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Editorial

The interface among human, livestock and wildlife is increasing with changing social and economic dimension in world. Rapid urbanization, intensification in livestock and poultry production, and encroachment into forest are opening door for close contact among these vital players on earth. The balance in ecosystem of different flora and fauna as well as microbes is forced to achieve another state of equilibrium. It is currently undergoing into transformation in their state. New microbes are jumping form wildlife to domestic livestock and poultry and to human population and vice versa. New diseases are becoming evident in livestock, poultry and human being. Changing climate is augmenting the process of transformation. Our old understanding to disease epidemiology and their prevalence is becoming obsolete. A systematic research on epidemiology and prevalence of different diseases needs to be carried out locally for better understanding. Diversified terrains of Nepal, starting from foothills of mountains to high Himalayas, result into many kinds of geography and climate. Ultimately, different kinds of disease pattern exist within a small country. Most of the papers in this issue of Nepalese Veterinary Journal reflect research and investigation on changing livestock and poultry disease epidemiology in Nepal. Nepal Veterinary Association is continuously putting its efforts to bring out quality research in field of one health, with its focus on animal health, through Nepalese Veterinary Journal. This issue will serve the purpose and leaving room for improvement in future issues.

Editorial Board

Nepal Veterinary Association

A REVIEW ON STATUS OF FOOT AND MOUTH DISEASE AND ITS CONTROL STRATEGY IN NEPAL

V. C. Jha

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ABSTRACT

Foot and Mouth Disease (FMD) is a highly infectious viral disease affecting all cloven footed animals including cattle, buffalo, sheep, goat and pigs. 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%). The higher density of animals in the hills, greater movement for agricultural activities and mostly the common grazing and watering places might be the reason for more number of outbreaks in the hills. Similarly, species-wise distribution of FMD outbreaks from 2000-2009 was highest in cattle (42%) followed by buffalo (32%), goats (19%), sheep (4%) and swine (3%). 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. The compilation of FMD virus serotyping results from 1965-2011 has shown that O is the most predominant serotype (77 %) followed by Asia 1 (15.4 %), A (6.4 %) and C (1.2%). However, serotype C was observed only during the period from 1990-1996. Looking at the prevalence of serotypes of FMD virus, from 2001 to 2011, revealed that prevalence of serotype O is in increasing trend (83%) followed by serotype Asia 1 (14.3%) and serotype A (2.7%). 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 Pan Asia 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. High cost of vaccination, relatively short duration of immunity, antigenic variation within a serotype, lack of easy access to quality vaccine and limited resources to the national veterinary services of the country are major drawbacks in implementation of the FMD control programme.

Key words: Foot and Mouth Disease, Nepal, Outbreaks, Serotypes

INTRODUCTION

Nepal is a landlocked country, which shares national boundary with Tibet of China in the north and India in east, west & south. The total area of the country is 147,181 square kilometers with 26.6 million human populations. Nepal has diverse agro-climatic and socio-economic characteristics. Geographically country is divided into three agro-ecological regions namely mountain (high hills), hills and terai. Administratively, the country is divided into five developmental regions such as eastern, central, western, mid-western and far-western development regions with a total of 75 districts.

Livestock is an integral part of agricultural production system, which plays a vital role in national economy. This sector contributes about 13% to the national GDP and 27% to agricultural GDP (DLS, 2011). Nepal has one of the highest livestock populations per capita and per unit of cultivatable land in the region. There are approximately 7.22 million cattle, 4.99 million buffaloes, 0.80 million sheep, 9.18 million goats and 1.10 million pigs (DLS, 2011). The production of milk and meat in the year 2011 in Nepal was 1556 thousand MT and 277 thousand MT respectively (DLS, 2011). Within livestock, dairy accounts for 63 percent of the total value added, followed by meat (32 percent) and eggs (5 percent). Following the sustained economic growth and rising domestic income, the demand for livestock products has increased tremendously. Commercial livestock production is expanding fairly rapidly in areas close to large population centers. The milk sub-sector has been doing well overall, with marked seasonal differences in production and supply. The commercial pig industry is small but expanding.

Over half of the cattle, buffalo, goats and sheep are kept in the hills, and one third in the terai. Transhumant ruminant production is practiced in the temperate, sub-alpine and in the alpine regions; extensive ruminant production prevails at the lower altitudes of the mid-hills (900 -1000 m) utilizing the available forage in and around villages; semi-intensive ruminant is found in the low to mid-hills (400-900 m) and in the peri-urban areas (FAO, 2005). Mostly sedentary husbandry system is practiced in the terai.

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). Foot & mouth disease (FMD) is endemic in Nepal since many centuries. It is present in almost all parts of the country and occurs round the year. Approximately 23 million livestock are susceptible to FMD apart from wild ungulates in the country. The improved breed of cattle and buffalo are highly susceptible to FMD leading to heavy economic losses to the farmers.

This paper reviews the status of FMD including economic importance and epidemiological situation of FMD, distribution of FMD virus serotypes and current control strategy in Nepal.

Economic importance of FMD:

FMD causes substantial economic loss to the livestock industry of the country. Infection in draft cattle causes a serious problem in tillage on which the preparation of agriculture land is mostly dependent. In dairy cows and buffaloes sharp drop in milk production, abortion and chronic mastitis is very common. In suckling calves the mortality rate is even high. Though economic losses due to FMD in Nepal has not been systemically quantified, 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 draft 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). The impact of the disease is further worse for the poor and subsistence farmers whose livelihood is based principally on the income from livestock farming; reduction in animal production, productivity and working ability of these animals means a significant reduction on their farm income.

Mandal (2010) conducted a study on evaluation of economic losses due to FMD in 2009 in Saptari and Sunsari districts of Nepal. The economic loss was calculated taking into account the milk loss in 200 FMD affected cattle and buffaloes for a period of 1 month. The loss in milk production was found to be 31.13%.

Moreover, as Nepal is a member of World Trade Organization, the presence of FMD in the country is 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).

Epidemiological situation of FMD:

Foot and Mouth Disease is a highly infectious viral disease commonly affecting all cloven footed animals including cattle, buffalo, sheep, goat and pigs. Irrespective of seasons, the disease is being observed throughout the country in all the three agro-ecological zones including terai, hills and the mountains.

On an average from 2001 to 2011, about 803 outbreaks per year have been reported in the country (table 1). Time and again, outbreaks of FMD have been reported throughout the country irrespective of season and altitude. The number of outbreaks, number of cases, and number of death and case fatality rate of FMD from 2001 to 2011 is presented in table 1.

Table 1: FMD Outbreaks from 2001 to 2011

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
2011	139	6811	130	1.90

Source: Annual Epidemiological Bulletins (2001-2011) of Veterinary Epidemiology Center, Kathmandu, Nepal

It can be seen in table 1 that the highest number of outbreaks was recorded in the year 2003 and 2001. But in terms of number of animals affected, year 2009 was highest with more than 80,000 animals affected with only 646 outbreaks compared to around 57,000 in 2003 and around 51,000 in 2001. The higher number of death 1644 was recorded in 2009 and 1265 death in 2003.

According to VEC (2010), 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%). The higher density of animals in the hills, greater movement for agricultural activities and mostly common grazing and watering places might be the reason for more number of outbreaks in the hills. Similarly, species-wise distribution of FMD outbreaks from 2000-2009 was highest in cattle (42%) followed by buffalo (32%), goats (19%), sheep (4%) and swine (3%).

Spatial distribution of FMD shows that during 2000-2009 except one district all 74 districts reported FMD. Fifty seven districts had reported the FMD outbreak in 2009 alone. The lowest number of districts reported the FMD outbreak during the period was 21 districts in 2008. The highest number of districts reported FMD outbreak was 63 in 2003 when Pan Asia strain of 'O' serotype of FMD was involved in the outbreak (VEC, 2010). From the above findings it can be mentioned that FMD is highly endemic in Nepal and outbreaks are reported throughout the country.

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).

Uncontrolled animal movement within the country and unrestricted importation without quarantine of animals (goat, sheep and buffaloes) from neighboring countries pose additional threat to spread of FMD. The extensive movement of animals for agricultural activities during the rainy season and unrestricted importation of large number of food animals across the border during October and November leads to higher number of outbreaks during these months. In fact, movement of livestock takes place across all the borders of Nepal. Mostly buffaloes and goats are brought into the country all the year round from India and goats and sheep from Tibet, China especially during the religious festival (Dashami) during September and October. In these instances there is large scale movement of livestock in the major cities as well. Most probably, the most important factor in the continued and high frequency of occurrence of FMD in Nepal is the extensive movement of livestock. Apart from this common grazing and watering places and movement of animals in the weekly livestock markets are additional contributing factors for the spread of the disease.

Distribution of FMD virus serotypes:

The laboratory based FMD diagnosis was started in 1965 when specimens from suspected cases of FMD were sent to the World Reference Laboratory for FMD (WRLFMD), Pirbright, UK for confirmatory diagnosis. Since then serotyping of FMD virus is done on regular basis. The serotyping of FMD virus was started in the country at National FMD laboratory from 1987 however for further laboratory confirmation and virus strain characterization the representative specimens have been sent to the WRLFMD as well. The serotyping results of FMD virus samples collected from FMD outbreaks in Nepal from 1965 to 2011 is presented in table 2.

Table 2: Serotyping results of FMD virus samples

Year of collection	Number of samples tested	Serotype				No virus detected
		O	A	C	Asia 1	
1965	2	2	-	-	-	-
1966	3	3	-	-	-	-
1967	5	-	4	-	-	1
1968	3	1	1	-	-	1
1975	6	3	-	-	-	3
1978	1	1	-	-	-	-
1983	6	5	1	-	-	-
1984	41	12	8	-	7	14
1985	68	37	-	-	7	24
1986	31	10	3	-	-	18
1987	54	19	-	-	5	30
1988	113	25	5	-	6	77
1989	53	14	3	-	8	28
1990	81	42	-	2	2	35
1991	67	23	-	-	1	43
1992	105	20	-	2	3	80
1993	150	7	-	-	-	143
1994	107	11	-	3	1	92
1995	121	8	-	-	6	107
1996	77	27	3	1	-	46
1997	97	8	-	-	13	76
1998	52	7	1	-	4	40
1999	82	18	6	-	2	56
2000	110	9	-	-	2	99
2001	95	17	2	-	2	74
2002	119	20	-	-	5	94
2003	125	6	-	-	-	119
2004	101	22	1	-	10	68
2005	105	4	-	-	-	101
2006	102	19	1	-	3	79
2007	105	11	1	-	8	85
2008	129	15	-	-	2	112
2009	128	29	1	-	2	96
2010	147	25	-	-	-	122
2011	102	15	-	-	-	87
Total	2693	495	41	8	99	2050
Percentage of total positive samples		**77%**	**6.4%**	**1.2%**	**15.4%**	

Source: World Reference Laboratory for FMD, Pirbright, U.K. and National FMD and TADs Laboratory, Budhanilkantha, Kathmandu, Nepal.

It can be seen in table 2 that FMD virus serotypes O, A, C and Asia1 have been reported in Nepal. The compilation of FMD virus serotyping results from 1965-2011 has shown that O is the most predominant serotype (77 %) followed by Asia 1 (15.4 %), A (6.4 %) and C (1.2%). However, serotype C was observed only during the period from 1990-1996. Looking at the prevalence of serotypes of FMD virus from 2001 to 2011 revealed that prevalence of serotype O is in increasing trend (83%) followed by serotype Asia 1 (14.3%) and serotype A (2.7%).

A pandemic strain of FMD virus serotype O, which was named Pan Asia was first identified in northern India in 1990. In 1993 it was found in Nepal and later in Bangladesh in 1996 and in Bhutan in 1998 (Knowles *et al*, 2000). Pan Asia strain was continuously isolated from FMD outbreaks from Nepal during 1993-1996 (Knowles *et al*, 2000). Moreover, the results of genetic and antigenic typing of FMD virus isolates from Nepal submitted to WRLFMD, Pirbright, UK (table 3) revealed that serotype O isolates from Nepal belong to the Middle East-South Asia (ME-SA) topotype. In 2003 both Pan Asia 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.

Table 3: Results of genetic and antigenic typing of FMD virus isolates from Nepal submitted to WRLFMD, Pirbright, UK.

WRLFMD reference number	Country of origin	Serotype identified	Topotype	Lineage/strain
Nep/1/2003	Nepal	O	ME-SA	Ind 2001
Nep/2/2003	Nepal	O	ME-SA	PanAsia
Nep/4/2003	Nepal	O	ME-SA	PanAsia
NEP/5/2003	Nepal	O	ME-SA	PanAsia
Nep/6/2003	Nepal	O	ME-SA	PanAsia
Nep/2/2007	Nepal	O	ME-SA	PanAsia-2
Nep/4/2008	Nepal	O	ME-SA	PanAsia-2
Nep/5/2008	Nepal	O	ME-SA	Ind2001
Nep/7/2008	Nepal	O	ME-SA	Ind2001
Nep/2/2009	Nepal	O	ME-SA	Ind2001

Nep/3/2009	Nepal	O	ME-SA	Ind2001
Nep/6/2009	Nepal	O	ME-SA	none designated
Nep/10/2009	Nepal	O	ME-SA	none designated
Nep/11/2009	Nepal	O	ME-SA	none designated
Nep/13/2009	Nepal	O	ME-SA	none designated
Nep/14/2009	Nepal	O	ME-SA	none designated
Nep/15/2009	Nepal	O	ME-SA	none designated
Nep/3/2010	Nepal	O	ME-SA	Ind-2001d
Nep/5/2010	Nepal	O	ME-SA	Ind-2001d
Nep/6/2010	Nepal	O	ME-SA	Ind-2001d
Nep/7/2010	Nepal	O	ME-SA	Ind-2001d
Nep/9/2010	Nepal	O	ME-SA	Ind-2001d
Nep/11/2010	Nepal	O	ME-SA	Ind-2001d
Nep/12/2010	Nepal	O	ME-SA	Ind-2001d
Nep/15/2010	Nepal	O	ME-SA	Ind-2001d
Nep/16/2010	Nepal	O	ME-SA	Ind-2001d
Nep/17/2010	Nepal	O	ME-SA	Ind-2001d

The molecular epidemiology result from WRLFMD revealed that, FMD virus serotype O isolates PanAsia-2 strain (NEP/2/2007) and NEP/4/2008 from Nepal were most closely related with the serotype O from Bhutan (O/BHU/11/2007) and Iran (O/IRN/56/2006) respectively. Likewise serotype O isolate Ind2001 strain NEP/7/2008 from Nepal was most closely related with the O/BHU/7/2002 (DQ164864). In 2009 the Ind2001 strain NEP/2/2009 from Nepal was most closely related with Indian isolate (O/IND/83/01 IVRI). In South Asia, there is extensive cross-border movement of cattle, buffaloes and goats in between Nepal and India and also to Bhutan and Bangladesh. Therefore it is quite obvious that such similar strains are circulating in the livestock population. Because of the porous borders and mostly informal livestock trade a regional FMD control strategy is of paramount importance.

According to the results obtained from WRLFMD (table 4), based on virus neutralization test (VNT), serotype O isolates from Nepal best match against O IND R2/75 vaccine strain. Based on liquid phase blocking (LPB) ELISA, among the tested vaccine strains O Manisa is best match for protecting against serotype O isolates from Nepal. Although the vaccine matching of the FMD virus O serotype isolates from Nepal has been done regularly at the

WRLFMD but since 1998 there is no information available on the vaccine matching for serotype A and Asia 1 isolates from Nepal.

Table 4: Results from WRLFMD on the FMD vaccine matching strain differentiation

	VNT				LPBE						
Vaccine / Field Isolate	VNT	O Manisa	O Bfs	O lnd R2/75	ELISA	O 4174	O BFS 1860	O lnd 53/79	OHkn 6/83	O Tai 189/87	O Manisa
ONep 2/2007	Mean	0.28	0.60	>1.0	Mean	0.54	0.42	>1	1.00	0.50	>1
O Nep 7/2008	Mean	0.29	0.47	>1.0	Mean	0.50	0.29	DNT	1.00	0.50	>1
O Nep 6/2009	Mean	0.15	0.60	>1.0	Mean	0.44	0.32	DNT	DNT	DNT	>1
O Nep 15/2009	Mean	0.17	0.40	>0.94	Mean	0.75	≥ 1	0.84	0.75	0.63	>1

According to OIE/FAO (2009) the vaccine strains that may be suitable for use in the South Asia region is presented in Table 5.

Table- 5: Vaccine strains that may be suitable for use in the South Asia region

Serotype	Internationally available	Locally available
O	O_1 Manisa	IND R2/75
A	A_{22} Iraq	IND 40/2000, Turkey 1/2006 (A Iran 05 lineage)
Asia 1	Shamir	IND 63/72

Control Strategy of FMD in Nepal:

The socio-economic situation in the country is not conducive to adopt a slaughter policy in the control of FMD. As outbreaks of FMD occur everywhere irrespective of altitude and climatic variation which results possibly from extensive movement of animals throughout the country. Regular vaccination measure as a prophylactic measure alone could be the option for which a potent vaccine with a long duration of protective immunity is required.

Department of Livestock Services initiated mass vaccination programmes in some of the dairy pockets since 1998 but it's not on regular basis. FMD vaccine is not produced in the country. At present, FMD vaccine which includes serotype O, A and Asia 1 is being imported in small quantities from India and used to routinely immunize improved and crossbred animals and ring vaccination in the face of outbreaks. 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.

Although continuous efforts have been made to study the epidemiological situation of the disease, identification of the field serotypes and creation of public awareness regarding the nature and control remedies of the disease but due to lack of implementation of national FMD control programme several outbreaks of FMD occur every year. High cost of vaccination, relatively short duration of immunity, antigenic variation within a serotype, lack of easy access to quality vaccine and limited resources to the national veterinary services of the country are major drawbacks in implementation of the FMD control programme.

The national FMD control programme has not been initiated because of various reasons including resource constraints. The FMD control strategy plan aims mainly to consider following aspects:

1. Creation of mass vaccination zones based upon animal population size, disease prevalence and animal movement activities.
2. Stratification of each region into high, medium and low endemic zones to minimize the cost of operation.
3. Animal movement control in low endemic zones - high mountains.
4. Use of preferably monovalent vaccine for ring vaccination so that the cost is affordable for farmers
5. Sero-surveillance programmes
6. Strengthening the vaccine production capacity or importing of appropriate vaccine
7. Strengthening of FMD diagnostic capacity

CONCLUSION

Irrespective of seasons, the disease is being observed throughout the country in all the three agro-ecological zones including terai, hills and the mountains. Although the vaccine matching of the FMD virus O serotype isolates from Nepal has been done regularly at the WRLFMD but since 1998 there is no information available on the vaccine matching for serotype A and Asia 1 isolates from Nepal. Therefore it is necessary for Nepal to study the local virus strains to select candidate strain for vaccine production. At the same time, vaccine production needs to be established in order to provide mass vaccination required in controlling the disease. An alternative would be to import concentrated inactivated viral antigens in bulk which could then be formulated, adjuvanted, bottled and labeled locally. There is a need for the formulation and implementation of a national control programme for

FMD with legislative powers to make FMD a notifiable disease and enforce animal movement restrictions. FMD eradication should be the final goal that is difficult to achieve at the national level. Due to the nature of the disease it is obvious that any single country alone would face difficulties in eradicating FMD without coordinated actions with its neighboring countries. Nepal has fairly established livestock trade with India, China and Bangladesh with movement of livestock in borders of the country. The recently launched progressive control pathway of FMD control (PCP-FMD) programme in the SAARC region is a very good initiation which will help to develop a common methodology and strategy for FMD control and eradication at the regional or sub-regional level.

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SERO-SURVEILLANCE OF JAPANESE ENCEPHALITIS VIRUS IN PIGS OF KATHMANDU VALLEY

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ABSTRACT

Japanese Encephalitis (JE) is a disease of priority in Nepal which has major public health significance caused by Japanese Encephalitis virus (JEV). This study is aimed at finding out the evidence and prevalence of JE in pigs by detecting antibody against JE virus in pig sera. A serological study for JE was conducted by collecting 102 sera from pigs of Kathmandu valley from February to July 2007 and examined at Central Veterinary Laboratory, Kathmandu to know the status of JE. The sera were tested by Competitive Enzyme Linked Immunosorbent Assay (C-ELISA) for the detection of antibody against Japanese encephalitis. Of the total number of tested sera, 42 were found positive for the presence of antibodies against JE virus infection in Kathmandu valley of Nepal. The highest percent of seropositivity was found in Kathmandu (52%) followed by Lalitpur (46.67%) and Bhaktapur (21.67%) indicating the people of Kathmandu district at high risk.

Keywords: Japanese Encephalitis (JE),Serum, ELISA

INTRODUCTION

Japanese Encephalitis (JE) is a vector-borne infectious viral disease that affects pigs, horses, donkeys, humans including other domesticated animals like cattle, sheep, goat, dogs and cats as well as wild mammals and birds. This disease is caused by Japanese Encephalitis Virus (JEV), an arbo virus belonging to the genus Flavivirus and family Flavivirida (Iowa State University [The Center for Food Security & Public Health], 2007). This zoonotic disease is widespread throughout Asia and is spreading beyond its traditional habitat (Joshi, Banjara, Bhatta & Wierzba, 2004). The JEV virus can cause irreversible neurological damage (Erlanger, Weiss, Keiser, Utzinger & Wiedenmayer, 2009). This virus is transmitted by the mosquito Culex *tritaeniorhynchus*, which usually breeds in irrigated rice paddies, plays crucial role in spreading the virus to humans and domesticated animals (Burke & Leake, 1988; Erlanger, et al., 2009). Other mosquito species involved in the transmission of JEV are C. *vishnui*, C. *fusocephala* and C. *gelidus* (Nitatpattana *et al*., 2005). Animals are infected when they are bitten by an infected mosquito. Pigs act as the most important amplifiers and reservoir hosts (World Organization for Animal Health, 2012). In addition, Wading ardeid water birds (e.g. herons and egrets) and ducks also serve as the reservoir host of the virus (Erlanger *et al*., 2009). Humans and horses do not transmit the viruses to biting mosquitoes

because of low titres and short duration of viraemia. These are the dead end hosts (World Organization for Animal Health, 2012).

The history of the clinical recognition and recording of JE dates back to the 19th century. The first clinical case of JE was recorded in 1871 in Japan. However, half a century later, in 1935 Japanese Encephalitis Virus (JEV) was first isolated and has subsequently been found across most of Asia (Solomon et al., 2000). Japanese Encephalitis virus (JEV) is the most important cause of epidemic encephalitis worldwide, with an estimation of 35000- 50000 cases and 10000 deaths (Tsai, 2000). In Asia, the case fatality rate varies across regions, ranging from 10% to 30% (Solomon, 2004; Tsai, 2000). In Nepal, JE was first recorded in 1978 as an epidemic in Rupandehi district (Bista & Shrestha, 2005). This disease was imported from Gorakhpur district and surrounding areas of Uttar pradesh of India (Takashi, 1996). In Nepal, JE has been reported from all eco-regions of the country (Pant, 2009), however, the disease is confined to endemicity in lowland region, especially in 24 districts of Terai (Joshi, et al., 2004). JE is seasonally endemic in Kathmandu valley (Partridge, Ghimire, Sedai, Bista, & Banerjee, 2007). The three viral strains namely B-2524, B-9548 and Nep-1/90 have been so far isolated in Nepal (Singh & Gurung, 2002) and serological diagnosis has shown that pigs and ducks are the main reservoir hosts of JE (Joshi, et al., 2004).

