45±0.69 and 5.6±0.52 in the chicks of groups 1, 2, 3 and 4 respectively. On day 14 post post first vaccination, HI titre were 2.5 ± 0.61, 5.7 ± 0.86, 6.55 ± 0.69 & 5.4 ± 0.69 for the groups 1, 2, 3 and 4 respectively. Again the HI titres of chickens after 16 days of vaccination at the age of day three were derived to be 2.4 ± 0.59, 5.7 ± 0.92, 6.55 ± 0.89 and 5.4 ± 0.97 for the groups 1,2,3 and 4 respectively. It was thus found that the titre levels of group 2, vaccinated with B1 strain on day 3 of age of the chicks, were highly significant (p=0.01) and the titre levels of group 1 and 3, vaccinated with F and LaSota strain respectively on same age, were significant (p=0.05) when compared to the non vaccinated group of chicks-group 1.

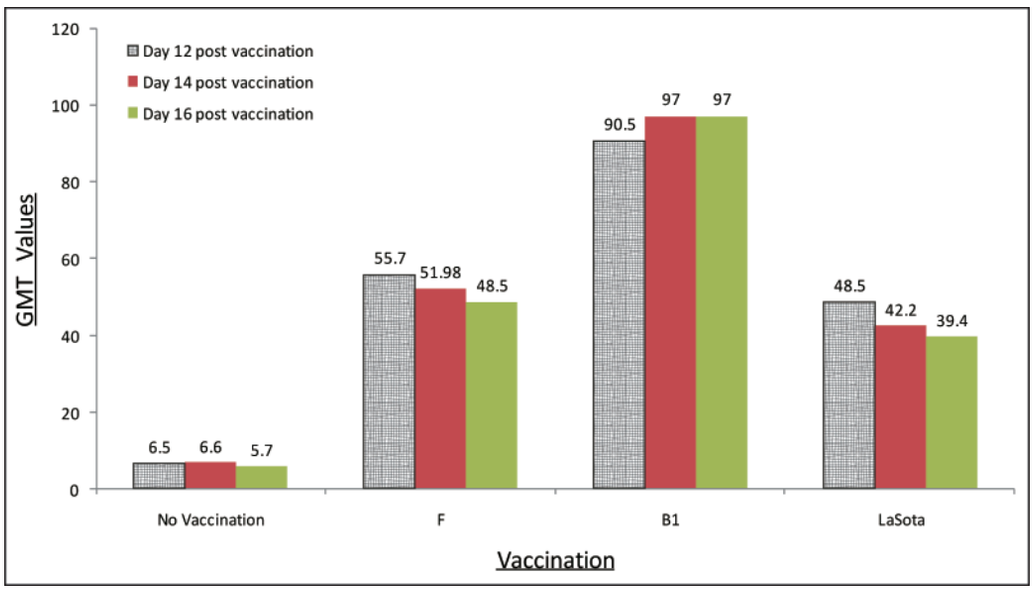

Fig. 1: Geometric Mean Value of HI titres after 12, 14, 16 days of first Vaccination

On day 23 of age, the chicks were vaccinated and on the days 12, 14 and 16 after that vaccination, HI test were carried out in order to find out the serum antibody level. On day 12 the values of HI titre were 2.2±0.42, 6.4±0.70, 7.6±0.70, .6.1±0.99, 6.1±0.99, 7.2±0.79 and 2±0 regarding groups

1, 2, 3, 4, 5, 6 and 7 respectively. It was observed that the serum antibody level of chicks for the group 3 and 6 were highly significant (at p=0.01), and the GMT values of groups 2, 4, and 5 were significant (at p=0.05) when compared to the unvaccinated group. Similarly HI titre on day 14, post secondary vaccination were 2±0, 6.4±0.69, 7.6±0.70, 6±0.94, 6±0.81, 7.4±0.69 and 2±0 respectively, for the groups 1, 2, 3, 4, 5, 6 and 7 respectively. The antibody level of groups 3 and 6 were highly significant (at p=0.01), and that of group 2, 4, and 5 were significant (at p=0.05). On day 16, after second vaccination, HI titres were 2±0, 6.3±0.67, 7.5±0.71, 5.9±0.99, 5.9±0.88, 7.3±0.67 and 2±0 for the groups 1, 2, 3, 4, 5, 6 and 7 respectively. Likewise in cases of day 12 and day 14, the titre level of group 3 and 6 were highly significant (at p=0.01) and titre levels of group 2, 4, and 5 were significantly different at p=0.05 (Table-5).

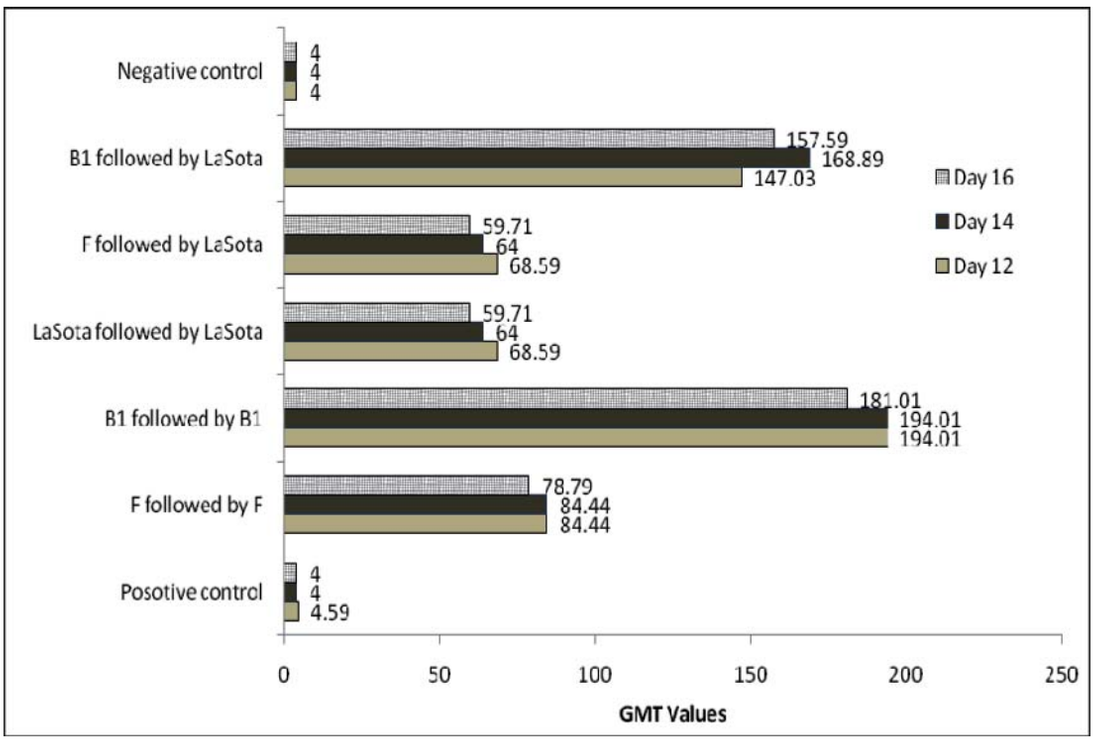

Fig. 2: Geometric Mean Titre Values for different groups after secondary vaccination

Clinical signs and post mortem lesions

Most of the dead chicks showed severe pathological lesions with haemorrhages throughout the intestinal, especially in small intestine and caeco-cloacal area. Almost all of the dead chicks showed lesions in the caecal tonsils with button-like ulcers and haemorrhages in the tip of proventricular glands, whereas few chicks had congestion in the lung and trachea. Spleenomegaly was also observed.

Morbidity and mortality after challenge

Morbidity and mortality were recorded for 14 days after challenge. There were no signs of sickness of chicks until 24 hours of infection. After 24 hours of disease challenge, chicks from Group 1 started showing prominent respiratory signs. After 36 hours after challenge, some chicks in the same group started nervous signs like marked depression, greenish diarrhoea, head tremor and twisting, paralysis and occasional lateral recumbency. In treatment group first sign of sickness was prominent in group 4 after 72 hours of challenge. Then chicks from all the groups slowly developed noticeable signs of sickness. All the chicks in the control group and the group 4 went sick, that is 100% morbidity. Least morbidity were recorded in groups 3 and 6, where 20% chicks did show clinical sign in group 3 and 30% chicks showed clinical signs in group 6. In Group 2 and

5 the morbidity rates were 70% and 80% respectively.

All the dead chicks were subjected to postmortem examination and tissue samples were collected for egg inoculation for confirmative diagnosis. Tissue samples from one chicks were considered as one unit and one unit was inoculated in one, 10 day old, embryonated chicken egg. HA and HI test were carried out all the samples and all of them showed positive results. For HI test, standard chicken antiserum for Newcastle disease virus was used for confirmative diagnosis.

Death of the chicks came only after 3 days of challenge. At first, mortality occurred in the control group, followed by group 4. In the positive control group, all the chicks died within 6 days after challenge. On group 4, first mortality was recorded on day 5 post challenge. 40% of the chicks died in the group 4, whereas none of the chicks died in the groups 3 and 6. Mortality rate in group 2 and 5 were 20% and 40% respectively.

In Group 7 (Negative control Group), there was no morbidity and mortality, because the chicks in Group 7 were not challenged with Newcastle Disease Virus and the chicks of this group were reared in different poultry shed.

Morbidity and Mortality of chicks in different groups vaccinated with different combination of vaccines

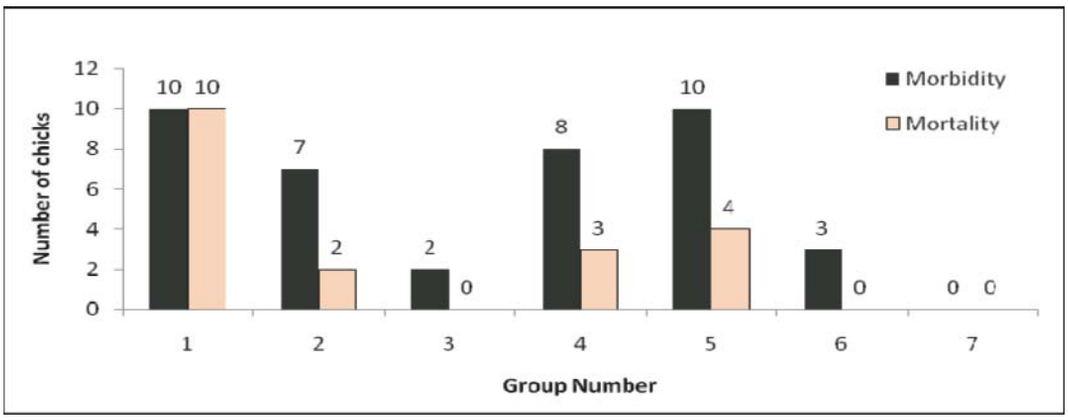

Fig. 3: Morbidity andmortality of chicks after challenge

DISCUSSIONS

Maternal antibody

Spradbrow et al (1978) and Schalkoort & Spradbrow (1980) considered a HI titre of >8 indicative of immunity to virulent NDV. Mean HI titre level up to 32 should prevent from infection to Newcastle disease (Boktor, S. N. 1979, Schmidt and Schmidt 1955). Bwala (2002), reported that the more than 80% of chicks having serum antibody HI titre above 32, at the age of day 3, resisted the virulent Newcastle disease challenge on day 7, but the chicks having serum antibody titre below 32 couldn't survive the challenge.