Pig farming has been increasing in urban areas because it is a profitable enterprise, which requires a comparatively small investment and returns come rather quickly. Pig is highly prolific breeder and cheap source of protein (Dietze, 2012). High fertility in pig gives an assured income to farmer round the year. Pig farming as a business provokes a demand for research on JE because pigs are the reservoirs and amplifying hosts of this disease. The virus reproduces in pigs and infects mosquitoes that take blood meals. Mosquitoes are the main vector of JE virus. Consistent development of viraemia in susceptible pigs ensures a continued supply of infected mosquitoes (Iowa State University [The Center for Food Security & Public Health], 2007). In Nepal, sero prevalence of JE virus in pigs was reported 57.25% in 2005 (Pant, Shrestha, Ratala, & Mahato, 2005). The disease is transmitted to humans by the bite of infected mosquitoes. It is a zoonotic disease of importance.

Hence, in 2007 this study was conducted with an aim to detect antibody against JE virus in pig sera, to find out the evidence of JE among the pigs at the site and to find out the prevalence rate of JE among those pigs. Though this study was done 5 years ago, its data can be used for the various epidemiological and analytical studies in the future.

MATERIALS AND METHODS

Questionnaire survey

Before collection of sample, observation was carried out for presence of stagnant water or pond near farm, hygienic condition of farm, and distance of home near farm.

Collection of serum samples

A total of 102 serum samples were collected purposively from pigs of Kathmandu, Lalitpur and Bhaktapur in between February and July 2007. Blood was collected from each of the unvaccinated animal aseptically from the ear vein. The blood containing tubes were kept in slanting position at room temperature for two hours and centrifuged at 3000 rpm for 10 min. The separated sera were then preserved at -20^{o} C till further use.

Reagents and Materials

JE antigen, monoclonal antibodies and horseradish peroxides conjugate, TMB substrate and ELISA plate, ELISA reader, Incubator

Buffers and Solutions

Coating buffer (pH=9.0), phosphate buffered saline, washing buffer (pH=7.2), TMB substrate and stopping solution (1 M H_2SO_4) were used.

Procedure

Serum samples were tested in Central Veterinary Laboratory (CVL), Tripureshor, Kathmandu following the standard guidelines of Australian Animal Health Laboratory (AAHL, 2002). The protocol of C-ELISA used in this study for the detection of specific antibodies (IgG) against JE virus infection in pigs has been established standardized at CVL, Kathmandu. JE antigen was diluted at the ratio of 1:100 with coating buffer (pH 7.4). Plates were coated with 50 µl diluted antigens per well, covered and incubated for 1 hour at 37^{0}C. Test sera were diluted at 1:10 dilution in ELISA diluent with pH 7.2. Control positive sera were diluted at 1:100, 1:800, 1:1000 and 1:2000 dilutions in ELISA diluent. Diluted test sera were kept in duplicate wells and then control sera were located in the last columns of the plate. Antigen coated plates were washed thoroughly with wash buffer (pH 7.2). 50 µl of control and test serum was added in duplicate to the plate wells according to C-ELISA record sheet. Plates were covered and incubated for 1 hour at 37^{0} C on the plate shaker. While serum incubation was in progress, monoclonal antibody was diluted in 1:15000 dilutions in ELISA diluent. After the serum incubation ELISA plates were not washed and 50 µl of prepared monoclonal antibodies was added in all well of the plate except H (11 and 12) and incubated at 37^{0} C for half an hour on the plate shaker. During incubation period, HRPO anti-mouse conjugate was prepared in 1:2000 dilutions in ELISA diluent. ELISA plates were washed three times with PBS at the end of the serum-Mab incubation and 50 µl of conjugate was added per well. Plated were again re-incubated for 30 minutes at 37^{0}C. Plates were washed after incubation and 50 µl of TMB substrate prepared with hydrogen peroxide was added to each well. Reaction was stopped by adding 50 µl of 1M sulphuric

acid to all wells after 10 minutes. Plates were read at 450 nm in ELISA reader to get OD values. Percentage of inhibition of each serum was calculated by the formula:

100-{100(OD of mean test sera/OD of mean negative control sera)

Sera resulting in level of less than 40% inhibition were considered negative whereas sera resulting in level of greater than 40% inhibition were considered positive.

RESULT AND DISCUSSION

Out of 102 samples, 42 samples (41.17 %) were found positive to JE antibodies (IgG) whereas the rest were negative. The results were presented in table 1 to table 4. The numbers of positive and negative sera were distinguished on the basis of percentage of inhibition on the development of colour in each well. More than 40 percent (41.17%) of tested sera were found positive (Table 1). This study confirmed presence of JE virus in pigs of Kathmandu valley. The highest percent of sero-positive was found in Kathmandu (52%) followed by Lalitpur (46.67%) and then Bhaktapur (21.67%) (Table 1).

The study of Pant 2006 showed the sero-positivity of 75% in Bhaktapur which is very higher compare to the findings of this study. The minimal sero-positivity of JE virus in pigs of Bhaktapur in this study could be due to the absence of reservoir water near pig shed. Further the samples were collected in the month of Falgun which is the non-breeding season of the vector mosquito.

Table 1: Sero-prevalence of JE virus in pigs of Kathmandu valley

District	**Place**	**samples collected**	**Positive sample**	**% of positive**	**Mean %**
Kathmandu	Balaju	10	6	60	52
	Dharmasthali	30	15	50	
Lalitpur	Khumaltar	4	4	100	46.67
	Bungmati	2	-	0	
	Balkumari	24	10	41.67	
Bhaktapur	Dadikot	21	4	19.07	21.67
	Srijananagar	11	3	27.27	
Total		102	42	-	41.17

In Lalitpur, the sero-positive was 46.67% which is lower than that of the findings of Pant and colleagues (Pant, et al., 2005). However, a study done in 2004 reported the sero-positivity to be 91.4% in Lalitpur (Pant, 2006). The findings of sero-positivity of 42% by Pant (2006) in Kathmandu is slightly below the findings of this study (52%). This shows

that the sero-prevalence varies at different times and should be done regularly for the epidemiological study and development of effective control measures.

After this study, no any sero-prevalence study has been done in Kathmadu Valley. In 2010, Thakur et al.conducted a study only in four high altitude mountainous districts of Nepal (outside Kathmandu valley) which showed the sero-positivity of 16.7% (Thakur et al., 2012) . Therefore the findings of this study could be a landmark in conducting future studies in Kathmandy Valley.

Antibodies against JE infection have been detected in Nepal in pigs and ducks in the past (Joshi & Gaidamovisch, 1981/82) by performing haemaglutination test. The incidence of JE in humans from 2004 to 2006 in Kathmandu, Lalitpur and Bhaktapur is 25, 10 and 4 respectively (Japanese encephalitis vaccination, 2064) which could be co-related with the sero-positive of JEV virus in pigs of Kathmandu valley. This indicates that people of Kathmandu district is at high risk.

Age wise analysis of data showed that, pigs of 2-4 months age had high sero positivity (50%) followed by 0-2 months (35.48%) (Table2). The sexwise data revealed that high sero-positivity was found in female pigs (51.9%) compared to the sero-positivity in male pigs (30%) (Table 3).

Table 2: Sero-positivity of JE virus in pigs of different age groups of Kathmandu valley

Age(months)	**Total sample**	**positive**	**% positive**
0-2	31	11	35.48
2-4	46	23	50
4-6	25	6	24

Table 3: Sex wise distribution

Sex	**Total sample**	**positive**	**% positive**
Male	50	15	30
Female	52	27	51.9

In a sero-survey conducted in Nepal in 2004, 40% of pigs from the sample are found to be positive for JE antibody (Pant, 2004). In India, 44% pigs tested had antibody to JE.

JE is known to occur mainly in Southern Terai districts of Nepal, however JE outbreak had been reported in southern part of Lalitpur district in 1996 and 1997 which was confirmed both serologically and virologically (Joshi, Bista, Joshi, & Sharma, 1998).

Circulation of JE virus is affected by animal management practices and close location of pigs and human dwellings is an obvious risk factor for human disease. Presence of stagnant water and mosquitoes near the pig farming are the risk factors for Japanese Encephalitis which is prevalent in Kathmandu valley (Table 4). In Nepal, pigs are generally animals that will be sold for meat after relatively short periods (1-2 years). Annual farrowing, together with removal of immune animals, ensures non-immune pigs will exist each year, heightening the risk of the disease (Pant, et al., 2005).

Table 4: Relationship of management factors in JE

S.N	Variable	Categories	Sample Size	Positive	Percent
1	Presence of stagnant water or pond	Yes	64	31	48.43
		No	38	10	26.31
2	Presence of mosquito	Yes	72	35	48.61
		No	30	6	20

JE is a disease of zoonotic importance. So, further epidemiological study in depth is very essential to adopt effective disease control measures.

CONCLUSION AND RECOMMENDATIONS

The sero-prevalence of JE virus infection in pigs of Kathmandu valley was found 41.17%. Thus the following recommendations are made to combat JE in the population:

1. Immunization against JE in pigs especially in breeding stocks reared by either government or private sectors.
2. More and continuous epidemiological surveillance studies should be done in Kathmandu valley and other probable areas.

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PREVALENCE OF *E. COLI* 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 E. coli in goat meat from different meat shops of Kathmandu valley. Bacterialflora was isolated from samples according to the methods described in theBacteriological Analytical Manual of the Food and Drug Administration. The conventional culture in agar plate method, Gram's staining and appropriate biochemical tests were done for the identification of bacteria. Out of 101 samples, 30.69% were positive for E. coli. The prevalence of E. coli indicates the unhygienic handling of meat, unsatisfactory sanitary condition in the local meat markets of Kathmandu valley and risk to the consumers.

Key words: Food borne diseases, Microorganisms, Contamination, lactose fermenters, ISO standard

INTRODUCTION

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

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.

MATERIALS AND METHODS

Altogether 101 dressed goat meat samples from different meat shops of Kathmandu, Bhaktapur and Lalitpur districts of Kathmandu valley were collected randomly. All the laboratory analysis was undertaken at the microbiology laboratory of Agriculture and Forestry University (Former- Institute of Agriculture and Animal Sciences, T.U.), Rampur, Chitwan. A quantity of about 200 gm of cut meat samples were collected and labeled accordingly from the different regions of the carcass, 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).

Enumeration of TVC and TCC

For the determination of TVC, 0.1 ml of each ten-fold dilution was transferred and spread on

triplicate in NA agar using a sterile pipette. The diluted samples were spread quickly on the surface of the plate with a sterile glass spreader. One sterile spreader was used for each plate. The plates were then kept in an incubator at 37^0C for 24 hours. Following incubation, platesexhibiting 30-300 colonies were counted. A plate without inoculum was kept as control. After incubation, the colony counting was done by using microbial digital colony counter. The average number of colonies in a particular dilution was multiplied by the dilution factor to obtain the TVC. For the determination of Total Coliform, TVC method was employed. For TCC method Mac Conkey agar plates were used as above and the procedures of sampling, dilution and streaking was similar to those followed in TVC of bacteria. The colonies of non-lactose fermenters on BGA plates were also counted as above. The stocks of the organisms from all the positive samples were also maintained accordingly.

Isolation of *E. coli*

25gms of meat samples were minced in mincing machine aseptically into small pieces. 5gms of the homogenized sample with 25ml buffer peptone water was incubated at 37^0 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 37^0 C for 24 hours for enrichment.

One loopful of the enriched sample from Selenite- F broth was inoculated in selective media i.e. Mac Conkey agar and one Petri dish was kept as control. Then Petri dishes were incubated at 37^0C for 24 hours. After overnight incubation, the typical lactose fermenting (pink) colonies were picked up from Mac Conkey agar plates and pure culture was done in Nutrient Agar and incubated at 37^0C 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

Various biochemical media were inoculated and the results were observed on following day. The typical lactose fermenting colonies for the confirmation of *E. coli* were used from different selective media. The In dole production test, Motility test, TSI test and Citrate utilization test of more than one colony were done separately from single plate.

RESULTS

Altogether 101 goat meat samples were collected from different meat shops of Kathmandu valley. In this study, different parameters were studied so as to know about the bacteriological quality of the goat meat of Kathmandu valley. Out of 101 samples, 97.02% (98/101) were found to be positive for TVC, 66.33% (67/101) were found to be positive for Coliform/lactose fermenter, 67.32% (68/101) were found to be positive for non-lactose fermenter and 30.69% (31/101) were found to be positive for *E. coli*. The mean values of

TVC and TCC of goat meat in Kathmandu valley were found to be $1.92x10^7$cfu/g and $3.50x10^5$cfu/g respectively.

DISCUSSIONS

The mean value of TVC and Coliform of dressed goat meat in Kathmandu valley was found to be $1.92x10^7$cfu/g and $3.50x10^5$cfu/g respectively which were more than the standard prescribed by the ISO. The prevalence rate of *E. coli* of goat meat in Kathmandu valley was 30.69% which was found to be close to the prevalence rate given by NARC (1999), i.e. 39.44% and lower than the prevalence rate given by Maharjan (2009), i.e. 73% and Hanglombe *et al.,* (1999), i.e. 41.7%. According to Pearce *et al.,* (2004), *E.coli* higher than 10^2/g indicate dangerous contamination of food. Hence, the meat from Kathmandu valley was found to be insecure for consumption. As *E. coli* is one of the major indicators of fecal contamination, the carcass might be contaminated with fecal materials of human or the particular animal or the water which has been already contaminated with fecal materials. This finding 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, 97.02% were positive for TVC, 66.33% were positive for Coliform and 30.69% were positive for *E. coli*. 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 equipments to be used in the slaughter slabs.

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PROXIMATE AND MICROBIAL ANALYSIS OF FRESH, DRIED AND FRIED NAINI FISH (*CIRRHINUS MRIGALA*)

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ABSTRACT

This study was carried out to investigate the nutritional status and microbial load in naini fish (Cirrhinus mrigala) under different state of preservation. Proximate analysis (moisture, protein, fats, total ash and carbohydrate) were made for fresh, experimentally hybrid solar dried, smoked dried and commercially fried flesh of naini while food-spoilage and pathogenic organisms such as mold, Coliform, Escherichia coli, Staphylococcus, Total plate count (TPC), Salmonella and rancidity were screened in the same samples. Highest protein content (58.3±2.6%) was recorded in commercially smoked dried fish while the least (17.6±0.3%) was observed in fresh fish samples from the village fish pond. Proximate composition of commercially dried and hybrid solar dryer dried fish were not significantly different (P>0.05) but differed with fresh and commercially fried fish. Present analysis suggests that fresh fish from pond and commercially dried fish which is marketed in highway corridor are less hygienic at current state of drying sincemold, Coliform, Escherichia coli, Staphylococcus, were detected at concentration above recommended norms. The need of training and capacity building program for fish processing to the fish vending communities have been suggested. The use of hybrid solar dryer could be the available best option for efficient drying to produce relatively hygienic and nutritive dried fish product.

Key word: *Cirrhinus mrigala*, microbial load, fresh fish, dried fish, pathogenic organisms

INTRODUCTION

Fish constitute a very important component of the diet for many people and often provides the much needed nutrient that is not provided in cereal based diets. Fish is rich in protein with amino acid composition very well suited to human dietary requirements comparing favorably with egg, milk and meat in the nutritional value of its protein. Smoked or dried fish is a traditional part of the diet of a large section of the world's population. Drying is a traditional and widely used method of preserving fish in tropical countries especially in the absence of refrigerated storage facilities. In Nepal, usually fish is eaten fresh and surplus fish

is preserved or processed. Smoked drying and deep frying are common traditional practices of fish processing widely used in different parts of Nepal. Locally processed indigenous fish are delivered to the consumers by fisher and other communities settled nearby wetland (rivers, lakes, etc).

Fish is, however, susceptible to damage as soon as it harvested. Fish being a quickly perishable commodity, chemical breakdown of protein, fat and water contribute to quick spoilage. Some factors responsible for this include the prevailing high temperatures and the facilities for processing, storing and distributing. Therefore, nutritive and microbial quality assessments are necessary to ensure the food safety of any processed products. A number of microbiological tests of fish and fish products are used by authorities elsewhere to check that the microbiological status is satisfactory. However, information on the nutritional and microbial status of fresh and preserved fish and their food safety values are meager in the country. An attempt was made by Fisheries Research Division, Godawari in 2009 to study the microbial load of smoked dried fish product of Kalanch (*Labeo calbasu*) and sahar (*Tor putitora*) of Karnali Chisapani. Presence of total plate count and *Staphylococcus* at the level of too numerous to count suggest that locally processed fish at Karnali Chisapani were poorly hygienic at current state of drying. Smoke drying is one of the crude methods of preservation which often do not last longer, as a result involved farmers, traders and broker might lost their investment and benefits. For these often microbes, whether, incomplete drying, moisture, humidity, thickness of fish muscle etc are blamed, however, the clear picture has not yet been sorted out in local socio-economical situation. Therefore, a detail study and quality assessment is requiring to address the problem Therefore, present study was made to assess the nutritive value and examination of microbial load (*Salmonella, Staphylococcus* spp., *E. coli*) or other types of general contamination or poor handling practices (*Coliform* bacteria, total viable count*)* in different forms of fish (fresh and preserved) marketed in the country. Findings of the study have been interpreted in alternative measures of fish preservation for food safety and the need for capacity building of fish vending community.

MATERIALS AND METHODS

The commercially valued indigenous fish *C. mrigala* was selected for the present study. Commercially dried and commercially fried fish were collected from three different hotels of highway corridor of Dhading district and packed in plastic bags. Similarly, fresh fish samples were collected from three different fish ponds of Dhanusa district. After harvest, the fish were immediately placed in cooler box with ice packs. Both types of samples after

collection were immediately refrigerated and preserved in deep freezer at Fisheries Research Division, Godawari (FRD) until send to National Food Laboratory for analysis.

Some of the fresh fish brought from Dhanusa district were washed properly with tap water and degutted. The scales were removed with a sharp knife. The fish were dried in hybrid solar dryer at FRD Godawari. The hybrid solar drier consists of fire chamber with a great and gate for controlling the air inlet. There are 7 flue gas pipes passing through the drying chamber to exchange heat to the drying chamber. It also consists of the air inlet and exhaust chimney with valve connected to the drying chamber as in the case of normal solar dryer. It consists of 5 trays with steel nets to keep the products for drying. It uses fuel wood or agricultural residue such as corncob as a fuel for drying along with the solar radiation**.** Fish were removed from the dryer when 80% weight was lost.

The samples (commercially smoked dried, commercially fried, fresh and experimentally hybrid solar dried) were send to Food Research Unit of Nepal Agricultural Research Council at Khumaltar for proximate analysis of moisture, ash, fat, protein and carbohydrate. The same samples were sent to Soil Test Laboratory at Koteswar for enumeration of Mold, *Coliform*, *Escherichia coli (E-coli*), *Staphylococcus*, *Salmonella*, and total plate count.

Data and information collected from the fish sampling sites and respective laboratories were used to analyze any significant differences on nutritive value and microbial load among fish preservation methods using software Statgraph version 3 plus. Also the values were compared with WHO norms for food fish.

RESULTS AND DISCUSSION

Proximate analysis (moisture, protein, fats, total ash and carbohydrate) were not significantly different between experimentally hybrid solar dried and smoked dried flesh of *naini* (Table 1). There was significant differences among fresh, commercially fried, experimentally hybrid

Table 1. Proximate composition of fresh, commercially dried, commercially fried and experimentally dried fish in hybrid solar

Treatment	Moisture (%)	Total ash (%)	Total fat (%)	Crude protein (%)	Carbohydrate (%)
Commercially dried fish	12.6 ±3.0^{a}	12.5±0.4^{c}	15.7±0.3^{b}	58.4±3^{c}	1.0±0.4^{a}
Commercially fried fish	44±0^{b}	6.1±0.6^{b}	21.4±4^{c}	22.53±1^{b}	6.±3^{b}

Fresh fish	78.4±0.6[c]	2.4±0.3[a]	0.4±0.1[a]	17.6±0.3[a]	1.3±1.0[a]
Experimentally hybrid solar dried fish	11.6±0.8[a]	13.3±0.6[c]	16.83±0.4[b]	57.5±0.7[c`]	0.6±0.1[a]

The microbiological analyses also showed variations among the samples. Enumeration of total viable bacteria (Total Plate Count, TPC), total mold count, *E. coli*, *Salmonella sp.* rancidity were assessed and the results are given in Table 2.

Table 2. Microbial load in raw, commercially dried, commercially fried and experimentally hybrid solar dried fish

Treatment	Mold/g	*Coliform*/g	*E-coli*/g	*Staphylo coccus*/g	*Salmon ella*/g	TPC /g
Commercially dried fish	2253 ±3108[a]	236.6 ±225[b]	80 ±138.5[a]	TNTC[c]	0.0[a]	2506670 ±496622[b]
Commercially fried fish	3.0±6[a]	0.0 [a]	0.0[a]	1444 ±1308[b]	0.0[a]	4200 ±1539 [a]
Fresh fish	2611 ±1230[a]	>2400[c]	>2400[b]	TNTC[c]	11600[b]	TNTC[a]
Experimentally hybrid solar dried fish	0.0 [a]	0.0 [a]	0.0[a]	62.±9[a]	0.0[a]	2615±124[a]

The International Commission on Microbiological Specifications for Foods (ICMSF, 1982) indicated a limit of acceptability as less than 1.0 x 10^6 cfu/g for the total viable count in any food to be safe for consumption. Such counts records in the commercially fried fish and experimentally hybrid solar dried fish were therefore within the limits of acceptability.

Except *Salmonella* the microbial loads in commercially dried fish were above the recommended level of ICMSF (1978) (Table 3). In fresh fish all microbial loads were above recommend International guideline for microflora ICMSF (1978). Hence these 2 items (fresh fish and commercially dried fish) were poor in microbial quality.

Table 3. International guideline for microflora (ICMSF, 1978)

Total viable count	Not to exceed 1000,000 cfu/g
Salmonella	Not to be detected
E. Coli	Less than 10 cfu/g

Staphylococcus aureus	Less than 100 cfu/g
Fecal *Coliforms*	None
Yeast and mould	10000 cfu/g

Mould counts were 3.0±6 cfu/g in commercially fried fish (Table 2). The ICMSF (1982) has a specification of less than 1.0 x 10^4 cfu/g, which indicated that the commercially fried fish met the specified standard requirement for safe food. While commercially dried fish exceed the permissible standard.