Thus the chickens appeared to have a good maternal immunity on day three. But on successive tests for the presence of serum antibody level of the unvaccinated group, it declined sharply and HI titres of unvaccinated group at the age of day 15 was below 8 and it was decreasing continuously. Thus the chicks in control group seemed vulnerable to NDV infection before the age of 15 days.

Clinical signs after vaccination

A number of authors considered about development of clinical signs of Newcastle disease after vaccination with different strains Newcastle disease vaccines. But here in this study clinical sign, regarding Newcastle disease was not observed. This may be because the shed where the chicks

were kept was thoroughly disinfected, feed given to them was ad-libitum, and temperature of the room was maintained.

Serological response

The result of serological study shows that the B1 vaccine is better among the three vaccines used in the study (Fig. 1 and Fig. 2.) The HI titre level for the group of chicks that were vaccinated with B1 strain were statistically higher ($p=0.01$) as compared to the other groups.

These results of serological results support the finding of Rahman (2002), who reported higher antibody level of chicks vaccinated with B1 strain than F and ND clone 30 strain. Miah et al., (1989) also documented better immune response of chicks after vaccination with B1 strain than LaSota strain in Pakistan.

On revaccination with killed mesogenic R2B strain, the serum titre level of the chicks those were previously vaccinated with F strain was significantly higher as compared to the chicks those were previously vaccinated with LaSota strain (Barman, L. R., 2002). Here in this study also comparison between Group 4, vaccinated with F strain on day 3, and group 5, vaccinated with LaSota strain on day 3, shows that upon revaccination with LaSota strain on day 23, HI titres is higher in the Group 5.

But contrasting result was documented by Parede & Young (1990), Rehmani (1996), Bwala (2002), OIE (2008) where LaSota strain gave better titre level than other strains of Newcastle disease vaccines including B1 strain. The reason behind the low antibody level of LaSota strain may due to superior quality of other strains, degeneration of the strain during manufacturing, transportation or storage etc.

4.4 Challenge study

In this study there was 100% mortality in case of control group, whereas in any other treatment groups mortality was not 100%. It can here be discussed that in unvaccinated group, the protection level is zero. This is in accordance with an experiment of vaccination of chicken with six different strains of Newcastle disease vaccine; all vaccines except the control gave 90% protection to clinical signs and mortality (Miller et al, 2007).

Schmidt and Schmidt (1955) studied the relationship between HI titres and protection capacity for a 10 months period after vaccination. They found, that a variable percentage (8.5-95%) of chicks having HI titres up to 16 fail to resist the challenge infection against virulent NDV and those having titres 32 and above resisted the challenge infection.

The level of protection against challenge virus in the groups 3 and 6 were found to be highest. In these two groups, at least for once, B1 vaccine was applied. Although there are some morbidity in those groups, but none of the chicks died in those group. The reason behind the low morbidity may be due to the significant high level of serum antibody level in these two groups of chicks. Kapczynski and King (2005) demonstrated that a positive correlation exists between the presence of higher level of antibody titres and the subsequent protection offered post challenge. The result of the present study are also in agreement with those of Parede and Young (1990), who determined that in chicks with high antibody titres, mortality after challenge with the virulent field strains is also low.

Mortality in different treatment groups were different, which is in accordance to Liu et al., (2003) who reviewed that the ND vaccines may not produce adequate protection against velogenic challenge. Work done by Beard et al., (1993), Shilva et al., (2004), Perozo et al., (2004, 2008) using different strains of vaccine against different isolates of velogenic Newcastle disease virus, reported level of protection with different strains of vaccines.

The absence of post challenge mortality in the Group 3 and Group 7 could be attributed to the

protection offered by vaccination. The high level of protection from mortality demonstrated in Group3 and Group 7 shows that the chicks can be protected from ND-related deaths when compared with the deaths in the unvaccinated chicks.

The protection result emanating from the present study concurs with quite a number of ND vaccine trials (Asplin, 1952; Parede & Young, 1990; beard et al., 1993, perozo et al., 2004; Miller et al., 2007), all of which demonstrated that the proper application of vaccine can protect chicks against Newcastle disease.

The present study also confirmed that the application of B1 strain can confer protection against Newcastle disease, since none of the chicks manifested death form ND. This agrees with the report by Rehmani (1996), where a single application of B1 vaccine at 12 days of age was sufficient to offer responsible protection against ND challenge.

The chicks in the unvaccinated control group were not protected and all died from the challenge. And there was no mortality seen in Group 3 and Group 6. All the control chicks in either experiment died within the six days after challenge which met the OIE requirements for such challenge trials, which demands at the end of 10 days, 90% of the vaccinated chickens survive, but all controls die within 6 days.

CONCLUSION

This can be concluded here that Newcastle disease is a serious fatal disease of poultry and in can cause mortality up to 100%. Vaccination is an effective measure to protect chicks from this disease. Vaccination at early age should be practiced in order to maintain adequate serum antibody level, which is enough to protect chicks from mortality due to ND.

For primary vaccination of chicks against Newcastle disease, B1 produces highest serum antibody level, which was statistically defined here in this study. For secondary vaccination of chicks against Newcastle disease, both B1 and LaSota vaccines produce highly significant immune response. Between these two strains B1 vaccine produces higher serum antibody level. So B1 vaccine should be used in both primary and secondary vaccination. This is proven by the 100% level of protection of chicks, immunized with B1 strain, from death due to Newcastle disease challenge.

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IDENTIFICATION OF POISONOUS PLANTS IN WESTERN MID HILLS OF NEPAL

R. P. Sah[1], K. P. Dhungana[1], D. R. Khanal[2] and H. R. Paudel[3]

ABSTRACT

Poisoning of animals occurs frequently in rural areas of Nepal, when animals are mainly fed with forest fodders which may accidentally consist of poisonous plants resulting economic loss in livestock production. Investigators made an attempt to identify poisonous plants with their toxic effect in goats and sheep available in western hills. A semi-structural questionnaire survey format was developed, distributed to a total of 96 goat and sheep raising farmers in Ghanpokhara of Lamjung, Dangsing of Kaski and Ghara of Myagdi districts. Farmers were asked verbally to get information on available poisonous plants found in their location, toxic effects of poisonous plants after ingestion, duration of death after ingestion. Required plants sample were collected from the above sites with their local name and were subjected to National Herbarium and Plant Laboratories (NHPL), Godawari, Lalitpur. Six months of age was reported the most susceptible age for deleterious effect of poisonous plants followed by two years and more than two years. Several plants had been found poisonous for sheep and goats. Among these Angeri (Lyonia ovalifolia sp.), Bikh (Aconitum spicatum.), Anke (Calotropis gigentica), Pauli (Reinwardtia indica), Hanuman jhar (Ageratum conyroides), Ghurilo (Cynanchum sp.), Banko (Arisaema sp.) etc. were the most commonly found poisonous plants available in western hills. Amilo, Chuk, Pakhanbed (Bergenia ciliata), Nirmasi (Acotinum gammiei), Panchaule (Dactylorhiza sp), Timur (Zanthoxylem armatum) and khoto have been found widely used antidote for the treatment of poisoning case in their sheep and goats.

INTRODUCTION

Nepal has diversified physiographic zones with different types of vegetation. Some types of these Plants contain a large number of biologically active chemicals. Some of these have been found to be extremely useful for treating various human and animal diseases; however, some plant constituents produce adverse health effects following exposure. The onset of these adverse effects can be quite sudden or take some time to develop. Fortunately, among the thousands of plants in the environment of animals, relatively few cause acute, life-threatening illnesses when ingested. The magnitude of economic losses due to ingestion of poisonous plants varies considerably between geographic regions. Losses include not only those due to mortality but also those due to poor productivity such as decreased weight gain or decreased milk production. In addition, the economic cost of controlling poisonous plants needs to be considered. Although the value of livestock varies from year to year, the total annual economic loss in these states is estimated to be nearly a quarter billion dollars. Ingestion of a potentially toxic plant is the number one route of poisoning in animals. It is important to emphasize that many, but certainly not all, toxic plants are not very palatable. Therefore, if given the choice, animals will avoid ingesting them even though they may be prevalent in the environment of the animal. In these situations, animals will

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often eat such plants only when other suitable feedstuffs are unavailable or when the animal is not able to selectively avoid the plants. In Nepal, very little study has been carried out regarding the poisonous plants along with its toxic effect. Therefore this study aims to explore and identify the poisonous plants in the western hills.

In order to obtain information on toxicity of poisonous plants, a semi structural questionnaire format was developed and distributed to goat and sheep raising farmers in three sites viz. Ghanpokhara of Lamjung, Dangsing of Kaski and Ghara of Myagdi districts as per suggestion made by respective DLSOs. Farmers were asked verbally to get information on available poisonous plants found in their location, toxic effects of poisonous plants after ingestion, duration of death after ingestion, toxic parts of the plants, effective age of animal, their indigenous practice reducing the toxicity of plants as well. Required plants sample were collected from the above sites with their local name and were subjected to National Herbarium and Plant Laboratories (NHPL), Godawari, Lalitpur. Furthermore, some plants were identified with their botanical names. Collected information was analyzed through SPSS statistical package.

RESULTS AND DISCUSSION

Farmers were questioned to provide information of available poisonous plants along with toxicity of these plants in their sheep and goat.

All of respondents reported prevalence of toxic effect in their animals due to ingestion of poisonous plants sporadically.

Duration of death after ingestion

Majority of farmers (54.2%) reported the duration of death of animals within 12 hours of ingestion of poisonous plants followed by death within 2 hours and 24 hours after ingestion. This finding is in accordance with the finding made by Aryal and Singh (1999).

Table 1: Duration of death after ingestion

Duration of death	Frequency	Percent
2 hours	24	25
12 hours	52	**54.2**
24 hours	20	20.8
72 hours	0	0
Total	96	100

Toxic parts of the plants

Shoot was the most toxic parts of plants (87.5%) followed by leaf (12.5%), while stem had no any toxic effect as reported by farmers. The young leaves of Laligurans are poisonous and young leaves and buds of Angeri were found poisonous in goats. This finding is similar to findings made by Aryal and Singh (1999), Anonymous (1970) and Joshi (1991).

Table 2: Toxic parts of the plants

Toxic parts	Frequency	Percent
Shoot	84	87.5
Stem	0	0
Leaf	12	12.5
Total	96	100

Age of animal

Six months of age was reported the most susceptible age for deleterious effect of poisonous plants followed by two years and more than two years. It may be due to low immunity power against poisoning. The older animals might tolerate the toxicity level to some extent.