Fresh fish sample exceeded all limit of acceptability specified standard requirement for safe food according to the guideline of ICMSF, 1982. Hence fish caught in waters not polluted by human or animal wastes are free from intrinsic microbiological hazard when handled according to good commercial practice. Indeed fish and other free-swimming aquatic animals do not usually carry those organisms generally considered to be typical of the mammalian microflora, including *Escherichia coli,* the 'faecal coliforms. The presence of human enteric organisms on aquatic food products is clear evidence of contamination from a terrigenous source. Fish and shellfish products are a minor source of bacterial food borne disease in North America, the United Kingdom, and Australia (Todd, 1978), but there is a continuing high relative incidence of bacterial food-borne disease from fish products in Japan and probably in Southeast Asian countries where fish are commonly eaten raw or with little cooking (Sakazaki, 1979). Raw or processed aquatic foods are in general excellent substrates for the growth of most common bacterial agents of food-borne disease if held at improper temperatures. It is important therefore to avoid contamination of these foods during preparation and storage and to hold them at chill temperatures. Coliforms and *Escherichia coli* (*E. coli*) were also not detected in the commercially fried and experimentally hybrid solar dried fish sample. ICMSF (1982) specified the levels of *E. coli* to be less than 1.0 x 10^1 cfu/g. The non-detection of these food poisoning organisms indicated that the fish was safe for consumption. *Salmonella* species were not detected in any samples (Table 3) except fresh fish. ICMSF (1982) stipulated levels of zero cfu/g of these organisms in the food to be considered safe for human consumption. The cured fish are liable to deteriorate during storage due to mould growth. Experimentally hybrid solar dried fish was properly handled and well treated with heat and it dried quickly and the end product was clean and hygienic. Rancidity is caused by a higher rate of lipid oxidation, which consequently affects the flavor and general acceptability.

CONCLUSION

Present analysis suggests that fresh fish from pond and commercially dried fish which is marketed in highway corridor are less hygienic at current state of drying. The need of training and capacity building program for fish processing to the fish vending communities have been suggested. The use of hybrid solar dryer could be the available best option for efficient drying to produce relatively hygienic and nutritive dried fish product.

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RICE MILLING CO-PRODUCTS: POTENTIAL FEED INGREDIENTS FOR LIVESTOCK AND POULTRY IN NEPAL

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ABSTRACT

A study was conducted at Probiotech Industries Pvt. Ltd, Birgunj to evaluate the nutritional composition of rice milling co-products. Samples of rice polish (127), broken rice (15) and deoiled rice bran (33) were analyzed using MPA-FT-Near Infrared Reflectance (NIR) Spectrometer. The results showed that the crude protein (12.47%), crude fibre (5.56%) and total ash (7.73%) content of rice polish were reasonably uniform. Major variation occurred in oil content of rice polish which ranged from 13.47% to 22.10%. Acid insoluble ash (AIA) content of rice polish was 1.07 to 1.19% whereas deoiled rice bran (DORB) contained AIA in the range of 1.07 to 4.30% with a mean value of 2.81%. The AIA content in broken rice was minimal (0.029%). The mean dry matter content was higher in DORB (88%), medium in rice polish (87.67%) and lower in broken rice(85.60%). Crude protein (CP), crude fibre (CF), ether extract (EE) and ash content of DORB were 15.47%, 6.18%, 0.77% and 11.17%, respectively whereas broken rice contained CP-6.90%, CF-0.53%, EE-0.50% and ash-0.51%. It is concluded that rice milling co-products in Nepal are of reasonably good quality and could replace some portions of high cost grains and oilcakes in livestock and poultry ration.

Key words: Broken rice, crude protein, crude fibre, deoiled rice bran, dry matter, ether extract, rice polish, total ash

INTRODUCTION

Rice is one of the most important cereal crops in Nepal which shares around 60 per cent of total cereal crop production (NARC, 2012). It contributes nearly 20 per cent to the agricultural gross domestic products and is the major food crop of Nepal (NARC, 2012). Rice production in fiscal year 2011-12 was 5.07 million metric tones which was 13.7 per cent higher than the previous year (NARC, 2012). Rice milling coproducts used in livestock

and poultry feed in Nepal are rice polish or rice bran, broken rice and deoiled rice bran (DORB).

Rice polish is a byproduct of rice milling industry and is derived from the outer layers of the rice caryopsis and consists of fine particles of pericarp, seed coat, nucellus, aleurone layers, embryo and part of sub-aleurone layer of the starchy endosperm, and obtained from the polishing of brown rice (Juliano, 1988). Deoiled rice bran is produced from crushing industries by removing oil from rice polish through solvent (hexane) extraction process.

Rice polish is a good source of protein (13.2 to 17.1%), fat (14.0 to 22.9%), carbohydrate (16.1%), fiber (9.5 to 13.2%), vitamins and minerals (Vargasgonzalez, 1995; Aljasser and Mustafa, 1996; Ambashankar and Chandrasekaran, 1998). It contains 12 to 13% oil (NRC, 1994). It is rich in vitamins including vitamin B, vitamin E, thiamin, niacin, and minerals like calcium, iron, magnesium, phosphorus, potassium, copper, sodium and zinc (Saunders, 1990., Hu et al. 1996., Xu, 1998). The amino acid profile of the rice polish protein is generally superior to that of cereal grain proteins (Farrel, 1994). It is a good source of lysine and methionine compared to maize and wheat (Khalique et al., 2004).

Nepal is almost self-sufficient in rice milling coproducts such as rice polish (Sharma, 2012) and broken rice but depends partly on India for DORB as there are only a few (2 to 3) rice polish crushing plants in Nepal. There are possibilities of exporting these commodities in future. Rice coproducts produced in various parts of Nepal may vary in chemical composition due to varietal differences (rice), milling procedures, adulteration with hulls of little nutritive value and sand silica. It is therefore necessary to have knowledge on actual nutritive values of these feed ingredients available in the market before using them for formulating compound feeds.

The aim of this study was to assess the nutritional composition of rice polish, broken rice and deoiled rice bran that are produced in different parts of the country.

MATERIALS AND METHODS

Sampling method

Samples of rice polish (127), broken rice (15) and deoiled rice bran (33) were received at Probiotech Industries laboratory from forty six rice mill industries, crushing plants and traders/suppliers located at Siraha, Janakpur, Kailali, Banke, Bara, Parsa, Morang, Chitwan and Rupandehi districts of Nepal between March 2011 to November 2012.

The samples were ground to pass through 0.5mm sieve. Moisture content, dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF), ash, nitrogen free extract (NFE) and sand silica (SS) contents were determined using MPA-FT-Near Infrared Reflectance Spectrometer (NIRS) at the 12,500-4,500 nm wavelength range. Calibration databases used in NIRS included enough samples from all over Nepal to cover most of the possible spectral variability encountered during routine analysis. Metabolizable energy (ME, kcal/kg DM) was calculated by using prediction equation as provided by Janssen (1989). Two representative samples of rice polish with CP-12.69 % were selected and sent to Evonik lab, Mumbai, India to determine the amino acid content.

Statistical Analysis

Data on chemical composition of rice polish generated through NIRS were compiled by using Microsoft (MS) Excel 2007. The data were analyzed using descriptive statistics tool through MS Excel 2007. The mean value of amino acid content of two samples of rice polish with 12.69% CP was taken to determine amino acid content in rice polish.

RESULTS AND DISCUSSION

Chemical composition and metabolizable energy value of rice milling coproducts are presented in Table 1.

Table 1. Chemical composition of rice milling coproducts (% as fed basis)

Composition	Rice polish	Broken rice	DORB
Number of Samples	127	15	33
DM			
Minimum	85.16	84.27	84.27
Maximum	92.93	87.7	91.21
Mean	87.67	85.60	88
SE	0.10	0.13	0.24
CP			
Minimum	12.37	6.70	15.15
Maximum	12.53	7.06	15.67
Mean	12.47	6.90	15.47
SE	0.002		0.02
CF			
Minimum	5.46	0.20	4.84
Maximum	5.70	1.32	7.26
Mean	5.56	0.53	6.18
SE	0.003		0.11
EE			
Minimum	13.47	0.34	0.53
Maximum	22.10	0.65	0.92
Mean	16.93	0.50	0.77
SE	0.15	0.086	0.02
Ash			
Minimum	7.47	0.34	9.76
Maximum	8.17	0.6	11.84
Mean	7.73	0.51	11.17
SE	0.01	0.058	0.08
NFE			
Minimum	38.38	76.79	49.69
Maximum	53.08	79.23	59.22
Mean	44.97	77.61	54.40
SE	0.20	0.55	0.39
ME (kcal/kg DM)			
Minimum	2942		1807
Maximum	3365		2314

Mean	3137	-	2039
SE	7		22
AIA (Sand/Silica)			
Minimum	1.07	0.024	1.07
Maximum	1.19	0.04	4.30
Mean	1.12	0.029	2.81
SE	0.001	0.003	0.16

Note: DM- dry matter, CP- crude protein, CF- crude fibre, EE- ether extract, TA- total ash, NFE- nitrogen free extract, ME- metabolizable energy, AIA- acid insoluble ash, DORB- deoiled rice bran, SE-standard error

About 28% of rice polish, 22.86% of DORB and 98% of broken rice samples had less than 87% DM and 38% of rice polish, 51.43% of DORB and none of broken rice had more than 88% DM. Dry matter in the range of 87-88% was recorded in 34% of rice polish, 25.71% of DORB and 2% of broken rice. Major variation occurred in the oil content of rice polish (range: 13.47% to 22.10%) and AIA content of DORB (range: 1.07% to 4.30%). About 14.73% of rice polish samples contained less than 15% oil, 57.36% of samples contained oil in the range of 15 to 18 % whereas 27.91% of rice polish samples contained more than 18% oil.

The mean CP, EE and ME content of rice polish were more than the NRC (1994) values of 12.2% CP, 11% EE and 3090 kcal/kg DM metabolizable energy. Crude protein content of rice polish (12.47 % as fed basis or 14.22 % as DM basis) was more than the value reported by Tiwari et al. (2006) of 12.77 as % DM basis. Crude fibre content of rice polish was 1.46 percent higher than NRC (1994) value of 4.1% but this value was less than that recorded by Tiwari *et al*. (2006). Ash content of rice polish (7.73% as fed basis or 8.82% as DM basis) was also lower than that described by Tiwari *et al*. (2006) of 10.19% as DM basis. Oil content of DORB was less than 1% in all the samples. Broken rice contained 6.90% CP as fed basis (8.06% as DM basis) which was slightly less than that reported by Upreti (2006). He found 8.80% CP in broken rice on dry matter basis. Metabolizable energy values obtained for rice polish and DORB were higher but this may not give clear picture on accurate ME values as the prediction equation for ME is not derived from the samples that are produced and used in Nepal.

The amino acid content in Nepal rice polish samples are presented in Table 2. Amino acid content are compared with NRC (1994) values.

Table 2. Amino acid content of rice polish in Nepal as compared to NRC (1994)

Amino acids	Content (%) , CP-12.69%	NRC, 1994 value, (CP-12.2 %)
Methionine (M)	0.24	0.22
Cystine	0.26	0.10
M+Cystine	0.5	0.32
Lysine	0.52	0.57
Threonine	0.46	0.40
Tryptophan	0.16	0.13
Arginine	0.95	0.78
Isoleucine	0.42	0.41
Leucine	0.87	0.80
Valine	0.66	0.76
Histidine	0.35	0.24
Phenylalanine	0.56	0.46

Content of large particles (>1mm) and ash are indicators of milling standards and variation in these components can indicate adulteration, particularly hulls and broken rice grains (Spadaro et al, 1980). Maximum ash content of 8.17% in rice polish obtained in present study was lower than the maximum reported by Warren & Farrel (1990) of 11%. There is some variation in oil content of rice polish which may be due to varietal differences in rice.

CONCLUSION

Rice polish, broken rice and deoiled rice bran are rice milling coproducts that are extensively used in livestock and poultry ration in Nepal. Nepal is almost self sufficient in rice milling coproducts and these are potential local feed ingredients for future. This study analyzed nutritional composition of these ingredients and found that they were of reasonably good quality and could replace some portions of high cost grains and oilcakes in livestock and poultry ration. However, due to wide variation in some of the important nutritional parameters, it is recommended that the feed millers analyze the samples before accepting them to be used in the compound feed.

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VETERINARY STUDENTS AND VETERINARIANS PERCEPTION TOWARDS VETERINARY EPIDEMIOLOGY AND PUBLIC HEALTH

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ABSTRACT

A cross sectional questionnaire survey was done among 105 veterinarians and 105 university veterinary students during March-May 2012 to evaluate their perception towards VE&PH in Nepal. The significant findings reveled that though several modification have been made in the university curriculum for undergraduate veterinary studies, 32.86% opined that addition of courses on animal health & human welfare, emerging infectious diseases and food safety & security will enhance the graduates skill for interests in VE&PH. Similarly all veterinarians (100%) perceived the need of higher educational degree in VE&PH as well as need of more veterinary epidemiologists and public health specialist for jobs outside veterinary colleges in the country. The study showed that higher percentages (98%) of the veterinary students perceived that new veterinarians should have firm understanding of epidemiology. It is high time that the veterinarians put themselves in the frontline of epidemiology and public health professionals as an expert of comparative medicine.

Keywords: Veterinary epidemiology, Veterinary public health, Opinions, Veterinarians and students, Nepal

INTRODUCTION

FAO, WHO and OIE define veterinary public health (VPH) as "the contributions to the physical, mental and social well-being of humans through an understanding and application of veterinary science". Veterinary public health contributes to public health through the knowledge, skills and resources of veterinary science. This generally relates to the understanding, prevention and control of zoonotic diseases and food safety issues.

The scope of veterinary public health is clearly multidisciplinary, involving not only veterinarians in public and private sectors, but also other health and agriculture professionals, communication experts and scientists as well as paraprofessionals. An interdisciplinary team approach to problem solving, research, control programs and communication is essential for the improvement of human health in a significant and sustainable manner. Veterinary epidemiology deals with the investigation of diseases,

productivity and animal welfare in animal populations. It is used to describe the frequency of disease occurrence and how disease, productivity and welfare are affected by the interaction of different factors or determinants. This information is then used to manipulate such determinants in order to reduce the frequency of disease occurrence. Veterinary epidemiology is a holistic approach aimed at coordinating the use of various scientific disciplines and techniques during an investigation of disease or impaired productivity or even animal welfare. The field of veterinary epidemiology can be divided into different components. One of its essential foundations is the collection of data, which then has to be analyzed using qualitative or quantitative approaches in order to formulate causal hypotheses. As part of the quantitative approach to epidemiological analysis, epidemiological investigations involving field studies or surveys are being conducted and models of epidemiological problems can be developed. This discipline has evolved through three stages, beginning with the fight against animal diseases, moving on to include meat inspection and control of zoonoses and now encompassing a much broader health sciences education, with the goal of guaranteeing a safe and wholesome food supply, protecting human wellbeing and conserving the environment(Lipman & van Knapen, 2009).

Improvement and innovation in undergraduate teaching and learning requires understanding of student perception of discipline context, content and relevance. This is particularly so for veterinary public health and epidemiology which, due to its population focus, can appear to veterinary undergraduates to be less relevant than other disciplines. At the same time, department of the government of any particular country is equally responsible for policies regarding the development of livestock sector. Until and unless the veterinary medical college students perceive VE&PH as an integral part of the national and personal liability, they will not be able to work in line with the government for attaining the national livestock development goal. Veterinary medical colleges in Nepal do not have a uniform syllabus and the values given to the core courses like medicine and surgery far exceeds the values given to para-clinical courses like VE&PH. In order to direct improvements in course design and implementation, and to help in fulfilling the gaps, and for the recommendation, a study of veterinarians and university veterinary students' perceptions of veterinary public health and epidemiology was conducted in two veterinary colleges and different line agencies of Nepal in 2011 A.D.

The objective of the study was to learn the perceptions of veterinarians and university veterinary students’ towards veterinary public health and epidemiology in Nepalese context and to compare the perception of university veterinary students at the commencement and conclusion of an epidemiology course.

METHODOLOGY

Study design

A cross sectional study design was used.

Selection of research site

There are three veterinary colleges in Nepal out of which one college has not yet produced any graduates. The Himalayan College of Agricultural Sciences and Technology, HICAST (Purbanchal University) and Institute of Agriculture and Animal Sciences, IAAS (Tribhuvan University) are the two colleges whose graduates are out in the field. The practicing veterinarians were randomly selected from various organizations such as Nepal Agriculture Research Council (NARC), Central Veterinary Laboratory (CVL), National Avian Laboratory (NAL), Department of Livestock Service (DLS), District Livestock Service Office (DLSO) and private veterinary clinics in two districts (Kathmandu & Chitwan).

Sample size and sampling procedure

The list of veterinarians was obtained from Nepal Veterinary Association's Vet Directory. The list of students was obtained from respective veterinary colleges. The respondents were selected by simple random sampling method. Altogether 105 veterinarians and 105 veterinary students were selected. The sample sizes for this study by various institutions are as shown in Table 1.

Table 1. Sample size distribution by institutes

Institute	Respondents		
	Vets with graduate or higher degree	Vets with undergraduate degree	Students of veterinary medical school
HICAST	6	15	57
NPI	2	2	0
IAAS	15	19	48
NARC	5	7	0
CVL	6	3	0
DLS	4	4	0
DLSO	1	4	0
NAL	2	1	0
Private Practice	3	6	0
Total	**44**	**61**	**105**

Data collection

Data was collected by written personal interview using a pre structured questionnaire format. Written interview was undertaken to avoid the personal hesitation to answer the questions in face to face oral interviews and for completing the study within the specified time frame. Two sets of questionnaires containing both closed and open-ended questions, one for veterinary students and the other for veterinarians were developed. Questions were designed to obtain information on demography, interest, experience, perception and knowledge towards VE&PH and their importance in veterinary field. Closed-ended questions included those that required participants to indicate their responses according to a Likert scale (i.e., strongly agree, agree, unsure, disagree, and strongly disagree). The format for veterinary students consisted of 34 questions on demography and topics of veterinary syllabus. The questionnaire for veterinarians consisted of 33 questions on demography and topics of VE&PH.

Pre-testing of questionnaire

The interview format was pre-tested prior to administering to the actual respondents. Pre-testing was done at HICAST with four veterinarians and six veterinary students on 25th February 2012.Recommended adjustments were made on the interview schedule and were finalized.

Survey

Veterinarians' and veterinary students' survey was carried out between 7th March to 21st May 2012. Pre-tested questionnaire was distributed to the sampled respondents. Respondents were visited at their respective institutes for format distribution. The format was given to the respondents and the recipients were requested to fill up the format to the best of their knowledge but avoiding giving any private information. The format was collected after twenty four hours or later but within two days of distribution.

Data analysis

The information collected from the field was coded first and entered into a computer. Separate data sets were created for responses to the veterinary student and veterinarian questionnaires. Descriptive statistics like mean, percentage and frequency were used to describe demographic, interest, experience, perception and knowledge towards VPH&E. Data entry and analysis was done using Statistical Package for Social Science (SPSS) vs. 16 and Microsoft Excel vs. 7. Analyzed data was then presented in tables, graphs and pie chart.

Dichotomous (yes or no) outcome variables derived from responses to the veterinary student questionnaire included the following: whether a course related to public health was considered necessary for their veterinary career choice, whether a course in public health was listed as 1 of the 3 courses that will help most for their current career choice, whether they would like more courses in VPH&E offered in the veterinary curriculum, and whether a course in public health was listed as 1 of the 3 courses they expected to help their career the least.

Dichotomous (yes or no) outcome variables derived from responses to the veterinarian questionnaire included the following: whether veterinarians considered a course related to public health to be necessary in the veterinary curriculum on the basis of their career experiences, whether a course in public health was listed as 1 of the 3 courses that helped their career the most, and whether a course in public health was listed as 1 of the 3 courses that helped the least in their career. In addition, responses to questions that appeared on both the student and veterinarian questionnaires were compared.

RESULTS AND DISCUSSION

Gender and education distribution of the veterinarians by institutions

Respondents were asked to specify their formal level of educational in veterinary science. The educational level of veterinarians was studied in two groups (BVSc & AH or equivalent as 'veterinarians' and those with degrees beyond BVSc & AH as 'veterinarians with higher degree'). Among the total respondents 33.33% were male veterinarians with higher degree and 8.57%were female veterinarians with higher degree. Similarly, 40% were male veterinarians and 18% were female veterinarians. The analysis of educational degree of the respondents indicated that veterinarians with minimum degree (BVSc & AH) were higher (58%) as compared to veterinarians having higher degree (42%). The data shows that the percentage of female veterinarians with higher degree (8.57%) as well as female veterinarians (18.09%) is lower as compared to that of male. There were 22.22% female respondents with higher degree in veterinary CVL (22.22%) followed by 14.70% at IAAS and 4.76% at HICAST (4.76%)(Table 2).

Table 2. Gender distribution of the respondent veterinarians by institution

S.N.	Institutions	Veterinarians with higher degree				Veterinarians				Total	
		Male		Female		Male		Female			
		No	%	No	%	F	%	F	%	F	%
01	HICAST	5	23.8	1	4.7	9	42.8	6	28.5	21	20.0
02	NPI	2	50	0	0	2	50	0	0	4	3.8
03	IAAS	10	29.4	5	14.7	14	41.1	5	14.7	34	32.3
04	NARC	4	33.3	1	8.3	5	41.6	2	16.6	12	11.4
05	CVL	4	44.4	2	22.2	2	22.2	1	11.1	9	8.5
06	NAL	2	66.6	0	0	1	33.3	0	0	3	2.8
07	DLS	4	50	0	0	2	25	2	25	8	7.6
08	DLSO	1	20	0	0	3	60	1	20	5	4.7
09	Private Practice	3	33.3	0	0	4	44.4	2	22.2	9	8.5
Total		35	33.3	9	8.5	42	40.0	19	18.0	105	100

Gender distribution of the veterinary students by institutions

The gender analysis of the respondent students showed that among 105 veterinary students, 77.14% students were male and only 22.85% were female. The gender distribution of veterinary students by institutions showed that female students in veterinary science at HICAST (26.31%) were higher as compared to IAAS (18.75%). The percentage of male students at HICAST and IAAS was found 73.68% and 81.25% respectively.

Additional courses required for better knowledge in VE&PH

Among 210 sampled veterinarians and veterinary students, 32.86% (n=69)respondents suggested that there should be additional curriculums on animal health &human welfare, emerging infectious diseases and food safety &security for the better knowledge in VE&PH at the university level. Similarly, 26.67%(n=56) respondents preferred to adding courses on veterinary farm hygiene, meat technology and zoonoses courses as major three subjects which would help them with their career in VE&PH. The details of the courses as suggested by the respondents that would help them with their career in VE&PHare as shown in Fig. 2.