Table 3: Age of animal

Age	Frequency	Percent
6 months	52	54.2
2 years	40	41.7
> 2 years	4	4.1
Total	96	100

List of poisonous plants

Table 4 shows the list of plants which are reported to be toxic to the animals. Several plants had been found poisonous for sheep and goats. Among these Angeri, Bikh, Anke, Pauli, Hanuman jhar, Ghurilo, Banko etc. were the most commonly found poisonous plants available in sampled sites. Toxicity of these plants was also reported by Aryal and Singh (1999) in mid western region.

Table 4: List of poisonous plants

Poisonous plants	Botanical name	Yes (%)	No (%)	Total (%)
Angeri	*Lyonia ovalifolia*	**70.8**	29.2	100
Bikh	*Aconitum spicatum.*	**66.7**	33.3	100
Anke	*Calotropis gigentica*	**54.2**	45.8	100
Pauli	*Reinwardtia indica*	**50.0**	50.0	100
Hanuman jhar	*Ageratum conyroides*	**43.8**	56.3	100
Ghurilo	*Cynanchum sp.*	**35.4**	64.6	100
Banko	*Arisaema sp.*	**31.3**	68.8	100
Laligurans	*Rhododendron arboreum*	26.0	74.0	100
Khirro	*Sapium insigne*	22.9	77.1	100
Tunnee	*Cedrella sp.*	21.9	78.1	100
Pore	-	11.5	88.5	100
Aijaeru	*Scurrula parasitica*	11.5	88.5	100
Cardomum	*Amomum sp.*	8.3	91.7	100
Tatasiri	-	8.3	91.7	100
Ikro	-	4.2	95.8	100

Farmer's indigenous antidote measure

Table 5: Farmer's indigenous antidote measure

Common name	Botanical name	Yes %	No %	Total %
Amilo	-	**70.8**	29.2	100
Chuk	-	**65.6**	34.4	100
Pakhanbed	*Bergenia ciliata*	**50.0**	50.0	100
Nirmasi	*Acotinum gammiei*	**46.9**	53.1	100
Panchaule	*Dactylorhiza sp.*	40.6	59.4	100
Timur	*Zanthoxylem armatum*	33.3	66.7	100
Khoto	-	22.9	77.1	100

Farmers have developed their indigenous control measure against animal disease since time immemorial. Some of these may be regarded as superstition however they are found technically sound. Likewise, they have used Amilo, Chuk, Pakhanbed, Nirmasi, Panchaule, Timur and khoto for the treatment of poisoning case in their sheep and goats. Majority of respondents reported effectiveness of these antidote measures.

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CLINICAL STUDY ON IVERMECTIN AGAINST PSOROPTES CUNICULI IN RABBIT.

R.P. Sah[1] and B.R. Joshi [2]

ABSTRACT

The clinical study was carried out for 12 months to investigate the efficacy of ivermectin against ear mite (Psoroptes cuniculi) in meat type rabbits of Regional Agricultural Research Station, Lumle, Kaski, Nepal. A total of 27 skin scraping samples were collected from rabbits having ear lesions. Samples were examined for mites at Veterinary Investigation Laboratory, Lumle. All the samples were found positive for the presence of Psoroptes cuniculi. Rabbits, infected with ear mite, were injected subcutaneously with a single dose of ivermectin at 400 mcg/kg of body weight. The effect of the drug was evaluated clinically and parasitologically over 5 weeks. The ear lesions disappeared in treated rabbits after 7 days. The therapeutic efficacy of ivermectin was found 100% against Psoroptes cuniculi in meat type rabbit.

INTRODUCTION

Rabbits can be infested with ear mites Psoroptes cuniculi, fur mites (cheyletiellosis), or burrowing mites (mange). The ear mite is the universal parasite of rabbit. It has different life stages: egg, larva, protonymph and adult mite. The cycle lasts about 21 days, depending on environmental conditions, with eggs hatching after 4 days (Sanders and Wall, 2000). Psoroptes cuniculi is mainly found inside the rabbit ear pinnae, and it is not uncommon to discover that only one ear is affected. In older or sick animals, or if not treated properly, the parasite may spread and infest the head, neck, legs, ventral abdomen and perianal region (Cuttler, 1998). Clinical findings are itching ears, frequent shaking the head and scratching up to the stage of automutilation. In the beginning, small tightly adherent skin scales appear deep in the ear canal and the ear lobes and are surrounded by alopecic (balding) regions. Those yellow- gray scales can be rather thick; they carry large numbers of the parasite, mite feces, skin cells and blood.

Bowman and Fogelson (1992) found that efficacy of ivermectin against ear mite was 99.61% at the dose rate of 400 mcg/kg body weight subcutaneously.

Pandey (1989) reported that the animals of treated group became negative for mite 6 days after treatment. He also reported the regression of lesions was faster in rabbits administered 400 mcg/kg body weight of ivermectin.

For the treatment of ear mite in rabbit at the station, topical application of insecticides like malathion, cythin were used but there might be danger for rabbit as well as human to inhale during application. Ivermectin for treatment of ear mite in rabbit has not been used commonly in Nepal hence, this study was undertaken to investigate the efficacy of ivermectin against Psoroptes cuniculi.

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MATERIALS AND METHODS

The experiment was carried out for a period of 12 months from July, 2010 to June, 2011 at RARS, Lumle Rabbit farm. A total of 27 skin scraping samples were collected from meat type rabbits having ear lesions. The samples were examined at Investigation Laboratory of the station.

Collection of sample: The affected part of inner surface of the ear was moistened with mineral oil. The skin scrapings were collected with the help of sterile scalpel blade by scraping the affected skin until some blood might ooze out. The scrapings were kept in clean filter paper with label.