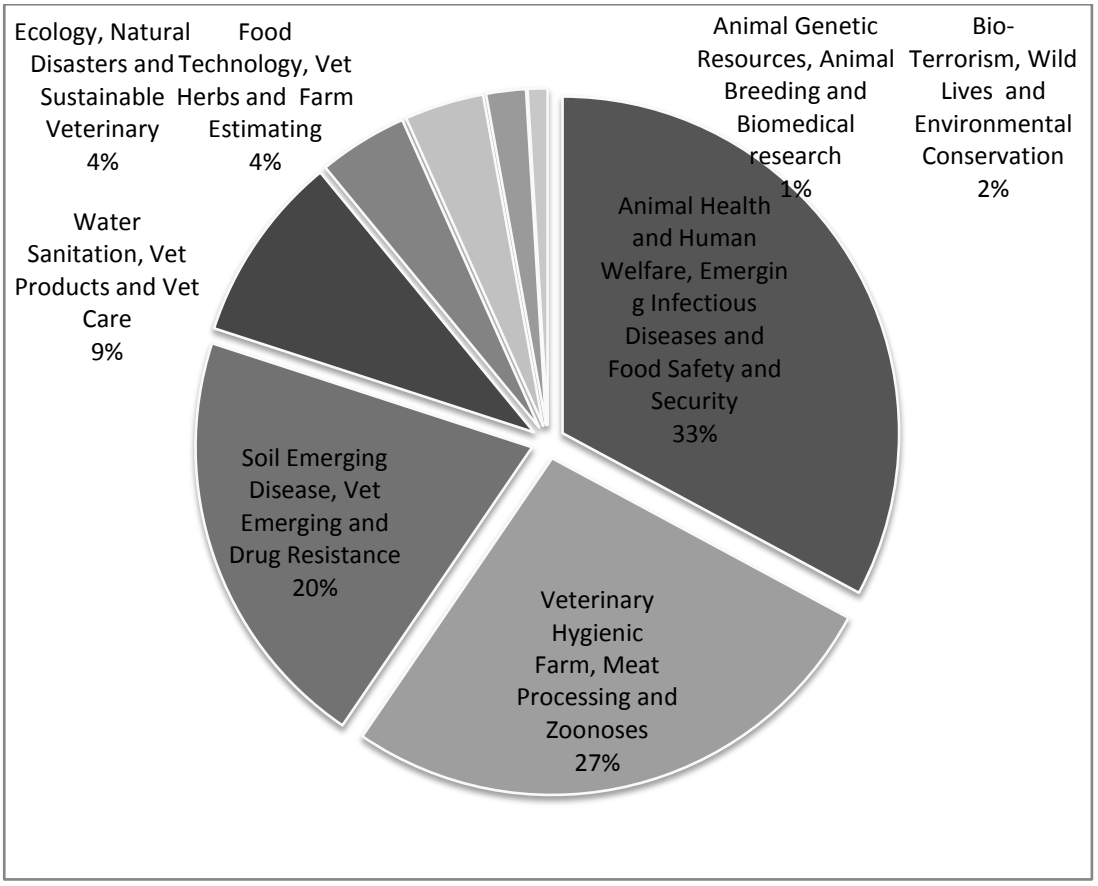

Figure 1. Additional courses as suggested by the respondents so as to help their career in VE&PH

Views on year of teaching VE&PH in university curriculum

Among 105 sampled veterinarians during the study 32.38% (n=34) veterinarians expressed their view that the courses on VE&PH should be taught in fourth year of the study duration while 25.71% (n=27) veterinarians thought it is better to teach this subject in third year. When the same question was asked to veterinary students, higher percentage 28.58 % (n=30) of the veterinary students preferred the fourth year of college for teaching VE&PH. This was followed by views of 25.72% (n=27) in third year and 21.9% (n=23) in fifth year. 1 veterinarian and 4 veterinary students thought that courses on VE&PH were unnecessary for their curriculum (Fig. 2).

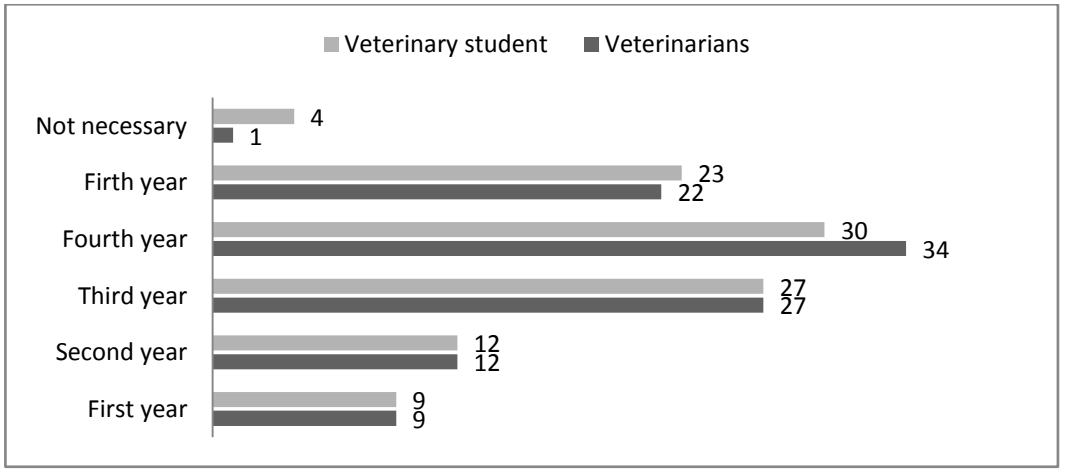

Figure 2. Number of vets and veterinary students suggesting the suitable study year for the courses on VE&PH

Major courses of veterinary curriculum which helped the most in personal careers

Major three subjects which help in career development of the veterinarians as well as veterinary students were studied. Open ended question was put forward to evaluate the most helpful subject that would contribute and that had contributed to building a successful personal career. Out of 210 of veterinarians and veterinary students, 23.9 %(n=23) respondents perceived epidemiology, public health and medicine as most important subject helpful in building their career. Higher percentages of respondents 21.4 %(n=45) perceived preventive medicine, pharmacology and microbiology as the most helpful courses for their career. Para-clinical courses like fodder, breeding and parasitology were also perceived by 19% (n=40) respondents as courses useful in their career. Other preferred courses are as given in Fig 3.

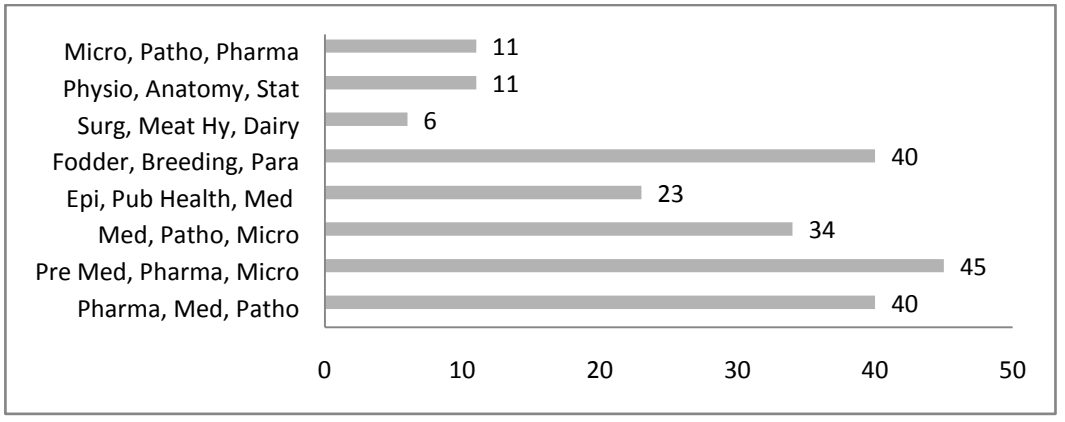

Figure 3. Major courses in veterinary science perceived as the most helpful in personal careers

Major courses of veterinary curriculum which helped the least in personal careers

Study showed that perception of respondents on least helpful subject in their career was highly variable. Among 210 sampled veterinarians and veterinary students,38% (n=80) did not wish to rank any courses they studies as the least helpful in their career. However, 19% (n=40) respondents described economics, aquaculture and surgery as the least helpful subjects in their career. The details of these responses are given in Fig. 4.

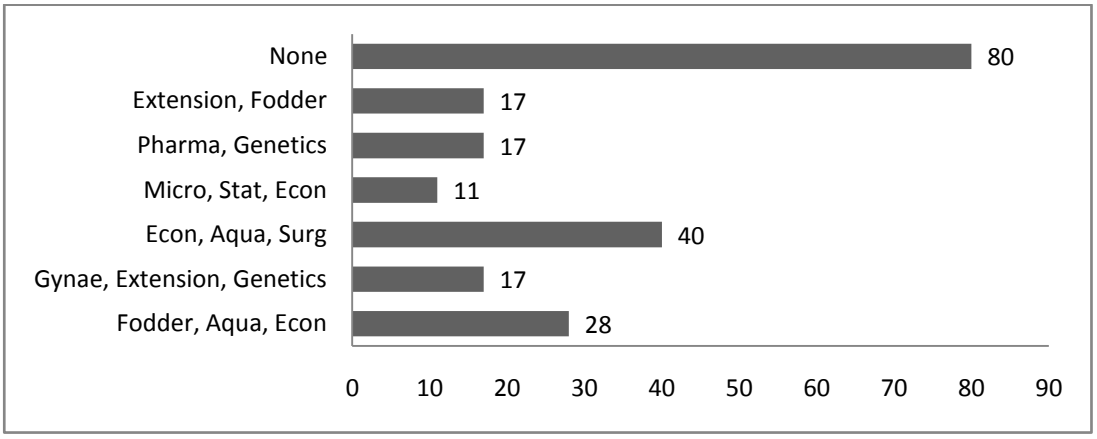

Figure 4. Major courses in veterinary science perceived as the least helpful in personal careers

Attitudes toward veterinary epidemiology and public health

To understand attitudes of veterinarians and veterinary students towards VE&PH, eight different statements related to VE&PH were put on the questionnaire and five options were given to classify the intensity of specific given argument in a Likert scale as strongly agree, agree, unsure, disagree, and strongly disagree on the basis of which attitudes towards VE&PH predicted.

Attitudes of veterinarians toward VE&PH

The study showed that 100% veterinarians strongly agreed with the notion thatgraduating veterinarians should be able to consider environment and other predisposing factors in addition to etiologic agents while diagnosing a disease while78.3%strongly agreed that graduating veterinarians should be aware about antimicrobial resistance to help them prescribe antimicrobials. It is noteworthy that 19.5% of the veterinarians strongly disagreed that the vets graduating from Nepalese veterinary college get enough understandings of epidemiology, public health and zoonoses. The details of the responses for the veterinarians are given in Table 3.

Table 3. Veterinarians with particular attitudes toward VE&PH (in percentage)

Statements	SA	A	U	D	SD
Graduating veterinarians should have firm understanding of basic epidemiologic measures of diseases including incidence, prevalence, odds and risk ratios.	69.6	30.4			
Graduating veterinarians should be able to consider environment and other predisposing factors in addition to etiologic agents while diagnosing a disease.	84.8	15.2			
Graduating veterinarians in Nepal currently get enough understanding of epidemiology, public health and zoonoses to competently work in the field.		6.5	37	37	19.5
Graduating veterinarians should be aware about antimicrobial resistance to help them prescribe antimicrobials.	78.3	21.7			
Graduating veterinarians need a thorough knowledge of food hygiene to advise farmers to produce safe milk and meat.	69.6	30.4			
Veterinarians are the most influential people to train farmers about zoonotic and public health issues.	63	37			
Representation of veterinarians in national level public health programs is very low.	45.7	37	17.4		

Key: SA-Strongly Agree, A-Agree, U-Unsure, D-Disagree, SD-Strongly Disagree

Attitudes of veterinary students toward VE&PH

Study on the attitudes of veterinary students towards VE&PH showed revealed that 81% of the student strongly agreed in graduating veterinarians being able to consider environment and other predisposing factors in addition to etiologic agents while diagnosing a disease. Similarly, there was no significant variation in the percentage of students who strongly agreed or just agreed with the statements; graduating veterinarians need a thorough knowledge of food hygiene to advise farmers to produce safe milk and meat, representation of veterinarians in national level public health programs is very low and graduating veterinarians should have firm understanding of basic epidemiologic measures of diseases including incidence, prevalence, odds and risk ratios. The other statements and the percentage of students' responses are detailed in Table 4.

Table 4. Veterinary students with particular attitudes toward VE&PH (in percentage)

Statements	SA	A	U	D	SD
Graduating veterinarians should have firm understanding of basic epidemiologic measures of diseases including incidence, prevalence, odds and risk ratios.	71.4	20	8.6		
Graduating veterinarians should be able to consider environment and other predisposing factors in addition to etiologic agents while diagnosing a disease.	81	12.4	6.6		

Graduating veterinarians in Nepal currently get enough understanding of epidemiology, public health and zoonoses to competently work in the field.	47.6	35.2	17.1		
Graduating veterinarians should be aware about antimicrobial resistance to help them prescribe antimicrobials.	0	4.8	53.3	23.8	18.1
Graduating veterinarians need a thorough knowledge of food hygiene to advise farmers to produce safe milk and meat.	75.2	15.2	9.5		
Veterinarians are the most influential people to train farmers about zoonotic and public health issues.	60.6	26	13.4		
Representation of veterinarians in national level public health programs is very low.	73.3	14.3	12.4		

Key: SA-Strongly Agree, A-Agree, U-Unsure, D-Disagree, SD-Strongly Disagree

Necessities for betterment of VE&PH as perceived by the veterinarians and veterinary students

Fifteen different statements of VE&PH with yes/no questions were administered to the selected respondents (veterinarians and veterinary students) to study their perceptions on necessities for betterment of VE&PH in Nepal.

Necessities for betterment of VE&PH as perceived by the veterinarians

All veterinarians involved in the survey perceived that knowledge in VE&PH is important to perform their necessary job functions; new veterinarians should have firm understanding of VE&PH and food hygiene to advice clients on issues of food safety issue. Similarly all veterinarians (100%) perceived the need of higher educational degree in VE&PH as well as need of more veterinary epidemiologists and public health specialist for jobs outside veterinary colleges in the country. Only 42% of the veterinarians thought that we have enough vets to teach public health in our veterinary college while 58% were either unsure or thought that we did not have enough vets to teach the courses on VE&PH in our veterinary colleges. The other details of the Likert outcomes are detailed in Table5.

Table 5. Necessities for betterment of VE&PH as perceived by the veterinarians (in percentage)

Statements	Yes	No	Unsure
Knowledge in epidemiology is important to perform necessary functions in my job	100		
Knowledge in public health is important to perform necessary functions in my job	100		
I would go for higher degree in public health or epidemiology if I get an opportunity to study	67	14	**19**
New veterinarians should have firm understanding of epidemiology	100		

New veterinarians should have firm understanding of public health	100		
New veterinarians should have understanding of food hygiene to advice clients for food safety issue	100		
We need more courses in public health in B.V.Sc& AH curriculum	86		**14**
We need more courses in epidemiology in B.V.Sc& AH curriculum	78		**22**
We have enough veterinarians to teach public health in our veterinary college	42	29	**29**
We have enough veterinarians to teach epidemiology in our veterinary college	46	23	**31**
We need higher degree programs (MS, PhD etc.) in public health in Nepalese colleges.	100		
We need higher degree programs (MS, PhD etc.) in epidemiology in Nepalese colleges	100		
Higher degrees (MS, PhD etc.) in Nepalese colleges can address our needs better than that by foreign universities	78		**22**
We need more epidemiologists for jobs outside veterinary college	100		
We need more public health specialists for jobs outside veterinary college	100		

Necessities for betterment of VE&PH as perceived by the veterinary students

There was more variation in perceptions of veterinary students towards necessities for betterment of VE&PH as compared to the veterinarians. The study showed that higher percentages (98%) of the veterinary students perceived that new veterinarians should have firm understanding of epidemiology. Similarly, 95% of the veterinary students agreed with the statement that knowledge in epidemiology and public health is important to perform necessary functions in the future. Only 24% of the student believed that we need more courses on VE&H at undergraduate level. This might be due to the reason that these days more and more students are either attracted for abroad studies after graduation or they are likely to prefer courses on small animal practice, medicine and clinics rather than food animal and herd health management. Veterinary students' perceptions to VE&PH as found during this study are presented in Table 6.

Table 6. Necessities for betterment of VE&PH as perceived by the veterinary students (in percentage)

Statements	**Yes**	**No**	**Unsure**
Knowledge in epidemiology is important to perform necessary functions by a veterinarian	95	3	**2**
Knowledge in public health is important to perform necessary functions by a veterinarian	95	3	**2**
I would go for higher degree in public health or epidemiology if I get an opportunity to study	65	16	**19**
New veterinarians should have firm understanding of epidemiology	98	2	
New veterinarians should have firm understanding of public health	89	11	**10**

New veterinarians should have understanding of food hygiene to advice clients for food safety issue	81	10	**9**
We need more courses in public health in BVSc&AH curriculum	24	62	**14**
We need more courses in epidemiology in BVSc&AH curriculum	27	62	**14**
We have enough veterinarians to teach public health in our veterinary college	40	25	**35**
We have enough veterinarians to teach epidemiology in our veterinary college	24	41	**35**
We need higher degree programs (MS, PhD etc.) in public health in Nepalese colleges.	57	9	**34**
We need higher degree programs (MS, PhD etc.) in epidemiology in Nepalese colleges	60	12	**28**
Higher degrees (MS, PhD etc.) in Nepalese colleges can address our needs better than that by foreign universities	58	21	**21**
We need more epidemiologists for the jobs outside the veterinary college (eg government, NGOs)	61	20	**19**
We need more public health experts for jobs outside veterinary college (eg government, NGOs)	**60**	**12**	**28**

DISCUSSIONS

The results of the demography of the veterinarians and the veterinary students show higher number of males as compared to the females. This higher number of males is not only the situation in veterinary professions but in others as well. The secondary school dropout rate is equal at 6.9% for both females and males. However, the promotion rate is lower at 89% in females as compared to89.4% in males and the repetition rate is higher at 4.1% for females as compared to 3.4% to males in secondary level education thus leading to less number of qualified females at higher education level (MOE, 2012).Similar was the trend in 2000 in the USA as reported by (Slater & Slater, 2000) who found a difference in the gender representation among the veterinarians and the veterinary students.

Being one of the least developed countries in the world, the national focus is on the development of sustainable livestock in all parts of the nation with emphasis on courses of animal husbandry like fodder, nutrition, breeding which is clearly reflected in the veterinarians' choice of courses that helped the most in their career. Similarly, epidemiology and public health is ranked at the fourth place among the most useful courses in their careers. This shows that in the near future there will be less and less veterinary epidemiologists to work at the field level. Reports across the world also predict a shortage of veterinarians trained to address the future needs of public health (Fostgate, 2008).The opportunities for VPH are boundless, but the challenge is to be able to apply the plethora of available research results and knowledge. What we will need is a new breed of veterinarians who will lead and provide us with a vision (Arambulo, 2008)

Veterinarians have been found more likely to report that a course in VE&PH was necessary in their careers rather than the students. Although 100% of the veterinarians believed that knowledge of VE&PH is important to their line of work, that firm understanding of VE&PH is necessary for field practice, and they have felt the need for postgraduate degree in VE&PH in Nepal, 62% the veterinary students did not think that they need more courses in VE&PH but 60% believed that they see a future need of postgraduate degrees in VE&PH in Nepal.

This might be an indication that a true differences exists in the intensity of personal interests of the current students versus the past students or this disparity may be attributable to some unmeasured factors such as social and peer pressures, in societies like ours where social and peer pressure play a major role in selection of undergraduate courses for many youngsters. This might be also due to the fact that various modifications have been made in the courses of BVSc&AH with the commencement of semester system at IAAS and establishment of veterinary colleges at the private level. So this disparity is obvious. But now what is evident is that there is a strong urge for postgraduate degrees in VE&PH in Nepal. Around 60% of the students and 100% of the vets believed that there is a need for more epidemiologists in jobs outside the veterinary college or discipline which clearly unveils the scope of VE&PH as being a multidisciplinary science. There are opportunities to foster and encourage an interest in the VE&PH via some adjustments in the veterinary curriculum. Such courses could be pivotal to prepare the students for careers in VE&PH. In the context of current of revolutionary information technology, various collaborative online platforms and social interfaces for sharing educational contents can be used for attracting the students for education in VE&PH. One of the most novel and comprehensive implementations of collaborative online sharing of educational content in epidemiology and public health is the Epidemiology Supercourse (http://www.pitt.edu/~super1/). More than 9,000 faculties from 118 countries have contributed to an online library of more than 700 lectures with quality control and adherence to accepted pedagogic principles. The goal is to improve teaching and research in epidemiology and public health worldwide. Although the focus is on human medicine, the concepts, methods, and principles can easily be applied to veterinary medicine. The Association for Veterinary Epidemiology and Preventive Medicine (AVEPM) seeks to heighten awareness of issues in veterinary epidemiology and public health education among veterinary educators through various forums, symposia, and workshops. The AVEPM Web site (http://www.cvm.uiuc.edu/avepm/) includes a listing of educational software and Web sites supporting epidemiology and public health education (Smith, 2003).

Animal diseases are known to be the origin of many human diseases, and there are many examples from ancient civilizations of plagues that arose from animals, domesticated and

wild. Records of attempts to control zoonoses are almost as old. The early focus on food-borne illness evolved into veterinary medicine's support of public health efforts. Key historical events, disease outbreaks, and individuals responsible for their control are reviewed and serve as a foundation for understanding the current and future efforts in veterinary public health. Animal medicine and veterinary public health have been intertwined since humans first began ministrations to their families and animals (Steele, 2008). As seen in this study, 63% of the veterinarians and 60% of the veterinary students believed that veterinarians are the most influential people to train the farmers about zoonotic and public health issues. Similar reports have been made globally by various authors where they opine that veterinarians have a unique ability to bridge the fields of human and animal medicine and agriculture because of their education in comparative medicine. The veterinary profession deals not only with the animal as an individual but also with the herd health and zoonotics (Fostgate, 2008; Kelly & Marshak, 2007; Walsh et al, 2003; Baker et al, 2006 and Noah & Crowder, 2002). Veterinary medicine and the veterinary profession have a social responsibility too. We must care about the society we serve and live with. This responsibility is even more significant when one considers that we are the only medical profession trained in comparative medicine. We are more able than other medical professions to construct the complex picture of disease dynamics among diverse populations of animals and people (Blackwell et.al., 2006).Undergraduate and postgraduate training courses must promote a greater understanding of the importance of zoonoses and of how to investigate and control them. A good example has been seen at Auburn University, USA where they have been able to increase the students' exposure to the role of the veterinarian in public health and to develop a dual-degree DVM/MPH programs to augment their training in public practice(Wenzel, Nusbaum, Wright & Hall, 2008).

Though outside the scope of this report, some report suggest that around 20% of the students will change their career focus during their veterinary education (Becker, 2003) so we can expect some subtle changes in the personal career choice but the study still reveals that as 45.7% of the veterinarians and 73.3% of the veterinary students strongly agree in the notion of poor representation of the vets in national level public health programmes, we can expect more veterinary students choosing this line of career in future. Many variables may influence the skills and knowledge required to be a successful and competent veterinary practitioner. Expectations for care of small animal patients may vary with hospital and city size, between veterinarians practicing exclusively on small animal patients and those practicing predominantly on small animal patients, and with the geographic location of the practice. Recent graduates from veterinary school may use some skills or areas of knowledge with a different frequency than do seasoned practitioners and may have different expectations.