The skin scrapings were kept in 10% potassium hydroxide (KOH) and gentle heat in waterbath was provided to the sample for digestion process. The scrapings were transferred to centrifuge tubes and kept for centrifuge at 3000 rpm for 10 minutes. The supernatent was discarded and one drop of sediment was placed on middle of a clean and dry glass slide. It was covered with coverslip and was examined under low power of the microscope (10X).

Rabbits, infected with ear mite, were injected subcutaneously with a single dose of ivermectin (Kepromec 10%w/v, KEPRO BV, Netherland) at 400 micrograms/kg of body weight. The effect of Kepromec® was evaluated.

Collection of samples and Laboratory test for presence of mite were done 7 days after injection. The effect of the drug was evaluated clinically and parasitologically over 5 weeks.

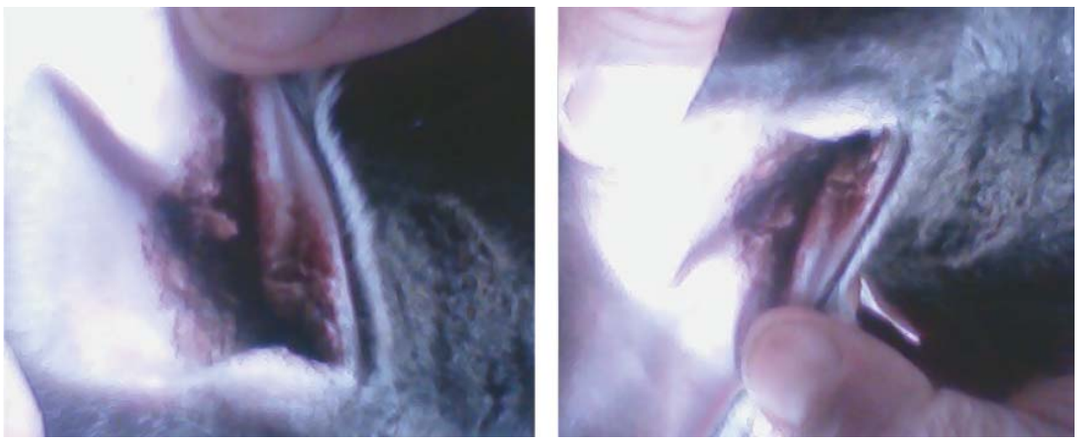

Fig.1: Lesions of ear mange

Fig. 2: Ivermectin- subcutaneous injection

RESULTS

All the samples were shown positive for ear mite. There were two kinds of breed viz. Hyline california and Soviet chinchilla. All the affected rabbits were above six months old. The results are present in the following table:

Month	Affected cases			Lab test	Lab test after 7 days of treatment
	Hyline California	Soviet chinchilla	Total		
July, 2010	0	0	0		
August	1	1	2	positive	Negative
September	2	0	2	positive	Negative
October	2	1	3	positive	Negative
November	1	1	2	positive	Negative
December	2	1	3	positive	Negative
January, 2011	2	0	2	positive	Negative
February	2	0	2	positive	Negative
March	3	2	5	positive	Negative
April	1	1	2	positive	Negative
May	1	0	1	positive	Negative
June	2	1	3	positive	Negative
Total	19	8	27		

Out of 27 positive cases for ear mite, 19 were belonged to Hyline california and 8 were belonged to Soviet chinchilla.

The rabbits, infested with ear mite, were injected with Ivermectin injection subcutaneously. No mites were found 7 days onwards treatment. The treated rabbits were observed for 5 weeks. No clinical signs as well as no any adverse effect of drug were reported over 5 weeks of treatment.

The effect of drug was found 100% effectiveness against ear mite in rabbits.

DISCUSSION

In this study the efficacy of ivermectin injection against ear mite in rabbit was found 100% at the dose rate of 400 mcg/kg body weight single injection. This finding is similar to the findings of Bowman and Fogelson (1992) and Pandey (1989).

The regression of lesions was faster and found negative for mite after 7 days of treatment. This is similar to finding of Pandey (1989).

It highly recommended for using Ivermectin injection subcutaneously for control mange infestation in rabbits.

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दशौं राष्ट्रिय भेटेरिनरी सम्मेलन

२०६८ चैत्र १५ देखि १७ बाट पारित

सुझाव तथा कार्यक्रमहरु

काठमाण्डौ घोषणा पत्र

क. गरिबि निवारण तथ ग्रामीण विकास

१. खाद्यान्नको चर्किदों मूल्यलाई नियन्त्रण तथा खाद्य सुरक्षा सुनिश्चित गर्न उपयुक्त प्रविधिको छनौट गरी पशुहरुको उत्पादकत्व बढाउने कार्यक्रमहरुलाई अभियानको रुपमा संचालन गरिनु पर्ने । खाद्य सम्प्रभुतामा खाद्यान्न वालि मात्र रहेको हुँदा सोमा दूध, फुल र मासुलाई पनि समावेश गरी सोही अनुसार कार्यक्रम तय हुनु पर्ने ।

२. ग्रामीण युवाहरु कामको खोजीमा विदेशिई रहेको सन्दर्भमा बेरोजगार युवाहरुलाई व्यवसायीक पशुपालन तथा पशुजन्य उद्योगमा आकृष्ट गर्न नयाँ प्रविधि, शीप, ज्ञान र आवश्यक सेवाको सुनिश्चितता गरी पशुपालन पेशालाई आयमुलक र मर्यादित बनाउन जरुरी छ । यसको लागी भेटेरिनरीयनहरुको सेवा ग्रामीण क्षेत्रसम्म पुऱ्याउनको लागी सेवा केन्द्र तहसम्म भेटेरिनरीयनको व्यवस्था गर्नु जरुरी भएको छ ।