Other factors that may come into play include the internal personal demands of the veterinarian on himself or herself, client expectations, facilities and equipment available in the practice, and outside resources that are readily available to the veterinarian. Although the influence of all of these factors cannot be determined, the effects of certain demographic variables on the frequency of use of certain procedures and areas of knowledge and the proficiency expected for new graduates can be investigated.

VE&PH is a population concept rather than individual concept so the students need to become familiar and comfortable at the same time with animal level medicine before embarking on a population medicine. This concept is truly represented in the Fig 2 & 3, which shows the appropriate years for commencement of courses on VE&PH. Both groups of respondents agree that the primary concept of basic VE&PH can be started in the third year of veterinary college education which can be continued to the fourth year with the advanced courses that can be learnt easily after completion of individual level medicine in the third year. Veterinary training in most parts of sub-Saharan Africa has focused on producing veterinarians to serve the livestock sector although socio-economic changes and privatization of Veterinary Services have caused curriculum adjustments, as have globalization and the increased risk of the spread of transboundary diseases. In South Africa, undergraduate veterinary training is more clinically oriented than in other regions (Swan, Coetzer, & Terblanche, 2009). Electives were considered an important didactic or experiential component of the curriculum to strengthen existing core curriculum. Electives or selective, though not required for graduation, serve to broaden skills and knowledge on the basis of individual needs, interests, and professional goals. They permit students to investigate alternative career options, often affording real world clinical experiences, and give students the opportunity to pursue focused, advanced, and in-depth material ona particular topic. Electives also provide a mechanism for faculty to introduce emerging or ancillary topics to interested students and to assist in development of new courses that may be incorporated into core curriculum. In many instances a strong elective program is used by a particular school as a recruiting tool. It could be said that veterinary public health education has literally moved from the local abattoir to the global community(Lipman & van Knapen, 2009). The future of veterinary public health relies on the opportunities available in education to teach and encourage students to pursue a career of public service.

CONCLUSION

From this study we concluded that there is higher representation of males in the veterinary education system of Nepal. The respondents believed that addition of courses on Animal

health & human welfare, emerging infectious diseases and food security & safety will enhance the knowledge of the current veterinary students so as to prepare for the future in the field of VE&PH. Both groups of the respondents, the veterinarians as well as the veterinary students believed that third and fourth year of veterinary schooling is the best years for education on VE&PH. By these years students will have had knowledge of herd health as well as medicine which will ease them to be familiar with the principles and practices of VE&PH. Preventive medicine, pharmacology and microbiology were regarded as the three most useful courses in personal careers. This reflects the trend of the veterinary students where they are attracted more towards clinical practice in small animal rather than the herd health or the large animal practice. Most of the vets working in the extension sector regarded fodder, animal breeding and parasitology as the most useful three courses for their career. The response was variable for the least useful courses but beside these, some ranked economics, aquaculture and surgery as the least useful courses for their career. Though the veterinarians as well as the students thought that we need more vets in VE&PH in Nepal, the sufficiency of the course credits were perceived differently. Several modifications have been made in the veterinary curricula after the commencement of semester system at IAAS and with the establishment of private veterinary colleges in Nepal.

The study concludes that the veterinarians as well as the students see a good future in developing careers in VE&PH but opine that the scope of VE&PH must be taken to the wider audience of human health, zoonotic, bioterrorism food and safety.

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STUDIES OF CHRONIC AFLATOXICOSIS IN RABBITS

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ABSTRACT

A total of six rabbits of 3-6 months of age with body weight of 1-1.5 kgs were kept on a uncontaminated diet for a 7 months period and base values of haematological and biochemical parameters were taken before they were subjected to 0.025 ppm of aflatoxin in the diet for a period of 7 months. The rabbits showed reduction in feed intake, emaciation and dehydration and icterus was marked in one rabbit. The haemogram in this study revealed reduction in haemoglobin, packed cell volume and total erythrocyte count which were significantly reduced during the seventh month. The total serum protein recorded a gradual reduction from third month onward. Similarly the serum albumin also showed downward trend, while the globulin values revealed an upward trend showing decrease at the seventh month of treatment. The blood glucose showed a gradual reduction with the lowest value at the seventh month. In the present study cholesterol showed a gradual increase with peak value recorded during seventh month. The ALT values also showed marked increase. The mean values of AST showed an increasing trend. The mean values of GGT also showed a marked increase. The activity of GGT in liver tissue was markedly increased and distributed diffusely in liver with increased GGT. The changes in liver were atrophy with reduction in size. The distention and thickening of the gall bladder was pronounced during the later stages of treatment. Microscopically hydropic and fatty changes as well as focal necrosis were seen. In some cases focal hepatocytomegaly, diffuse kupffer cell hyperplasia, bile duct hyperplasia and lymphocytic infiltration were observed towards the end of the trial. Perivenular edema and fibrin exudation were observed. Cholangitis was often present. Diffuse cholangiofibrosis with widened portal areas and isolation of groups of hepatocytes in the form of islands were present and multiple granuloma were observed. The lungs showed emphysema, edema, and septal infiltration. The kidney showed glomerular swelling, tubular degeneration and fibrosis and hyalinization while lymphoid necrosis and depletion was observed in the spleen. The intestine revealed desquamation of epithelial layers and increased catarrhal activity.The testes showed marked dissociation and atrophy

of spermatogonial cells and the brainrevealed neuronal degeneration, gliosis and frequent perivascular cuffing.

INTRODUCTION

Aflatoxicosis as a separate disease entity had its first report by Blount in 1960 in turkeys from England (turkey"X" disrease), when the exact etiology was not known. Subsequently many many others reported on aflatoxicosis from different parts of the world affecting several species of animals. The disease is due to the mycotoxin commonly called "Aflatoxins" which are the toxic metabolites of certain strains of the moulds *Aspergillus flavus* and *Aspergillus parasiticus.* This is one of the major causes of economic losses to the poultry and livestock industry. The fungi producing aflatoxins are known to be the usual contaminants of feeds and fodder such as ground nut, cotton seed meal, maize sorghum etc. Due to their ubiquitous presence in nature, these fungi can effect growing crops secondary to primary infections with bacteria or viruses during growth, harvest or storage and produce toxins. The presence of mycotoxins in grains, pulses and feed poses serious problems in quality control usually encountered by producers, manufacturers and handlers. The growth of moulds in the food stuffs is often influenced by environmental factors such as season (climate), humidity and temperature. Therefore, the magnitude of aflatoxicosis varies with geographical and seasonal factors, as in tropical and subtropical areas where optimal conditions in the form of high humidity and temperature favour the formation of toxins.

The hepatotoxic, teratogenic, mutagenic, immunosuppressive and carcinogenic characters of the aflatoxins drew the attention of scientific world. However, acute or chronic aflatoxicosis may occur depending on the level of toxins in the feed and species of the animals affected more often resulting in moderate to severe hepatic damage. Ducklings and rabbits are reported to be more sensitive to these toxins. Poor growth rates and moderate to high death rates have been encountered depending on the species susceptibility and the concentration of toxin uptake.

The rabbits as a species once grouped under experimental animals has now assumed the status of food source and listed in commercial livestock industry. They are bred for their valuable fur and meat owing to its rapid and high fecundity potentials and rabbitary on commercial lines is expected to ease the demand pressure on chicken and mutton. In Nepal, rabbit farming is now gaining recognition as an economic livestock industry.

Aflatoxicosis in rabbits will now assume greater importance with the use of commercial feeds, which may contain varying amounts of toxins. This study was undertaken by feeding

mash feed containing various levels of aflatoxins to experimentally produce sub acute and chronic aflatoxicosis in rabbits and study the pathology.

This study was designed to experimentally induce aflatoxicosis in rabbits and record the pathogenesis with suitable paramete4rs at low levels of the toxin incorporated in the feed. The study considered to assess the following aspects.

a) Haematological changes comprising of haemoglobin, Packed Cell Volume, Total erythrocyte count, Total leucocyte count, Total platelet count and clotting time.
b) b) Biochemical profile comprising of total serum protein, albumin, and globulin, glucose, cholesterol, aspertate amino transferase, alanine amino transferase and gamma glutamyl transpeptidase.
c) Gross and histopathological findings in the internal organs comprising of liver, kidney lungs, stomach, intestine, pancreas, spleen and brain.

MATERIALS AND METHODS

Culture of *Aspergillus parasiticus* NRRL2999 was received from Food and Drug Toxicology Research Centre, National Institute of Nutrition, Hyderabad. *Aspergillus parasiticus* (NRRL 2999) culture was maintained and subcultured once in15 days in potato dextrose agar (Shotwell *et al.,* 1966). Aflatoxin was produced on rice) Shotwell *et al.*, 1966). 100 g of rice was taken in 500 ml of Erhlenmeyer flasks, and soaked in 40 ml of distilled water for two hours with frequent shaking. The flasks were autoclaved, cooled and inoculated with *Aspergillus parasiticus*(NRRL2999). They were kept at room temperature and hand shaken vigorously six to ten times a day. After 48 hrs of inoculation mould growth was seen as white spots on the surface of the rice, later turning to bright yellow in colour. Subsequently in about a week time the colour became brown. On the tenth day, mouldy rice was dried in hot air oven overnight at 60o.C.after which it was ground to affine powder. The powdered mouldy rice was analysed for the aflatoxin content by the following method.

Extraction of aflatoxin (Romer, 1975 method)

Slurry was prepared by blending 25g of mouldy rice powder with 100 ml of distilled water for three minutes. The extraction was done by blending with 150 ml of acetone for two minutes and filtering through Whatman No.1 filter paper. The extract was purified by taking 75 ml of filtrate in 250 ml conical flask and swirling it after adding 1.5g of cupric carbonate. In another 250 ml conical flask, ferric gel was prepared after adding 15 ml of ferric chloride solution(0.41M) to 85 ml of sodium hydroxide(0.2M) and swirled. The ferric gel was immediately transferred to the flask containing the extract and the contents were mixed thoroughly and allowed to stand for two minutes with occasional swirling. It was filtered through Whatman No.! filter paper and 100 ml of the filtrate was mixed with 100 ml of

Sulphuric acid(0.03%) in a 500 ml separating funnel and the extraction was done thrice using 20 ml. 20 ml and 10 ml of chloroform each time, collecting the lower chloroform layer. The combined extract was transferred to a 250 ml separating funnel containing 100 ml of Potassium hydroxide(0.02M) and Potassium chloride(1%) mixture and gently swirled for 10 seconds. The lower chloroform layer was collected in a 100 ml beaker through a funnel containing a bed of anhydrous sodium sulphate. The final extract was evaporated to near dryness in a water bath at 40^{o}C and transferred to a vial and dried completely. Known quantity of chloroform was added to dilute the extract and used for quantitation in thin layer chromatography(TLC).

Estimation of aflatoxin by Thin Layer Chromatography(TLC)

TLC plates were prepared (0.25mm thickness) by using Silica gel G: distilled water (2:1) slurry. Applicator was used for spreading the gel on the plate. They were activated in a hot air oven at 110ºC for an hour. The samples and standards were spotted on the plates and developed in Chloroform and Acetone (90:10) mixture for 45 minutes. The plates were then removed dried and examined under long wave(365 nm) ultraviolet light in a chromato-view cabinet. The aflatoxin content (B1) was calculated according to AOAC (1980) specifications.

Formula for calculation:

aflatoxin content in ppm= S×Y×V/Z×W

where,

S= Comparable volume of standard in µml

Y= Concentration of standard in ng/ml

V= Volume in ml of the solvent required

Z= Comparable volume of sample in µml

W= Effectiveweight(g) of the sample

Preparation of the diet:

Special rabbit feed was obtained from the Poultry Research Station, Nandanam, Madras. The feeds were analyzed to eliminate the presence of aflatoxin. Weighed amounts of powdered rice containing known amount of aflatoxin were incorporated into the aflatoxin free diet so that the dietary aflatoxin level was at 0.025 ppm by mixing thoroughly.

Experimental animals:

Six male rabbits of 3-6 months of age with body weight of 1-1.5 kgs were received from Livestock research Station, Kattupakkam and from the Department of Laboratory Animal Science, Madras Veterinary College. The rabbits were housed in individual cages with *ad libbitum* supply of water. Animals were kept on a control uncontaminated diet for a 10 days

period and base values of haematological and biochemical parameters were taken before they were subjected to different treatments.

Experimental design:
The experiment consisted of six rabbits fed with diet containing 0.025 ppm of aflatoxin for a period of seven months.

Clinical symptoms:
The clinical signs exhibited by rabbits during the experimental period were recorded.

Clinical pathology:
Collection of blood samples:
Blood samples (2ml) were collected before starting the experiment and after every month, in vials containing EDTA for haematological studies and for estimation of glucose.For biochemical parameters, 3 ml of blood was collected and serum was separated and stored at -20º C.

Haematological Praameters:
Haemoglobin (Acid Haematin method), packed cell volume (Micro-hematocrit method), total erythrocyte count, total leucocyte count, total platelet count (Rees- Eccker method), clotting time (capillary tube method)

Biochemical parameters:
Blood glucose(Cooper and Mc Daniell,1970), total serum protein (Biuret method), total serum albumin, serum globulin, blood cholesterol-Ferric chloride method(Zak *et al*.,1954), Aspartate Amino Transferase(AST)- Reitman and Frankel(1957) method, Alanine Amino Transferase (ALT), Gamma Glutamyl Transpeptidase4(GGT, Szasz,1969 and Jacob,1971)

Pathology:
At the end of the experimental period the rabbits were sacrificed. A detailed post-mortem examination was conducted and gross changes were recorded. The animals which died during the trial were also subjected for post-mortem examination. Representative pieces of liver, lungs, heart, kidney, spleen, duodenum and pancreas were collected in 10% buffered formalin and embedded in paraffin for histopathological studies. S sections were cut and stained by Haematoxylin and Eosin.

Histochemical localization:

Histochemical demonstration of GGT was carried out in both cryostat sections and paraffin embedded sections as per the methods of Ruttenberg *et al* (1969) and Albert *et al* (1961). Tissue pieces were taken from all the lobes. Cryostat sections were prepared from fresh tissues stored in=20^{o}C mounted on slides, fixed with chilled acetone and stained.

For paraffin embedded sections tissues were fixed in 80% alcohol for 24-48 hours at 4^{o}C, dehydrated in two changes of absolute alcohol, cleared in benzene and impregnated with low melting paraffin at a temperature below 55^{o}C.They were imbedded in hard paraffin. Sections of 5 μm thickness were cut, mounted and de-paraffinised with benzene before staining. Sections were incubated at room temperature in a freshly prepared solution containing

γ - glutamyl-4-methoxy-B napthalamide-20mg
Glycyl glycine-10mg
Fast blue BBn-10 mg
Tris Hcl Buffer(pH7.4)-5-ml
Saline(0.9%)-14 ml

After incubating for 15-30 minutes, the slides were rinsed in saline for 2 minutes and then transferred to 0.1 M Copper sulphate solution for 2 minutes. After another saline rinse, the sections were rinsed in distilled water; counter stained with Haematoxylin, washed dried and mounted in glycerine jelly. Positive sites of enzyme were stained with yellowish red.

The data collected in the trial- I were subjected to least square analysis as per Harvey(1975) since the subclass numbers were unequal and disproportionate. The results of trial II were analyzed as per Snedecor and Cochran(1968).

RESULTS AND DISCUSSION

Clinical symptoms

The rabbits exhibited appreciable reduction in feed intake, emaciation and dehydration and icterus was marked in one rabbit. Clark *et al* recorded emaciation and dehydration in rabbits fed with 0.025 mg/kg body weight of aflatoxins, for 24 days. Clark *et al*(1982) and Abdel Hamid *et al* (1990) in their study on induced chronic aflatoxicosis in rabbits, reported decrease in feed consumption and loss of body weight. In present study icterus was observed in one animal, and diarrhea was evident in two rabbits. Clark *et al*(1980), observed icterus and dehydration in chronic aflatoxicosis in rabbits suggesting the possible hepatic injury in treated animals. Four animals died during treatment each on first, third, sixth and seventh month. Similarly, Clark *et al*(1980) and Clark *et al*(1982) observed death in a study of induced chronic aflatoxicosis. Progressive emaciation was seen in all the animals. In two animals diarrhea was evident.

Clinical Pathology

The haemogram in this study revealed reduction in haemoglobin concentration, packed cell volume and total erythrocyte count which were significantly reduced during the seventh month as was also reported by Clark *et al*(1980) who observed a gradual decrease in haemoglobin and packed cell volume in rabbits in chronic aflatoxicosis. Tung *et al*(1975) also reported low level of haemoglobin, packed cell volume and erythrocytes in chicken treated with greater doses of dietary aflatoxin. The total leukocyte count in this study recorded decrease which was persistent till the sixth month of treatment. Antyulov(1964) and Morrisse *et al* (1981) observed a decrease in leukocyte count following aflatoxin administration in rabbits. However leukocytosis was reported in chicken(Tung *et al*., 1975). The mean platelet count was found to decrease during the treatment from first to seven month, with a consistent increase in the clotting time. The defective coagulation which occurred in aflatoxicosis was attributed to reduced hepatic synthesis of clotting factors viz. factors V, VII and VIII apart from platelet number (Baker and Green, 1987).

Biochemical changes

The total serum protein recorded a gradual reduction from third month onwards during treatment. Similarly the serum albumin also showed downward trend from first to seventh month, while the globulin values revealed an upward trend til the sixth month, thereafter showing decrease at the seventh month of treatment. Reduction in total protein was recorded in aflatoxicosis in rabbits Clark *et al* (1980). The results suggested that the protein synthesis was interfered with in chronic aflatoxicosis, due to the direct inhibitory action of aflatoxin on protein synthesis (Wogan, 1966). Reduction of protein synthesis was attributed to AFB1 treatment. The protein synthesis was inhibited at elongation or termination stage i.e. at polysomal level during the initial periods of treatment. At later periods, inhibition occurred at the initiation stage itself (Salunkhe *et al.*, 1987). A reduction in albumin suggested the probable hepatic dysfunction. The initial increase in globulin level could be a relative Hyperlobulinaemia (Coles, 1986), while the reduction in globulin value during the terminal phase might reflect a direct action of aflatoxin (Salukhe *et al.*, 1987).

The blood glucose showed a gradual reduction from first to seventh month, recording the lowest value at the seventh month. Harvey *et al* 1991 also recorded a marked decrease in blood glucose in aflatoxicosis in goats while Gill *et al*(1985) reported increased glucose level in rabbits which received dietary aflatoxins. The decrease glucose level might be due to decrease feed intake and partial exhaustion of glycogen in the liver. Coles (1986) reported that hypoglycemia could be due to hepatogenic disorders apart from starvation.

In the present study cholesterol showed a gradual increase with peak value recorded during seventh month. Gill *et al*(1985) observed an increase in serum cholesterol in rabbits which received aflatoxins.. However Abdelhamid (1990) reported reduction in blood cholesterol following feeding molded diet.The increase in blood cholesterol could be reflection of liver injury(Coles, 1986). The mean values of AST showed an increasing trend as was reported in different domestic animals both in experimental and natural aflatoxicosis (Clark *et al*., 1980, Clark *et al*., 1982, Balachandran and Ramakrishnan, 1988; Bouda *et al.*, 1977, Bortell *et al*., 1983 and Cysweski, 1982). In the present study, the activity of AST was maintained at a higher level than normal through the trial indicating continuous hepatic injury during treatment (Baur *et al.*, 1974; Coles 1986)

The ALT values also showed marked increase from first to seventh month. Similar increased levels of ALT in aflatoxicosis in different animals were also recorded (Clark *et al.*, 1980, Abdelhamid, 1990, Balachandran and Ramakrishnan, 1988, Cysweski, 1982). The mean values of GGT also showed a marked increase from first to seventh month. Bortell *et al*. 1983, and Bouda *et al*., (1977) also observed an elevated level of GGT level following administration of aflatoxin in dairy cows and ponies respectively. The elevated GGT level was considered to be sensitive indicator of hepatic disorder (Rosalki, 1975)

Gross pathology

The changes in liver were marked in contrast to the lesions in other organs. Atrophy was consistent and prominent with reduction in sie and wrinkling of the surface. Initially pallidity of liver was also observed in the experimental rabbits, which in later stages showed numerous pin head sized raised nodules. The distention and thickening of the gall bladder was also pronounced during the later stages of treatment.. Microscopically hydropic and fatty changes as well as focal necrosis were seen. In some cases focal hepatocytomegaly, diffuse kupffer cell hyperplasia bile duct hyperplasia and lymphocytic infiltration were observed towards the end of the trial. The changes were intense and exaggerated at the end o the trial. In some cases perivenular edema and fibrin exudation giving a sheath like appearance were observed, while cholangitis was often present. Diffuse cholangiofibrosis with widened portal areas and isolation of groups of hepatocytes in the form of islands were charastically present. Occasionally glandular arrangement of hepatic cells, foci of altered hepatic cells and multiple granuloma were observed.

The proliferative changes of epithelial cells of bile duct appeared to be a characteristic future in cattle, duckling and turkeys while these changes were superimposed with destruction of hepatic parenchyma in chickens (Moreau and Moss, 1979). Clark *et al*., 1980 also observed

portal fibrosis and hyperplasia of biliary epithelium in rabbits while hepatic necrosis appeared to be insignificant. A gradual reduction in protein, albumin, blood glucose, hypercholesterinemia and increased serum levels of AST, ALT observed in all the treated animals correlated well with hepatic dysfunction associated with pathological lesions induced in the chronic trials. These indicated a failure of the hepatic compensatory mechanism as was evidenced by pronounced cholangiofibrosis with marked destruction and atrophy of hepatic cells. Microgranulomatous changes observed in this study was similar to changes induced by some organophosphorous compounds (Krishnan, 1991). The foci of altered cells that were considered as preneoplastic foci (Squire and Levit, 1975) were occasionally observed in this study. Since these foci had not progressed to neoplastic nodules in the period of study, they were not taken as preneoplastic foci.

The lungs showed emphysema, edema, and septal infiltration. The kidney showed glomerular swelling, tubular degeneration and fibrosis and hyalinization while lymphoid necrosis and depletion was observed in the spleen.The intestine revealed desquamation of epithelial layers and increased catarrhal activity.The testes showed marked dissociation and atrophy of spermatogonial cells and the brainrevealed neuronal degenration, gliosis and frequent perivascular cuffing. All these changes were the results of chronic intoxicationwith aflatoxin in feed (Newberne, 1973)

In this experiment the activity of GGT was not only markedly increased but also distributed diffusely in liver. In those cases serum GGT levels were too increased. The GGT activity in tissue was increased whenever there was damage to hepatocytes at their bile canalicular surface.

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PRODUCTION OF CELL CULTURE ANTI RABIES VACCINE FOR HUMAN USE IN NEPAL

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ABSTRACT

A trail batch of Cell culture inactivated rabies vaccine was produced at Rabies Vaccine Production Laboratory (RVPL), Kathmandu in 2012. Vero working cell line (164P) was used for propagation of cell that was infected with Pasture virus strain of rabbit fixed rabies virus (RV/RV) at 0.1ml working dilution into 169 serial passages of cells. Virus culture fluid was harvested and inactivated by beta propiolactone. Series of quality test were performed at RVPL and Nancy wild life laboratory in France. The result of quality test such as inactivation, toxicity, safety, specificity, sterility, ED50 and pH test were found satisfactory and titer of vaccine was 3.8 IU/dose. This is remarkable development toward the production of rabies vaccine in Nepal.

INTRODUCTION

Rabies is fatal viral endemic zoonoses in Nepal. The incidence has been reported throughout the year in 45/75 districts in Nepal. According to Epidemiology and Disease Control Division (EDCD), an estimated human case of rabies in Nepal is about 200 annually. In 2009, 37 hydrophobia cases were admitted at hospital and 41,200 people received post exposure vaccine from 48 hospitals. Health authority spends 1 million US $ annually to buy vaccine which is available to victims free of cost (Personal communication with EDCD). Cell Culture Rabies Vaccine (CCRV) for human use is imported in Nepal to date.

In Nepal, 1st batch of 5% and 20% Phenolised sheep brain rabies vaccines (nerve tissue origin) for animal use was produced in 1970. Then beta propiolactone inactivated 5% sheep brain vaccine for human use was produced in 1982 and was used in human from 1986-2006. 1st commercial batch of CCRV for animal use (NeJaRab) was produced by Rabies Vaccine Production Laboratory (RVPL) with the support of Japan International Cooperation Agency

(JICA) in 2006 and marketed. Currently RVPL produces 40,000 doses of rabies vaccine for animal use annually.

The production of CCRV for human use was started in 2007 however the potency of the first batch was only 0.01 IU/dose when it was tested at Nancy rabies and wild life laboratory in France. The production work was continued more effectively from 2009-2012 and eventually RVPL is able to produce the trial batch of vaccine for human use in 2012 according to the potency standard of World Health Organization (WHO). The objective of this research work is to produce vaccine for human being for sustainable control rabies in Nepal and to save the life of victim.

METHODOLOGY

1. Materials

Master cell bank and Working cell bank was established from Vero Seed cell imported from Institute of Pasture, Paris in 1999. Working cell was tested for mycoplasma at Australian Animal Health Laboratory, Australia in 2010. Cells were stored at -196 ℃ into liquid nitrogen until use.

Pasture virus strain of rabbit fixed rabies virus (RV/RV) strain imported from Institute of Pasture Paris in 2009 was propagated into Vero cell to prepare working seed rabies virus at the strength of 10^7 $TCID_{50}$/ml. Molecular characterization of working seed virus was performed at Centre of Disease Control, USA in 2011. Working seed virus (PV/RV) was stored at -80℃ until use.

Eagle's Minimum Essential Media (NISSUI-1) and Dulbecco's Modified Medium, NISSUI-2), Tryptose Phosphate Broth (Merck), L-Glutamine (Gibco), Penicillin Streptomycin (Gibco) Fungizone (Gibco), Kanamycin (Gibco), Fetal Calf Serum (Gibco), Trypsin (Himedia), PBS (NISSUI), Human normal albumin (Reliance) and Beta pripiolactone (Spectrochem)) were purchased or imported and stored at appropriate temperature.

2. Production procedure

Mycoplasma free Vero working cell lines (164P) were restored in growth media containing 10% Fetal Calf Serum and 2mM L-Glutamine. Cells were suspended at the rate of 2x 10^5 Cells/ml in growth media and incubated at 37℃ for 4-6 days. Cells were re-passaged again after 4-6 days. The size of flask and roller bottle (Greiner bio-one) was 25 cm^2, 75 cm^2, 175 cm^2 and 850 cm^2 containing 10, 30, 50 and 200 ml volume of growth media respectively. Cells were passaged up to 169 serial passages. Working seed virus was adjusted to 10^5 $TCID_{50}$/ml for inoculation into the cells at 0.1moi. Infected cell were kept at 34℃ for 7 days after adding maintaining media containing 0.3% Human albumin serum (Montagnon and Fanget,

1996) and 0.02% kanamycin. Virus culture fluid was harvested after the development of cythopathic effect into infected cell. Pooled sample of fluid from cell control and virus infected Roller bottle was tested by using Anigen Rapid Test Kit for the detection of rabies virus. Sample collected from infected cell was found positive for rabies virus antigen where as the fluid collected from control cell was negative for rabies virus. The harvested virus was then centrifuged at 10,000 rpm into continuous flow rotor and condensed for purification in pelliconmachine (milli pore) under Pump Master flex speed 0.5, feed pressure 0.2 Kg/cm^2 and pelican filter membrane 0.02 um pore size. Ten fold concentration of condensed virus fluid was prepared in pellicon machine. Finally this virus fluid was inactivated with beta propiolactone at the 0.025% dilution.

3. Quality test

After production series of quality control tests such as Specificity, Virus Titration in BSR cell line, Inactivation in BSR cell and Swiss albino mice, Sterility in Thioglycolate broth and Sabouraud broth, Toxicity and NIH test in mice and Safety test in dogs were performed according to standard operating procedure for the production of tissue culture rabies vaccine developed by RVPL (2010). The potency of vaccine was tested at Nancy Wild Life Laboratory in France and the efficacy of vaccine was also tested into mice by performing Rabies Fluorescent Focus Inhibition Test (RFFIT) at Changchun Veterinary Research Institute, China.

RESULT AND DISCUSSION

The titer of virus in vaccine fluid before inactivation was $10^{8.5}$ $TCID_{50}$/0.1ml. The ED_{50} of the titer of vaccine was 1:72 when challenged with CVS 500 LD_{50} /0.03 ml in NIH test. The titer of vaccine was 3.8 IU/dose when tested at Nancy rabies and wild life laboratory in France. Minimum potency of human vaccine should be 2.5IU/single human dose according to World Health Organization (WHO), 2005. Antibody titer in vaccinated mice was 1.5 IU/ ml serum on RFFIT test conducted at CVRI, China. WHO (2010) has recommended that virus neutralizing antibody titer in vaccinated human should be above 0.5 IU/ml of blood. The result of other quality test such as inactivation, toxicity, safety, specificity, sterility and pH test were found satisfactory. This is the first report of the remarkable development toward the production of CCV for human use in Nepal. Some improvement in protocol is necessary to produce safe, pure and potent rabies vaccine in Nepal. Now RVPL has to produce 3 batches of vaccine with similar potency according to WHO guideline. Commercial vaccine will be produced and available in the market after getting the satisfactory result of preclinical and clinical test of vaccine.

RECOMMENDATION

Technical and financial supports from national and international organization are required to produce vaccine at RVPL in Nepal to fulfill the national need for prompt availability of vaccine to the victim.

ACKNOWLEDGEMENT

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STUDY ON CHEMICAL EVALUATION OF SHRIKHAND PRODUCED FROM DIFFERENT TYPES OF MILK USING DIFFERENT LEVEL OF SUGAR

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ABSTRACT

A study on chemical evaluation of Shrikhand production process was conducted during June 2012 to November 2012 to comparethe physico-chemical attributes of Shrikhand produced from four different types of milk: Skimmed milk (0.2% fat), standardized pasteurized milk (3% fat), cow milk (4.1% fat) and whole buffalo milk (7.3% fat) and with three different levels of sugar (25%, 35% and 45%) on the weight basis of Chakka. The experiment was conducted using a completely Randomized Design (CRD) with 12 treatments with factorial combination of 4 types of milk and3 sugar level. The mean value of fat, protein, pH, total solids (TS), moisture content (MC) and acidity content of Shrikhand were significantly different ($p<0.001$) in different treatments both at room and refrigerated temperature. This study revealed buffalo milk with 25% sugar is the best for Shrikhand production having optimum fat and protein content.

Key words: Milk, Chakka, Shrikhand

INTRODUCTION

Shrikhand is Indian traditional fermented dairy product made from curd (dahi) which is partially strained through a cloth to remove the whey and thus produce a solid mass called *Chakka* (the basic ingredient for *Shrikhand*). The *Chakka* is mixed with the required amount of sugar, cardamom, saffron etc. to yield *Shrikhand*. This traditional product has been produced at many dairies in India where commercially production made at Sugam Dairy of Baroda, Sabar Dairy of Himatnagar and Amul of Gujrat. Increasing income level and changing food habits of consumers are some of the driving force for increasing demand for milk and milk products in India and Nepal. *Shrikhand* is a semi-soft, sweetish sour, whole milk product, prepared from lactic fermented curd. It shows characteristics flavor and taste due to addition of several ingredients. There is little information on *Shrikhand* production in Nepal and it has not been commercialized yet so it has been imported from India. Most of dairy industries have interested to introduce this new product in domestic market to substitute the import and promote the dairy industry. Therefore, the objective of this study was to determine the physico-chemical attributes of *Shrikhand* produced form different types of milk and sugar level.

MATERIALS AND METHODS

This research work was undertaken to analyze chemical attribute of *Shrikhand* prepared by different types of milk (skimmed milk, standardized milk, cow milk and buffalo milk) treated with different level of sugar (25% , 35% and 45%). Raw milk were collected from Baudh Bari Dairy of Ganessthan, Kathmandu (skimmed milk), Patan (standardized pasteurized milk), Chapagaon of Lalitpur (whole cow milk and buffalo milk) with quality reported 0.2%, 3.0%, 4.1% and 7.3% fat level respectively. The sample preparation and processing has been done according to the procedure mentioned in figure-1. The analysis was performed at various laboratories of NDDB, DDC and DLS. The fat content of *Shrikhand* was determined by Gerber centrifuge method as described in the DDC Quality control laboratory manual, 1998. Protein analysis was carried out following Kjeldahl method, using the Kjeldahl apparatus-DK6-DK20-DK6/48-DK20/26-DK42/26 digestion, distillation systems (AOAC, 1982) and finally followed by titration. The total solids were determined by AOAC method no. 925.23 (AOAC, 1990) and by following IDF standard - 151:1991 published by NDDB (2001). The pH values of different types of samples were measured by digital Hanna Instrument of S358236, IK-850-E11-185 pH meter. The titrable acidity percentage of different level of products was determined by titration method as mentioned in laboratory hand book NDDB, 2001. This experiment was conducted in completely randomized designed (CRD) with 12 treatments for the major parameters became 4x3 factorial combination like T1,T2,T3,….T12 and each type of milk were treated with 3 different level of sugar. The data were calculated by using Gen-stat Discovery 4 Edition with Analysis of Variance (ANOVA).

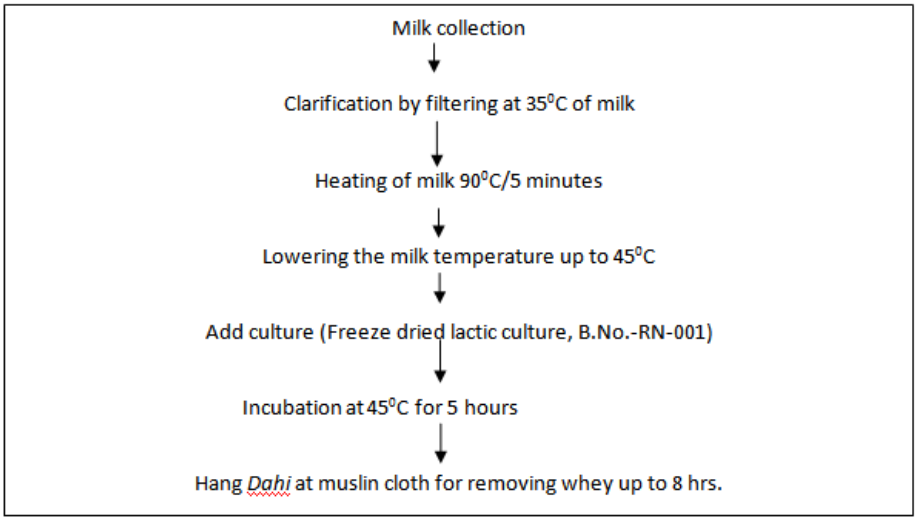

Fig:1 Follow process of *Shrikhand* preparation

RESULT AND DISCUSSION

Total Solid (TS) and Moisture Content:

The mean total solid (TS) content of *Shrikhand* made by different types of milk and sugar level and stored at different temperature (room and refrigerated) is presented in Table 1. The total solid content of *Shrikhand* was significantly different ($p<0.001$) among different treatment groups. The TS content of *Shrikhand* made from buffalo milk was higher compared to *Shrikhand* made from other types of milk. On the day of preparation, the TS content of *Shrikhand* made from buffalo milk with 45% sugar was the highest (52.8%) followed by *Shrikhand* made from standard milk with 45% sugar (50.4%) and buffalo milk with 35% sugar (49.7%). The TS content of the *Shrikhand* made from skimmed milk with 25% sugar was the lowest (35.1%). The TS content also differed significantly ($p<0.001$) among different treatment groups (*Shrikhand* types) stored for different period and different storage condition.

The total solid content of the *Shrikhand* made from different combination of milk and sugar were all below the specification of *Shrikhand* reported by (Gaur, 2010) in which it was stated that the TS of any *Shrikhand* should not be less than 58% by weight. (Kulkarni, Belsare, & Lele, 2006)and (Boghra & Mathur, 2000) have reported even higher level of TS (61.0%) content in *Shrikhand*. The PFA and BIS standards also states that the total TS content should not be less than 58%. The low level of total solid content in this study might have been observed either due to inefficient draining of whey during preparation of *Chakka* or due to addition of more milk with saffron during preparation of *Shrikhand* from *Chakka*. However, the present finding is in agreement with findings of(Nahar, Al-Amin, Alam, Wadud, & Islam, 2007) who reported that the average TS are highest in buffalo milk products.

The total solid content consistently decreased with advancement in the storage period both under storage at room and refrigerated conditions, however the rate of decline in TS content was higher in *Shrikhand* kept at room temperature. The mean TS content lowered to 39.31% from the initial 44.48% in normal room storage condition and to 41.4% from 44.20% under refrigerated condition (Table 1, Figure 2). The greater rate of decrease in TS content stored under room condition compared to products kept at refrigerated condition might be due to faster chemical reaction in product kept at room condition. Another cause might be that *Shrikhand* is fermented dairy product where lactic acid bacteria is reacting at faster rate with favorable condition at room temperature rather than refrigerated condition in which lactose converts into lactic acid resulting decreases in TS.

Table 1: TS content of *Shrikhand* stored at room and refrigerated condition

Parameters	Room Temperature (28±3^{0}C)				Refrigerated Storage (5±1^{0}C)			
	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Mean	44.8	43.0	41.3	39.3	44.2	43.5	42.6	41.4
SEM	0.11	0.57	0.11	0.13	0.05	0.07	0.06	0.07
F Prob	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.33	1.74	0.34	0.39	0.15	0.21	0.19	0.21
CV%	0.30	1.90	0.40	0.50	0.20	0.20	0.20	0.20

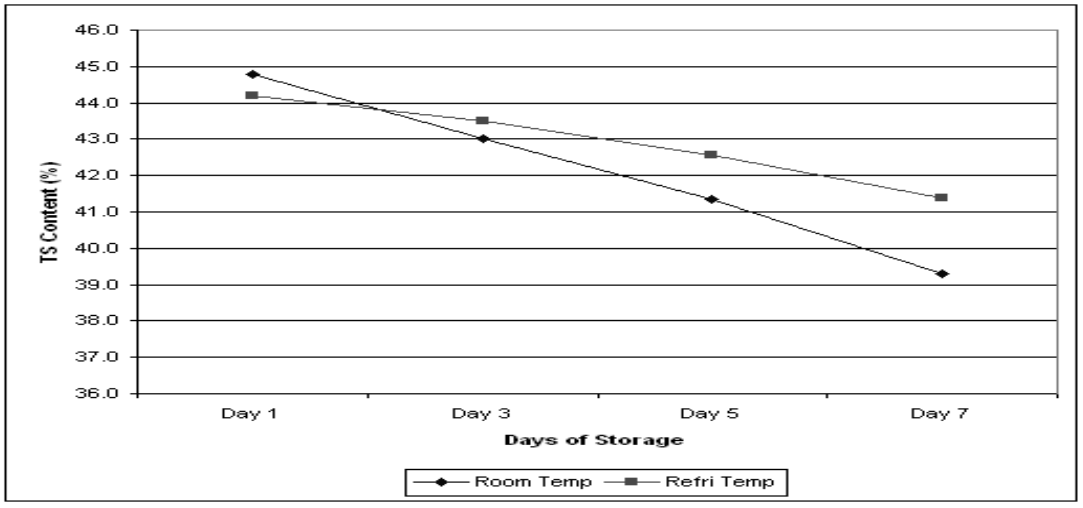

Fig. 2 Trend of TS of *Shrikhand* stored for 7 days under room & refrigerated condition

The moisture content of *Shrikhand* also differed significantly among different treatment groups ($p<0.001$). The difference remained significant for all storage period both under normal room and refrigerated storage conditions.

In contrary to our findings, (Sonawane, Chavan, & Pawar, 2007) had reported that the moisture content of *Shrikhand* during storage decreases and this decrease has been attributed to moisture loss from *Shrikhand* in the atmosphere. One reason for this contradictory result might be that atmospheric condition of our storage was not as dry as reported by the authors in India. However, they have also reported that the loss of moisture was lesser when the product was stored in refrigerated condition (Figure 3) compared to that kept at normal room temperature.

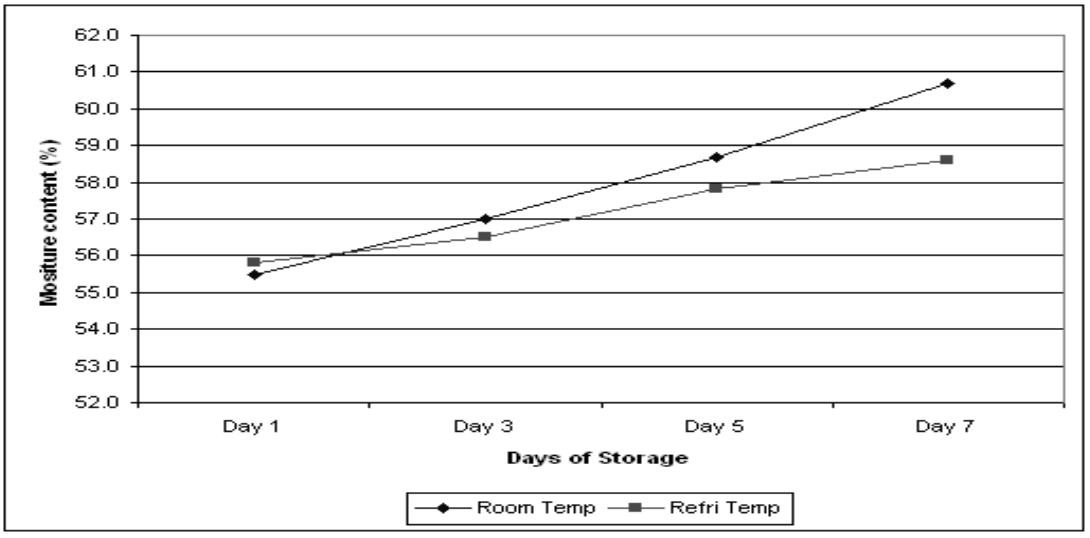

Fig. 3 Trend of MC of *Shrikhand* stored for 7 days under room and refrigerated condition

Fat and Protein Content in *Shrikhand:*

The fat content of *Shrikhand* made from different combination of milk type and sugar level is presented as Table 32. The fat content of *Shrikhand* significantly differed among different treatment groups ($p<0.001$). In general fat content of *Shrikhand* made from buffalo milk was higher compared to *Shrikhand* prepared from other milk. Addition of more sugar in general reduced the fat content of *Shrikhand* as there is no fat content in sugar. The fat content of *Shrikhand* made from buffalo milk with addition of 25% sugar was the highest (13.98%) and was (Figure 4) lowest in *Shrikhand* made from skimmed milk (1.08%).

Table 2: Fat and Protein content of different *Shrikhand*

Parameter	Fat (%)	Protein (%)
Mean	6.98	9.84
SEM	0.34	0.01
F Probability	<0.001	<0.001
LSD	0.11	0.04
CV%	1.00	0.2

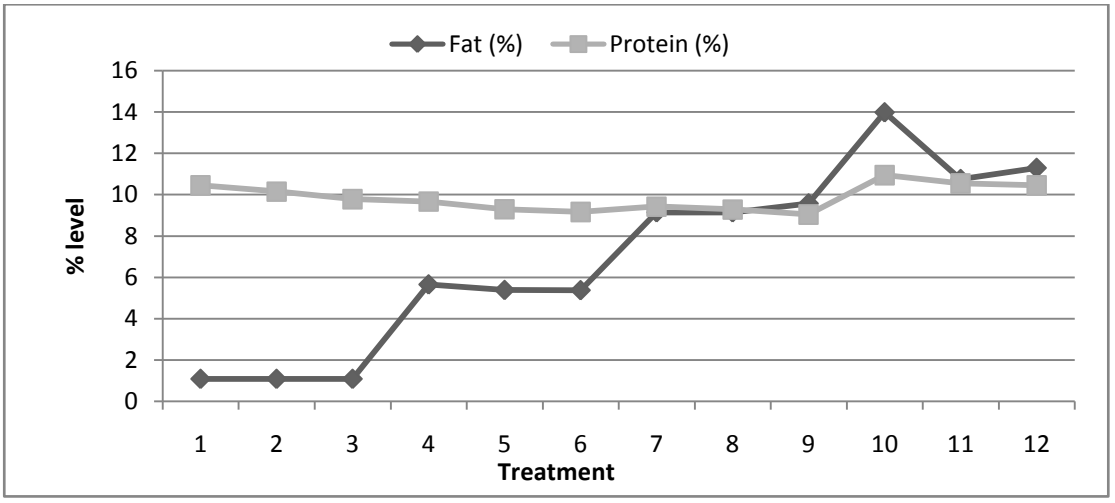

Fig:4 Fat and protein content of different treatment *Shrikhand* made by milk & sugar level

The specification for fat content of the *Shrikhand* in India reported by (Gaur, 2010) as set by PFA and BIS standards is not less than 8.5% on DM basis and not less than 5.3% on fresh basis. In this study, only *Shrikhand* made from buffalo and cow milk meet this standard. Milk fat/cream has to be added while making *Shrikhand* from standard and skimmed milk.

The protein content of *Shrikhand* was also significantly different ($p<0.001$) between different treatment groups and was highest for the product made from buffalo milk with 25% sugar (9.87%) and lowest for that made from standard milk with 45% sugar (9.03%).(Figure 4). The protein level specified by Amul *Shrikhand*, Prevention of Food and Adulteration (PFA) and Bureau of Indian Standard (BIS) is 10.5% on dry matter basis. The present findings suggest that only buffalo milk with all level of sugar added and skimmed milk with only 25% sugar level meet that standard. However, the product being new to Nepal has yet to establish minimum standard for the product.

pH and Titrable Acidity:

Both pH and titrable acidity of *Shrikhand* produced from different combination of milk and sugar level were found to be significantly different between different treatment groups ($p<0.001$) both at refrigerated (Table 3) and room temperature storage conditions and for different days of storage. The pH value of product declined significantly with increasing days of storage but rate of decline was higher for normal storage condition compared to the products stored at refrigerated condition. The rapid decline in pH of the product means the poorer the shelf life of the product.