ख. भेटेरिनरी शिक्षा तथा जनशक्ति विकास

३. पशुपालन क्षेत्रमा देखा परेको मानव संशाधनको कमीलाई परिपूर्ति गर्न तथा शिक्षा, अनुसन्धान र प्रसारलाई सवल बनाउन छुट्टै कृषि तथा वन विश्व विद्यालयको स्थापना भई सकेकोमा सो अन्तर्गत भेटेरिनरी साईन्स, एनिमल हजब्रान्ड्री र फिसरिज (Veterinary Science, Animal Husbandry and Fisheries Institute) को स्थापना हुनु पर्ने ।

४. पशुपंक्षीको उत्पादन तथा उत्पादकत्व वृद्धि गर्नको लागि आवश्यक विकास तथा अनुसन्धान (Research and Development) का लागि आवश्यक श्रोत र साधन उपलब्ध गराई नीजि, विश्व विद्यालय तथा सरकारी क्षेत्रबाट एकमुष्ठ रुपमा परिचालन हुने व्यवस्था गरिनु पर्ने ।

५. आवश्यक ज्ञान सीप सहज ढंगले ग्रामीण पशुपालकको पहुँचमा पुऱ्याउन पांचै विकास क्षेत्रमा भेटेरिनरी अनुसन्धान प्रतिष्ठानको विकास गर्न पहल गर्ने ।

ग. पशु स्वास्थ्य सेवा सुदृढिकरण

६. विश्व पशु स्वास्थ्य संगठन (OIE) को मापदण्ड अनुसार National Veterinary Service लाई सुदृढिकरण (Strengthen) गर्न तत्काल राष्ट्रिय योजना आयोगबाट प्राथमिकताकासाथ श्रोत र साधनको व्यवस्था गराईनुपर्ने । हाल उपलब्ध भेटेरिनरी जनशक्ति, संगठनात्मक संरचना, श्रोत साधनहरुको मूल्याङ्कन गरी सवल र सक्षमता तिर अग्रसर गराईनु पर्ने ।

७. वर्ड फ्लु देशमा भित्रि सकेको अवस्थामा यसको नकारात्मक असर बढ्न नदिन र पुनः संक्रमण हुन नदिन राष्ट्रिय तथा अन्तराष्ट्रिय दात्री संघ संस्थाहरुको सहयोग लिई एकिकृतरुपमा प्राथमिकताका साथ परिचालन गरिनु पर्ने ।

८. पशुपालन क्षेत्रको महत्व, उपलब्धता अन्तर्राष्ट्रिय स्तरमा प्रतिस्पर्धा र सम्भावनालाई अंगिकार गर्दै यस क्षेत्रको द्रुततर विकास तथा खाद्य सुरक्षाको लागि व्यवसायि क्षेत्रबाट समेत माग भई आए अनुसार छुट्टै पशुपालन तथा मत्स्य मन्त्रालयको स्थापना हुनु पर्ने ।

९. विचाराधिन रहेको भेटेरिनरी औषधि ऐन र पशु कल्याण ऐन संसदबाट पारित गराई अविलम्ब लागू हुनु पर्ने । विद्यमान पशु सेवा ऐन कानूनलाई समय सापेक्ष परिमार्जन गरिनु पर्ने ।

१०. वर्ड फ्लु, रेविज जस्ता खतरनाक रोगहरु विरुद्ध दिनरात खट्नुपर्ने भेटेरिनरी सेवालाई Emergency

Service को रुपमा सूचिकृत गरि जोखिम पूर्ण जुनोटिक रोग सम्बन्धि निदान तथा नियन्त्रणको काममा संलग्न पशु स्वास्थ्यकर्मीहरुको लागि विषेश जोखिम भत्ता र रु.२० लाखसम्मको जीवन वीमा गर्ने व्यवस्था अविलम्ब लागू गरिनु पर्ने ।

११. आम नागरिकलाई सुरक्षित खाद्य (food safety) उपलब्ध गराउनु राष्ट्रको दायित्व भित्र पर्ने हुँदा सम्माननीत अदालतबाट पनि मासु जाँच ऐन लागु गर्ने परमादेश जारी भैसकेको हुँदा यसको पुर्वाधार सहित नगरपालिका उप–महानगरपालिका तथा महानगरपालिकाहरुमा अविलम्ब पशु वधशाला तथा मासु जांच ऐन, २०५५ लाई तत्काल लागु गराईनु पर्ने ।

१२. भेटेरिनरी सेवाको विशिष्टतालाई मध्यनजर राख्दै विशेषज्ञताका आधारमा सरकारी सेवामा सेवा, समूह तथा उपसमूहहरु अविलम्व गठन गरिनु पर्ने । हालको संरचनामा रहेका पशु रोग निदान, उपचार, अनुसन्धान तथा नियमन कार्य गर्ने निकायहरुमा विषेशज्ञताको सेवा उपलब्ध गराउन सक्षम र सवल जनशक्ति र आधारभूत आवश्यकता परिपुर्ति गर्न विद्यमान सरकारी सेवा समुहलाई परिमार्जन गरी समय सापेक्ष बनाईनु पर्ने ।

१३. वन्यजन्तुहरु तथा Animal Origin का खाद्यतत्वहरुबाट विभिन्न जुनोटिक रोगहरु सर्नसक्ने सम्भावनालाई मध्यनजर राख्दै हरेक राष्ट्रिय निकुञ्जहरु त्था वन्यजन्तु संरक्षण सम्वन्धी अन्य निकायहरुमा तथा खाद्य गुण नियन्त्रण लगायतका निकायहरुमा भेटेरिनरियनहरुको यथेष्ठ दरवन्दी अविलम्व सिर्जना गरिनुपर्ने ।

१४. पशु बिकास तथा अनुसन्धान सम्बन्धि बोर्ड र पोल्ट्री बोर्ड अविलम्ब गठन गर्न पहल गर्ने ।

घ. पब्लिक प्राइभेट सहकार्य कार्यक्रम

१५. Livestock Industry को लागि आवश्यक पर्ने दाना सामाग्रीहरु उत्पादन गर्न सहकारी, करार तथा करर्पोरेट खेती प्रणालीको शुरुवात गर्ने नीति कृषि तथा सहकारी मन्त्रालयबाट व्यवस्था गराई अविल्म्व लागु गरिनु पर्ने ।

१६. कृषि बिकासको दिर्घकालिन विकासको रणनितीको लागी बन्दै गरेको Agriculture Development Strategy (ADS) मा पशुपालन क्षेत्रको विकासको लागी यथेष्ट र सान्दर्भिक दृष्टिकोण तथा श्रोतको सुनिश्चितताको लागी जोडदार माग राख्दछौं ।

१७. पशु तथा पंक्षीजन्य उद्योग तथा व्यवसायका लागि आवश्यक पर्ने सम्पूर्ण Input तथा यस्ता उद्योग तथा व्यवसायहरुबाट उत्पादित पदार्थहरुलाई प्रतिस्पर्धि बनाउन छिमेकी मुलुकहरुमा भएको व्यवस्था अनुरुप तथा विश्व व्यापार संगठनको प्रावधान अनुसार एकरुपता ल्याउन र व्यवसायिकरण गर्न, प्रोत्साहन गर्नका लागि Value Added Tax को प्रथा पुनर्विचार गरेर वर्गिकरण गरी लागू गरिनु पर्ने ।

१८. पशु पंक्षीजन्य उत्पादनको लागि आवश्यक पर्ने गुणस्तरीय उत्पादन सामाग्री तथा उत्पादित वस्तुहरुको आपूर्तिका लागि गुणस्तर कायम गर्ने व्यवस्था प्राथमिकताका साथ निजी तथा सरकारी क्षेत्रका प्रयोगशालाहरुलाई राष्ट्रिय तथा अन्तर्राष्ट्रिय संस्थाहरुबाट सम्बन्धन (Accreditation) गराई क्रियाशिल गरिनु पर्ने ।

१९. पशु स्वास्थ्य सेवालाई व्यापक र भरपर्दो बनाउन नीजि क्षेत्रमा PPP अन्तर्गत पशु चिकित्सालय स्थापना गर्ने रणनीति ल्याईनु पर्ने

ङ. ऐन कानून र नीति सम्बन्धमा

१९. भेटेरिनरी पेशासंग सम्बन्धित संसोधनका लागि प्रस्तुत ऐन नियमहरु अविलम्ब अघि बढाउने र मौजुदा ऐन नियमहरु कार्यान्वयनमा ल्याउन जोड दिने ।

२०. पशुपालन पेशा निर्वाहामुखि मात्र नभई व्यवसायिक बन्दै गएको र नेपाल WTO को सदस्य पनि भईसकेको अवस्थामा यो पेशालाई स्पष्ट दिशा निर्देश गर्ने उद्देश्यले पशुपालन नीति ल्याउनु पर्ने ।

२१. भेटेरिनरी औषधी ऐनको प्रस्तावित मस्यौदा सम्बन्धमा :-

(क) प्रेस्क्रिप्सन गर्ने अधिकार नेपाल पशुचिकित्सा परिषद्मा दर्ता भएका व्यक्तिले मात्र गर्ने

(ख) औषधि व्यवसाय गर्ने सम्बन्धमा ग्रा.प.स्वा.का. तालिम प्राप्त योग्यता भएका व्यक्तिलाई मात्र नभई पशु विज्ञान

विषयमा स्नातक योग्यता प्राप्त र पारा भेटेरिनरी प्रोफेसनलाई समेत निश्चित विषयमा निश्चित अवधिको थप तालिम दिई अनुमति दिनु पर्ने र दर्तावाल भेटेरिनरिएनलाई स्वत:औषधी व्यवसाय गर्न पाउने अनुमति दिनु पर्ने ।

(ग) भेटेरिनरी प्रयोगमा आउने औषधिहरुको प्रभावकारी व्यवस्थापन, नियमन र अनुगमन गर्न सम्बन्धित मन्त्रालय अन्तरगत भेटेरिनरी औषधि व्यवस्था विभाग स्थापना गर्न माग गर्ने ।

२२. दाना ऐनको कार्यान्वयन जिम्मेवारी पशु सेवा विभागले लिनु पर्ने ।

२३. पशुपालन व्यवसायलाई द्रुत आर्थिक आर्जन हुने विषयमा बढी भरपर्दो र सुरक्षित बनाउन पशु बिमा नीति अविलम्ब ल्याउनु पर्ने ।

२४. पशु चिकित्सा विज्ञानमा स्नातक योग्यता प्राप्त व्यक्तिहरुलाई पशुपालन व्यवसाय, उद्योग स्थापना र सञ्चालनमा आकर्षित गर्न सेवा सुविधाहरुको व्यवस्थापन गरी बढी से बढी संलग्न गराउन सहुलियत ऋण, अनुदानमा प्राथमिकता जस्ता नीति ल्याउनु पर्ने ।

धन्यवाद