Table 3: pH of different *Shrikhand* stored for different period

	Room Temperature (28±3^{0}C)				Refrigerated Storage (5±1^{0}C)			
parameters	Day 1	Day 3	Day 5	Day 7	Day 1	Day 3	Day 5	Day 7
Mean	4.31	4.27	4.13	4.00	4.45	4.39	4.33	4.26
SEM	0.02	0.02	0.02	0.02	0.010	0.010	0.019	0.01
F Prob	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
LSD	0.05	0.05	0.07	0.07	0.03	0.03	0.06	0.03
CV%	0.70	0.70	1.00	1.00	0.40	0.40	0.80	0.40

The pH value of traditional *Shrikhand* shouldn't be less than 4.1 (Aneja, Mathur, Chandan, & Banerjee, 2002) and all products (different combination of milk and sugar) in this study except stored for 7 days under room temperature were above this value. The decline in pH was slow while kept at refrigerated condition increasing the shelf life of the products.

Likewise, the mean titrable acidity of prepared products was also found to be significantly influenced ($p<0.001$) by types of *Shrikhand* stored for different days both under normal and refrigerated conditions. The acidity of product varies considerably depending on species, breed, individuality, stage of lactation etc called natural acidity. However, it may be varied due to lactic acid formation bacteria converts lactose into lactic acid called developed acidity (Aneja, et al., 2002) and the rate of formation of this acid influenced by temperature, lactic acid bacterial load, manufacturing process, and storage condition. Gaur (2010) indicated that the titrable acidity of *Shrikhand* should not be greater than 1.4%, and all the products in this study up to 7 days of storage had less (Figure: 5) than that level of acidity. The acidity increased sharply in the products kept at room temperature compared to the products kept at refrigerated condition.

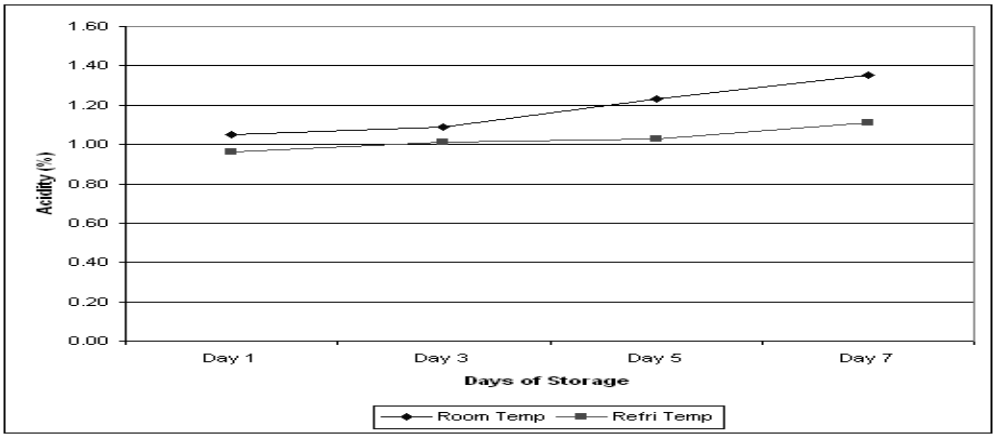

Fig:5 Increase in acidity of *Shrikhand* kept at room and refrigerated condition

CONCLUSION

The results of physico-chemical analysis revealed that overall acceptability on day 1st was highest for *Shrikhand* made from standard milk with 35% sugar and buffalo milk with 25% sugar. *Shrikhand* made of buffalo milk was of superior quality in terms of pH, Acidity, TS, MC, Protein and fat. The overall study of test parameters revealed that the product met the minimum mandatory legal standard. Product made by buffalo milk was superior in terms of sensory attributes due to increased fat, protein & lactose suggesting possible variation in type of milk used.

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SALMONELLA PENETRATION INTO EGGS WASHED WITH VARIOUS DISINFECTANTS AND STORED AT DIFFERENT TEMPERATURES AND DURATION

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ABSTRACT

A study was conducted in the laboratory of Veterinary Teaching Hospital, Rampur from November 2006 to April 2007 with an objective to establish the effective preventive measures for the control of egg-borne Salmonellosis. Salmonella free eggs, collected from IAAS poultry farm were disinfected with one of three egg-washing chemicals, including hydrogen peroxide (3%), alcohol (70%) and sodium carbonate (35 ppm). Seventy two intact-shell eggs were disinfected with each of three chemicals for 2 minutes but the eggs of control group were not disinfected. All the eggs were then inoculated by immersion for 5 minutes into an aqueous suspension of Salmonella typhimurium at 10^5 colony-forming units/ml and dried for 30 minutes. The eggs were stored at 4°C, 21°C or 37°C and Salmonella penetration was checked at one, five and nine day intervals. The results of microbial tests showed that higher percentage of Salmonella penetration into eggs was observed in control eggs (12.5% in day 1, 25% in day 5 and 45.83% in day 9) followed by eggs washed by sodium carbonate (8.3% in day 1, 25% in day 5 and 33.33% in day 9). Prevention of Salmonella penetration by hydrogen peroxide (90.28%) was significantly higher ($p<0.05$) over sodium carbonate (77.78%) and control (72.22%) but similar with alcohol (86.11%). Prevention of Salmonella penetration into eggs stored at 4°C (91.67%) was significantly higher ($p<0.05$) than those stored at 21°C (75%) and 37°C (78.13%). Prevention of Salmonella penetration on day 1 (92.71%) was significantly higher ($p<0.05$) over day 5 (82.29%) and day 9 (69.79%). Salmonella was isolated from albumen and yolk of 58% and 42% eggs, respectively. The possibility of becoming sick from egg borne Salmonellosis can be reduced by disinfection of eggs withhydrogen peroxide and storage at low temperature for short duration.

Keywords: Salmonella, Hydrogen peroxide, Alcohol, Sodium carbonate

INTRODUCTION

Eggs are one of the important human nutrition sources (Wang and Slavik, 1998). Eggs are also a good growth medium for bacteria. The natural defense of egg is generally not adequate to completely protect it from bacteria (Berrang *et al*., 1999). Salmonella on the surface of the shell can penetrate into the interior of the egg and contaminate the internal contents of the egg (Padron, 1990).

Although there is a possibility of transovarian transmission of *S. enteritidis* that results in internal contamination of intact shell eggs, horizontal bacterial contamination of eggs seems to be the major route (Wang and Slavik, 1998). Consumption of Salmonella contaminated eggs and egg products may cause outbreaks of human Salmonellosis (Musgrove *et al*., 2005) and consequently a dramatic decrease in the sale of eggs. So, Salmonellosis is a leading food borne disease, and is a serious threat to both poultry industry and public health.

The emergence of egg borne Salmonellosis has increased the interest to egg washing that might improve the microbiological safety of eggs. Egg storage temperature, storage duration and eggshell disinfection are the factors that are associated with the penetration of Salmonella into eggs. Heightened concerns about the microbial safety of eggs have led to the requirement of research on this matter. So, this study was carried out to define more clearly the effect of various factors on the penetration of Salmonella on shell eggs.

MATERIALS AND METHODS

Collection of Salmonella free eggs

Due to lack of specific pathogen free eggs, the experiment was carried out in Salmonella free eggs collected from IAAS poultry farm. In order to confirm that the eggs selected from IAAS poultry farm were Salmonella free, ten samples of cloacal swabs, eggs and fecal contaminated litter from selected flock were collected aseptically and kept in sterile plastic vials. The samples (egg, cloacal swab and litter) were kept in icebox and transported to laboratory of Veterinary Teaching Hospital, Rampur within 15 minutes. The samples were cultured for isolation and identification of Salmonella. Negative culture tests from all the samples confirmed that the selected flock was Salmonella free.

Disinfection of Salmonella-free eggs

A total of 288 fresh-laid, unfertilized eggs from Salmonellav free flock of IAAS farm were brought to lab of Veterinary Teaching Hospital (VTH), Rampur within 15 minutes. Three types of egg washing disinfectants including hydrogen peroxide (3%, pH 2.7, Central Drug House), alcohol (70%, pH 5.6, Bengal Chemicals, India) and sodium carbonate (35 ppm, pH 10.85, Qualigens Fine chemicals, Mumbai) were selected for disinfection of surface of eggs. The disinfection of eggs typically involved dipping in disinfectant solution for 2 minutes. The eggs of control group were not disinfected by any chemicals. The experiment was replicated two times. Total number of eggs used for disinfection by selected disinfectants is given in Table 1.

Table 1. Number of eggs washed by selected disinfectants

Disinfectants	pH	Manufacturer	No. of eggs		Total
			Rep. I	Rep. II	
Hydrogen peroxide (3%)	2.7	Central drug house (P) Ltd. New Delhi	36	36	72
Alcohol (70%)	5.6	Bengal chemicals, India	36	36	72
Sodium carbonate (35 ppm)	10.85	Qualigens fine chemicals, Mumbai	36	36	72
Control (No dipping)			36	36	72
Total			144	144	288

Artificial contamination and storage of eggs

After disinfection, all eggs were allowed to dry at 21°C for 30 min. *Salmonella typhimurium* in broth obtained from Microbiology laboratory of VTH, IAAS was diluted to 10^5 CFU/ml. The eggs were artificially contaminated by immersion for 5 minutes into Salmonella broth having 10^5 CFU/ml. During contamination, both the eggs and standard Salmonella broth were kept at room temperature (21°C). The eggs of control group were also inoculated by immersion for 5 minutes. All eggs were allowed to dry at 21°C for 30 min.

Egg storage

The 72 eggs, washed by each disinfectant, were divided into 3 groups with 24 eggs in each group and stored at 4°C, 21°C and 37°C respectively. The number of eggs stored at selected temperature is presented in Table 2.

Table 2. Number of eggs washed by each disinfectants and stored at selected temperature

Storage temperature (°C)	No. of eggs		Total
	Rep. I	Rep. II	
4	12	12	24
21	12	12	24
37	12	12	24
Total	36	36	72

Among 24 eggs kept at each storage temperature, number of eggs stored for various days is given in Table 3.

Table 3. Number of eggs kept at each storage temperature and stored for different days

Storage duration (days)	No. of eggs		Total
	Rep. I	Rep. II	
1	4	4	8
5	4	4	8
9	4	4	8
Total	12	12	24

After storage for fixed days, the egg-shells were sterilized by methylated spirit. Then the eggs were broken aseptically. Bacterial penetration into egg content (yolk and albumen) was checked by standard culturing method

Statistical analysis

The combined effects of three factors viz. storage temperature, storage duration and disinfection method on the penetration of Salmonella into the eggs were analyzed by factorial experimental design. In analysis of variance, the data was presented in prevention percentage. The prevention percentage was calculated as follows.

$$\text{Prevention percentage} = \frac{\text{Number of eggs not penetrated by Salmonella}}{\text{Number of eggs examined}} \text{ X } 100$$

The mean comparison of each factor was done by Duncan's multiple range test (Gomez and Gomez, 1984). Statistical analysis was carried out using the Microsoft Excel-2003 and MSTAT-C (Michighan state university, version 3.1).

RESULTS AND DISCUSSION

Salmonella penetration into eggs

Higher percentage of Salmonella penetration into eggs was observed in control eggs (12.5% in day 1; 25% in day 5 and 45.83% in day 9) followed by eggs washed by sodium carbonate (8.3% in day 1; 25% in day 5 and 33.33% in day 9) (Fig 1). In a similar study by Wang and Slavik (1998), higher percentage of Salmonella penetration (30% on day 1 and 76.7% on day 21) in eggs washed by sodium carbonate was reported. Facilitation of Salmonella penetration into eggs washed by sodium carbonate may be due to the destruction of cuticle layer of eggshell. Egg washing solutions having an alkaline pH produces a much greater damaging effect to the cuticle of the egg (Kim and Slavik, 1996).

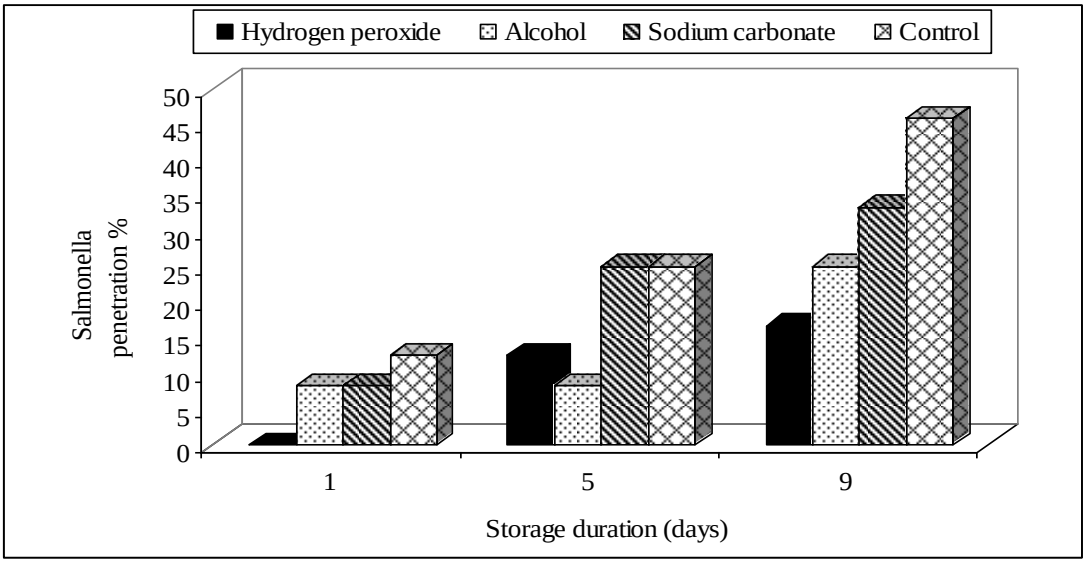

Fig 1. Percentage of penetration of Salmonella into eggs disinfected by selected chemicals

The percentage of Salmonella penetration was increased with increasing storage period of eggs. Higher percentage of Salmonella penetration was observed in eggs stored at 21°C. Salmonella penetration was higher in eggs (12.5%) kept at room temperature (21°C) for one day than in eggs (9.37%) stored under refrigeration (4°C) for 5 days (Fig 2).

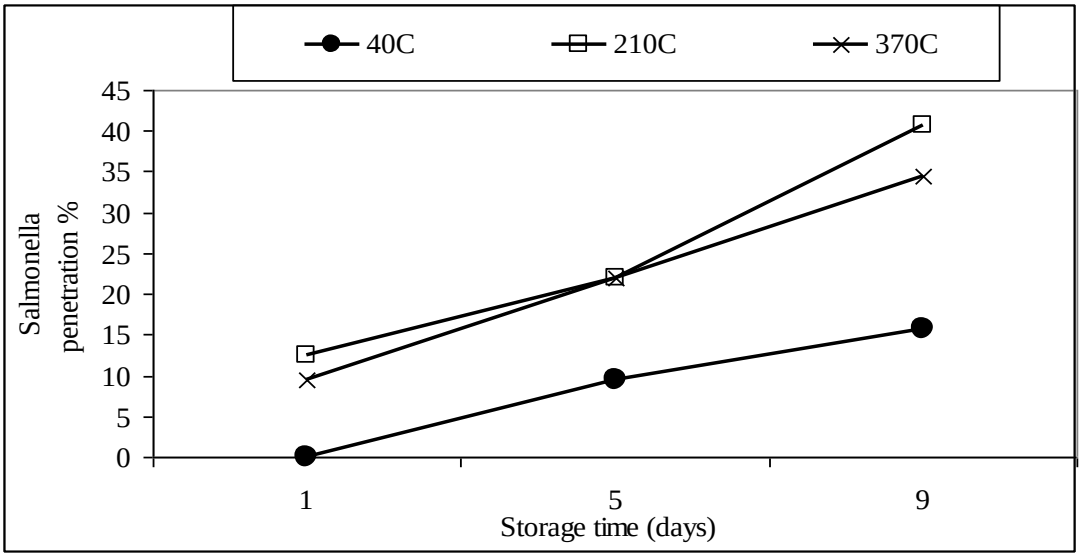

Fig 2. Percentage of penetration of Salmonella into eggs stored at different temperature and time

Disinfection of eggs

Prevention of Salmonella penetration by hydrogen peroxide (90.28%) was significantly higher ($p<0.05$) over sodium carbonate (77.78%) and control (72.22%) but similar with alcohol (86.11%) (Table 4). Padron (1995) also reported that dipping *S. typhimurium*-contaminated eggs twice in a 6% hydrogen peroxide solution reduced the number of *S. typhimurium* positive eggs by 55% compared with the infected untreated group. Food and Drug Administration (FDA) of USA affirmed hydrogen peroxide as GRAS (Generally Regarded as Safe) status (21 CFR 184.1366), and is approved for packaging and surface sterilization in the food industry (21 CFR 178.1005) (Schurman, 2001). So, there is no imminent danger to public health in disinfection of eggs by hydrogen peroxide.

Table 4. Prevention of Salmonella penetration into eggs by selected disinfectants

Disinfectants	No. of eggs	Salmonella penetration		Prevention percentage †
		Positive	Negative	
Hydrogen peroxide	72	7	65	90.28[a]
Alcohol	72	10	62	86.11[a]
Sodium carbonate	72	16	56	77.78[b]
Control	72	20	52	72.22[b]
Total	288	53	235	

† Values within a column followed by different lower-case superscripts are significantly different at 5% levels by DMRT; LSD = 5.589; Standard error = 1.947 at $\alpha = 0.05$

In comparison with control eggs, there was a 0.35, 0.5 and 0.8 times greater probability of Salmonella penetration into eggs disinfected by hydrogen peroxide, alcohol and sodium carbonate respectively (Table 5). Washing of eggs by hydrogen peroxide was significant ($p=0.005$). Washing of eggs by sodium carbonate was non-significant ($p=0.282$).

Table 5. Salmonella penetration into eggs washed by selected disinfectants

SN	Disinfectants	Salmonella penetration		Relative risk	Odd ratio	Pearson chi-square ($\chi 2$)	p †
		Positive	Negative				
1	Hydrogen peroxide	7	65	0.35	0.28	7.704**	0.005
	Control	20	52				
2	Alcohol	10	62	0.5	0.419	4.211*	0.032
	Control	20	52				
3	Sodium carbonate	16	56	0.8	0.742	0.593ns	0.282
	Control	20	52				

†: Exact probabilities calculated by Fischer's exact test.

ns means not significant. * and ** means significant at 5% and 1% level respectively.

Egg storage

Prevention of Salmonella penetration into eggs stored at 4°C (91.67%) was significantly higher ($p<0.05$) over 21°C (75%) and 37°C (78.13%) (Table 6). The present result is in agreement with the findings of Kim *et al*. (1989); Schoeni *et al*. (1995); Gast and Holt (2000) who reported quick multiplication of *S. enteritidis* in egg yolk at temperatures above 20°C, but is very slow below 10°C, and becomes negligible below 7°C. Rapid refrigeration of freshly laid eggs restricts the growth of pathogens (Chen *et al*., 2002). Gast *etal*. (2005) reported that Salmonella can penetrate into and begin to multiply inside the yolk of contaminated eggs at warm temperatures.

Table 6. Prevention of Salmonella penetration into eggs stored at selected temperature

Temperature (° C)	No. of eggs	Salmonella penetration		Prevention percentage †
		Positive	Negative	
4	96	8	88	91.67[a]
21	96	24	72	75.00[b]
37	96	21	75	78.13[b]
Total	288	53	235	

†:Values within a column followed by different lower-case superscripts are significantly different at 5% levels by DMRT; LSD = 4.84; Standard error = 1.686 at $\alpha = 0.05$

However finding of Wang and Slavik (1998) was in contrast with the present result, where the two different storage temperatures (4°C and 23°C) did not affect Salmonella penetration within 0 to 21 days storage periods. Similarly, Radkowski (2002) did not recover *S. enteritidis* from the egg contents up to 21 days at the storage temperature (2°C to 30°C).

More Salmonella penetration was observed in longer storage period. Prevention of Salmonella penetration on day 1 (92.71%) was significantly higher ($p<0.05$) over day 5 (82.29%) and day 9 (69.79%) (Table 7). This is similar to the finding of Wang and Slavik (1998).

Table 7. Prevention of Salmonella penetration into eggs at different storage duration

Storage duration (days)	No. of eggs	Salmonella penetration		Prevention percentage †
		Positive	Negative	
1	96	7	89	92.71[a]
5	96	17	79	82.29[b]
9	96	29	67	69.79[c]
Total	288	53	235	

†:Values within a column followed by different lower-case superscripts are significantly different at 5% levels by DMRT; LSD = 4.84; Standard error = 1.686 at $\alpha = 0.05$

Location of penetrated Salmonella

Salmonella penetration into yolk, albumen and both (albumen and yolk) was 16.98%, 39.62% and 43.4% respectively. Among 53 Salmonella penetrated eggs, Salmonella was isolated from albumen of 44 eggs (58%) and yolk of 32 eggs (42%). This may be due to inability of bacteriostatic substances to destroy all Salmonella during prolonged storage. Clay and Board (1991) found *S. enteritidis* to be resistant to the antimicrobial properties of albumen. Salmonella can persist or even grow in albumen (Baron *et al*., 1997; Gast and Holt, 2000; Cogan *et al*., 2001). Salmonella contamination in yolk of less number of eggs may be due to albumen viscosity and vitelline membrane integrity that prevent migration of Salmonella from albumen to yolk. Entry into the yolk could become more likely over time as albumen viscosity and vitelline membrane integrity decline, especially at elevated temperatures (Humphrey and Whitehead, 1993; Latimer *et al*., 2002).

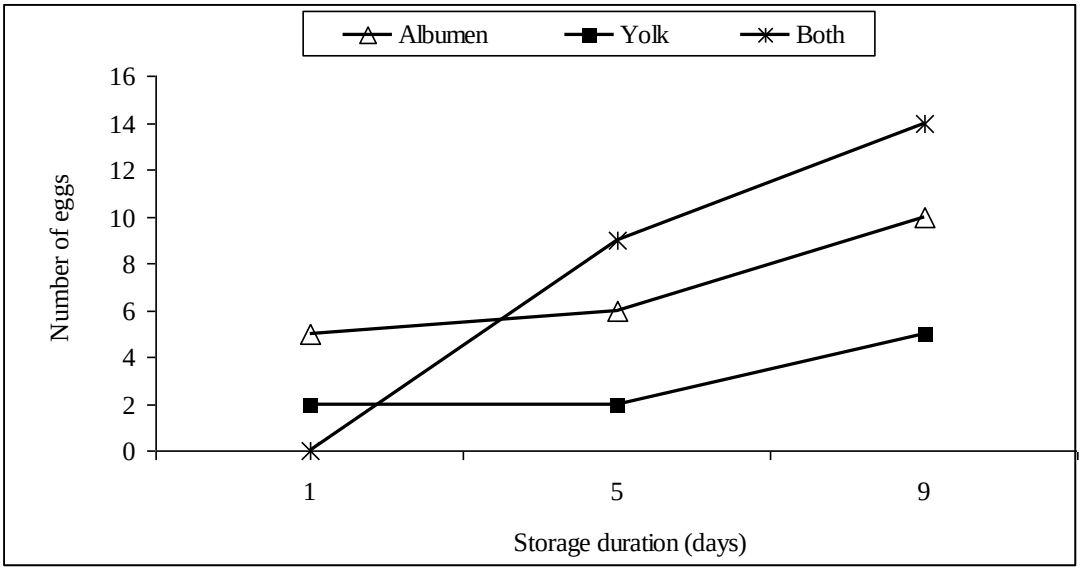

Fig 3. Location of penetrated Salmonella at different storage days

CONCLUSION

Disinfection of intact shell eggs by hydrogen peroxide or alcohol can significantly prevent Salmonella penetration into interior of eggs. However, the effects of hydrogen peroxide and alcohol should warrant further research with respect to the acceptable use of these disinfectants. Further researches with respect to evaluating the potential commercial value and easiness in application of these compounds would be necessary before recommending the technology to be adopted by the farmers.

Salmonella penetration into eggs can be significantly lowered by storage of them at low temperature for short duration. Thus, the practical implications of the present study emphasize the importance of refrigeration of the eggs during the whole chain, from laying until to consumption. Delay in transportation of eggs from producers to consumers should be avoided.

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EVALUATING THE USE OF EQUINE RABIES IMMUNOGLOBULIN (EQUIRAB) FOR POST EXPOSURE PROPHYLAXIS IN KATHMANDU, NEPAL

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ABSTRACT

Prevention of rabies following exposure to a suspected rabid animal requires thorough wound cleaning, antirabies vaccination, and in the case of severe (category III) exposures, rabies immunoglobulin (RIG). In this study, we reviewed the records of 1075 patients who presented to the Sukraraj Tropical and Infectious Disease Hospital (STIDH) for category III bites for the period of one year from May 2010 to 2011. The patient profile was predominantly male (70%) and children 10 years or younger accounted for 43% of patients. Of the 1075 patients, 1059 received equine RIG (ERIG), 15 received human RIG (HRIG), and one declined treatment. There were no adverse reactions to HRIG, and only a single mild allergic event to ERIG was observed. The results suggest that Equirab (Bharat Serums and Vaccines Limited, Mumbai), the ERIG currently used at STIDH, is a safe, potent, affordable alternative to HRIG, and we recommend the expansion of its use to other hospitals throughout Nepal.

Keywords: Rabies, Immunoglobulin, ERIG, Equirab, Post-Exposure Prophylaxis, Nepal

INTRODUCTION

Rabies is a viral disease that infects the central nervous system, and is fatal if not treated prior to the onset of symptoms. Transmission of rabies typically occurs via the bite or scratch of an affected animal, with bites from rabid dogs accounting for over 90% of human rabies cases (WHO, 1999). Despite the incurable nature of the disease, it can be prevented following contact with a rabid animal through the use of appropriate post-exposure treatment. Recommendations for treatment vary by the nature and severity of exposure to a

potentially rabid animal (WHO, 2004). While category II (or "mild") exposures can be treated solely with wound cleaning and vaccination, the World Health Organization (WHO) recommends that category III (or "severe") exposures are also treated with rabies immunoglobulin (RIG).

RIG provides passive immunization to the patient by supplying antibodies for the 7 to 10 days before the patient's body mounts an immune response to active vaccine immunization (WHO, 2004). Either human RIG (HRIG) or equine RIG (ERIG) can be administered, and though HRIG is the gold standard, its use is often limited due to production and financial constraints. The cost of ERIG is estimated to be one tenth that of HRIG, and is much more readily accessible (Wilde et.al, 1989). In Nepal, where tens of thousands of patients receive rabies post-exposure prophylaxis each year (Ministry of Health, Nepal), ERIG is the primary type of rabies immunoglobulin administered for category III exposures.

The safety of ERIG has been a topic of investigation over the past several decades, as the risk of adverse reactions associated with ERIG use is greater than that of HRIG. Early studies found that adverse reactions to ERIG occurred in 15 to 45% of patients (WHO, 2011). However, more recent work suggests the rate of adverse reactions in patients receiving ERIG is now as low as 1-2% (WHO, 2004), which is thought to be a result of improved purification techniques.

Table 1:WHO recommendations for post-exposure prophylaxis

Category	*Criteria*	*Recommended Treatment*
I (No exposure)	..Touching or feeding of animals ..Licks from animals on intact skin	. None, if reliable case history is available
II (Mild exposure)	..Nibbling of uncovered skin ..Minor scratches or abrasions without bleeding	. Administer vaccine immediately . Stop treatment if animal is proven to be rabies negative*
III (Severe exposure)	..Single or multiple transdermal bites ..Scratches or licks on broken skin ..Contamination of mucous membrane with saliva (i.e. licks) ..Exposure to bats	. Administer immunoglobulin and vaccine immediately . Stop treatment if animal is proven to be rabies negative[a]

[a] Treatment may be ceased if the animal remains healthy throughout an observation period of 10 days, or if the animal is proven to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques

In the present study, we reviewed the medical records of all patients receiving RIG from May 2010 to May 2011 at the Sukraraj Tropical and Infectious Disease Hospital (STIDH) in Kathmandu, Nepal to evaluate the characteristics of the at-risk population, and to determine the incidence of adverse reactions to ERIG.

MATERIALS AND METHODS

This study reviewed hospital records for patients who presented to STIDH with category III exposures from May 2010 to May 2011. STIDH is a 100 bed government-run referral hospital located in Teku, Kathmandu, and is reported to receive the largest number of rabies post exposure treatment cases of any hospital in Nepal, accounting for about 23% of post-exposure treatment cases nation-wide (Joshi et.al, 2000).

The category of exposure was determined by health care professionals in the rabies post exposure clinic, and patients diagnosed with category III bites were referred to the immunoglobulin clinic. Medical records from the immunoglobulin clinic were reviewed to collect information including the patients' age, sex, location of residence, body part bitten, weight, and amount of RIG received. The records were also reviewed for any report of adverse reactions to the intradermal skin test and/or the infiltration of RIG into the wound.

Of the 1075 patients who presented to STIDH for immunoglobulin treatment from May 2010 to May 2011, 1059 (98.6%) received ERIG, only 15 (1.4%) received HRIG, and one declined treatment. The ERIG used was Equirab, manufactured by Bharat Serums and Vaccines Limited (Mumbai). An intra dermal skin test with a 1:10 dilution of RIG was performed according to standard practice and read after 15 minutes, prior to infiltration of the wound with RIG. Patients who were to receive ERIG for wound infiltration received diluted ERIG for the intradermal skin test, and patients receiving HRIG for wound infiltration received diluted HRIG for the intra dermal skin test. Wound infiltration consisted of ERIG administered at a dose of 40 IU/kg, or HRIG at a dose of 20 IU/kg.

RESULTS

Patient Demographics

Of the 1075 patients who presented with a category III exposure from May 2010 to May 2011, 70% were male, and 30% were female. In terms of age, 43% were less than or equal to 10 years of age, 18% were 11 to 20, 10% were 21 to 30, 8% were 31 to 40, 8% were 41 to 50, and 13% were over 50 years of age (Figure 1). The majority of patients (75%) were from the Bagmati Zone, 10% of patients were from Lumbini, and 5% of patients were from

Janakpur (Figure 2). The remainder of the zones accounted for less than 10% of patients combined.

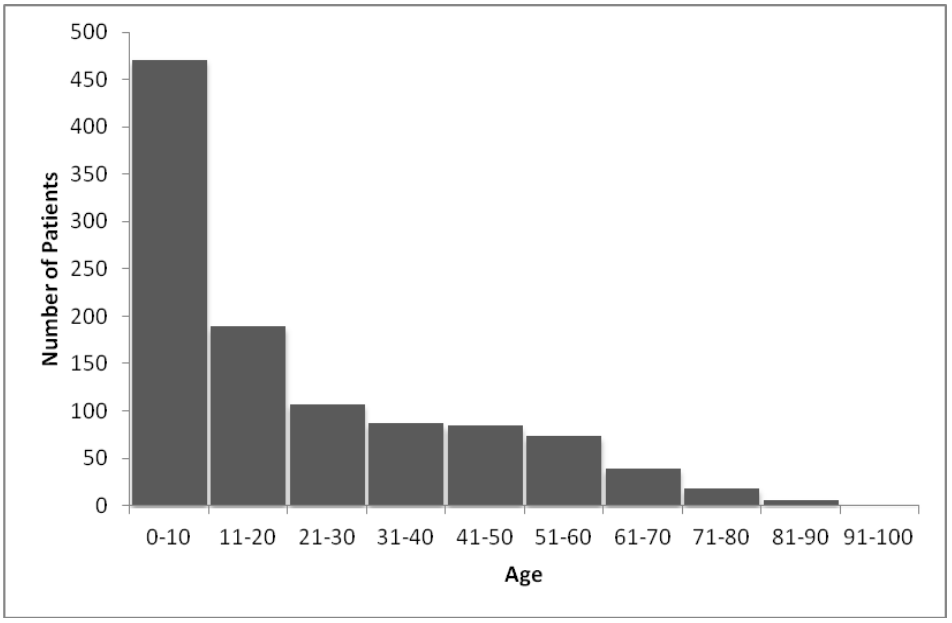

Figure 1: Age distribution of patients (n = 1075) presented with category III rabies exposures

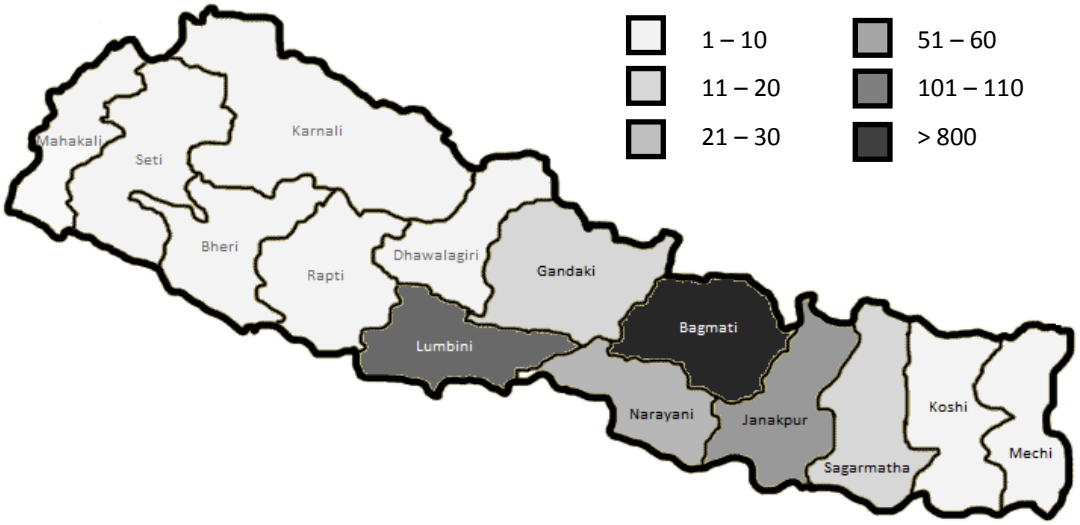

Figure 2: Geographic distribution of patients, by zone (n = 1075)

Ninety three percent (93%) of patients presented with a single bite, 6% presented with two or more bites, and the number of bites was not recorded for 1% of patients. For the 1075

patients, there were a total of 1135 bites. The largest proportion of bites (43%) occurred on patients' upper limbs, 26% occurred on the lower limbs, 23% occurred on the head or neck, 5.7% occurred on the trunk, and the body site was unknown for 1.4% of bites (Table 2).

Table 2: Body sites of 1135 bites incurred by 1075 patients

	Number	%
Head and neck	**265**	**23.3**
Head	51	4.5
Face	201	17.7
Neck	13	1.1
Trunk	**65**	**5.7**
Thorax, chest	17	1.5
Back, shoulder	16	1.4
Abdomen	15	1.3
Buttock	13	1.1
Groin	4	0.4
Upper limb	**491**	**43.3**
Arm	46	4.1
Forearm	3	0.3
Hand	442	38.9
Lower limb	**298**	**26.3**
Hip	6	0.5
Thigh	33	2.9
Leg	242	21.3
Foot	17	1.5
Unknown	**16**	**1.4**
Total	1135	100

Adverse reactions

None of the 15 patients who received HRIG experienced adverse reactions. Only one of the 1059 patients who received ERIG experienced an adverse reaction and it was reported to be minor. The patient, a 34 year old male, reported symptoms of nausea, weakness, pruritis, and mild dyspnea. He was administered 100 mg of hydrocortisone intravenously and kept under observation for three hours. There were no reports of a positive reaction to the intra dermal skin test.

DISCUSSION

The prevalence of rabies in dogs and other species in Nepal poses a substantial risk to humans who come into contact with these animals. For patients who suffer from category III exposures, RIG is a critical component of post exposure treatment for the prevention of rabies (WHO, 2004). Past studies have demonstrated that failure to administer RIG in addition to vaccine, or failure to adequately infiltrate the wound with RIG, can result in treatment failure and therefore rabies deaths (Devriendt et.al, 1982; Fangtaoet.al, 1988; Wilde et.al, 1996). In this study we attempted to define characteristics of the treatment population, and to estimate the incidence of adverse reactions to the administration of RIG.

The results revealed that there were more than twice as many males as females who received RIG treatment. Children up to 10 years of age were the largest group affected, accounting for 43% of patients in the study. These findings are consistent with the age and sex distributions seen in studies on post exposure prophylaxis from the Phillipines (Quiambao *et.al*, 2008) and Thailand (Chantanakajornfung *et.al*, 1999),as well as a previous study performed in Nepal(Joshi et.al, 2000).

Collection of information on patients' location of residence demonstrated that 75% were from the Bagmati zone included Kathmandu. The spatial distribution likely represents the greater practicality and feasibility of patients in the aforementioned locations receiving treatment at STIDH compared to patients from farther distances. This, in turn, emphasizes the need to have hospitals throughout the country equipped with RIG for treating patients with category III exposures.

Of the bites incurred, the upper limbs were the most commonly affected body part, followed by the lower limb, then the head and neck, and finally the trunk. Bites on the head and neck have been shown to pose the highest risk for the development of rabies (Shah &Jaswal, 1976), and here accounted for 23% of bites. Other highly innervated regions, such as the hands and feet, are also commonly affected body parts and high risk for rabies development. In this study, hands alone accounted for nearly 39% of bites.

As in many other developing countries, ERIG is the primary source of rabies immunoglobulin in Nepal. Of the patients treated with RIG from May 2010 to May 2011 at STIDH, 1059 (98.6%) were treated with ERIG, and only 15 (1.4%) were treated with HRIG. While the use of HRIG is considered ideal and is associated with a very low rate of adverse reactions (Chantanakajornfung *et.al*, 1999; Suwansrinon *et.al*, 2005), its exorbitant cost and limited production makes ERIG a more feasible treatment option.

The use of ERIG was previously under scrutiny after early reports detailed adverse reactions in 15 to 45% of patients (WHO, 1984). Adverse reactions may be local or systemic, immediate or delayed. Of most concern are immediate systemic anaphylactic or anaphylactoid reactions, which can manifest with urticaria, dyspnea, and hypertension (Bharat Serum). Delayed reactions may include inflammation at the site of administration, pyrexia, pruritis, urticaria, adenopathy, and arthralgia (Bharat Serum).

In the present study, there was only a single report of minor event, which occurred in a 34 year old male who received ERIG. This is consistent with other studies confirming the low incidence of adverse reactions and the relative safety of modern-day purified ERIG (Wilde *et.al*, 1989; Quiambao *et.al*, 2008; Wilde &Chutivongse, 1990; Sudarshan *et.al*, 2006). It should be noted, however, that the single reported reaction could be an underestimate of the actual incidence, as there may have been patients who failed to report delayed reactions occurring days to weeks after treatment.

Despite the possibility for under-reporting of reactions in this study, other sources provide substantial evidence for the safety and efficacy of ERIG (Wilde *et.al*, 1989; Quiambao et.al, 2008; Wilde &Chutivongse, 1990; Sudarshan *et.al*, 2006). Sudarshan *et al* (17), who used the same Equirab product as in the present study (manufactured by Bharat Serums and Vaccines Limited, Mumbai), found that while 6.1% of patients showed a positive reaction to the skin test, zero patients had immediate adverse reactions. Only two of 75 patients followed up were suspected of having delayed reactions. Most recently, Quiambao *et.al* (2008) found in their study that only 0.46% of patients receiving purified ERIG experienced local adverse reactions, and 1.36% experienced systemic adverse reactions. Protection against rabies in 143 patients exposed to laboratory confirmed rabid animals demonstrated the efficacy of ERIG when used in combination with vaccination and appropriate wound care (Quiambao *et.al*, 2008).

With over 1000 patients treated with ERIG at STIDH in the span of a year, ensuring the safety and efficacy of ERIG is of paramount importance in the prevention of rabies. The records from STIDH indicate a very low rate of adverse reactions to Equirab. In addition, the data highlights those children are among the most susceptible to category III exposures and males more so than females. The spatial distribution of patients emphasizes the importance of making ERIG available for people outside of central Nepal for whom treatment may be less accessible. For future studies, it would be beneficial to install added reporting mechanisms at STIDH for the collection of follow-up data on patients receiving ERIG.

CONCLUSION

Our study supports the finding that ERIG is a safe, affordable product appropriate for the treatment of category III exposures to potentially rabid animals. We recommend that the use of ERIG be expanded to other hospitals throughout Nepal in order to extend access to patients who cannot reach STIDH for treatment.

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REVIEW ON MEAT BORNE ZOONOSES – SERIOUS PUBLIC HEALTH RISK IN NEPAL

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ABSTRACT

Food borne zoonoses especially meat borne diseases have major public health impact throughout the world. In Nepal, consumption of meat has increased to the large numbers due to the increase in income, change in eating habit and many other factors. However, a hygienic standard of meat being sold is always questionable in a situation. This leads to ambiguity about safety of meat consumption in Nepal. The poor slaughtering and improper meat handling practices contribute greatly to the spread of meat borne diseases. Furthermore, the poor sanitary conditions in herd or flock, improper screening of diseases at quarantine offices and at lairage of abattoirs aggravate the situation and opens up risk of microbial contamination of meat. The meat which is highly perishable by its nature is susceptible to numerous microbial contaminations from slaughtering to cooking which exposes consumers to incidences of meat borne zoonotic diseases like Salmonellosis, Cysticercosis, Hydatidosis, Trichinellosis, Campylobacteriosis and other diseases. The Animal Slaughterhouse and Meat Inspection Act, 1999 and the Regulation, 2001 have provisions to ensure slaughter of healthy animals and to prevent contamination and/or adulteration of meat during and after slaughtering. However, due to various constraints like insufficient infrastructure, political instability, lack of awareness among all level stakeholders and other factors are creating obstacles in implementation of such acts and regulations. Unless these legislations are strictly enforced, the risk of meat borne zoonoses will remain as it is or even may increase.

Keywords: Meat, Zoonoses, Public Health, Risk, Nepal

BACKGROUND: meat, meat hygiene and safety

The term meat is widely used to define flesh and offal including their natural associates' skin and gristle, derived from carcass of any animal and bird normally used for human consumption. A meat product is defined as any food, which consists of meat or of which meat is an ingredient (Robinson, 2001). High level of hygiene is mandatory in any business that handles food for human consumption. Various microbial activities play major role in deterioration and spoilage of the meat. So, utmost precaution should be taken for producing hygienic meat. Meat should be free from any types of harmful chemicals that are generally used for preservation of meat and also make a good look for short period (Gracey &Collins,

2003). Since a range of legislation now exists which governs the management of livestock and their slaughter, and at all stages of the food preparation process, hygiene must be the high priority. The Nepal government should also impose Hazard Analysis and Critical Control Point (HACCP) principles for maintenance of food and meat safety and hygiene to safeguard the health of the consumers. There was one news published in Annapurna Post (2012), entitled "Dead goats' entry in the capital" shows the critical situation existing in the country and raises questions over quality meat, meat hygiene and safety. This also indicates the weak law enforcement of meat inspection regulations in Nepal which is to be strictly imposed for the welfare of the consumers. Concerning the meat hygiene, consumers are very much compelled to consume low quality meat and low hygienic meat in Nepal (Joshi, 2011).

Slaughterhouse practices, shortcomings and health risk

Meat has highly perishable nature. Owing this fact, much precaution should be taken to safeguard it right from bleeding during slaughtering animals till final consumption. However, in Nepal and many other tropical countries, shortcomings in meat production, processing and storage may contribute to too many health problems associated with meat borne zoonotic diseases and of food borne nature. (Gracey & Collins, 2003). The intrinsic factors that contribute to carcass and meat contamination are the physical and chemical properties of the meat. Examples are - availability of potential nutrients in the meat, its PH and buffering capacity, its redox potential and water activity. The extrinsic factors are those associated with the environment in which the meat is stored. Examples are - storage temperature and humidity, the composition of the gaseous atmosphere surrounding the meat, particularly if this is modified or controlled, and the length of the time of storage (Warriss, 2010). Additional Sources of cross contamination exist in slaughter process such as tools, equipments, human contact and carcass to carcass contact (Huffman, 2002). Prasai (2001) demonstrated that removing visible contamination by trimming and then washing was the practical and effective method for reducing microbial contamination of the carcass. That study emphasized that frequent sterilization of knives and other tools used in the trimming process was essential to reduce or minimize bacterial contamination, and that individual operative technique was the most important factor in the efficiency of trimming.

Right now there is lack of appropriate slaughtering facilities and unsatisfactory slaughtering technique still exists. This in turn, is causing adverse human health impacts besides the unnecessary losses in meat as well as in valuable by-products. In Nepal, still animals are slaughtered in the banks of river, just on the roadside in an open space or inside an old ruined house with poor sanitation. In such places the carcass is highly polluted with blood, intestinal contents, dirty effluents etc. Such places are not protected against flies, rodents and dogs.

The water used in slaughter places is obtained either from the polluted river where slaughtering is carried out, shallow wells, tube wells and similar places and practice of using water sanitizer is not existing which gives an extra load of bacterial and chemical contamination (Parajuli, 2007).

In recent study carried out in Chitwan district among the pig slaughter slabs and retail pig meat shops very poor hygienic status was found. No slaughter slabs had separate dirty sections; only 16.7% practiced chilling immediately after slaughtering. No anal plug method was used to avoid contamination of meat with intestinal content. Only 47% slaughter house workers used to wash hands regularly before and after handling pork (Ghimire, 2013). In one previous study in Kathmandu valley in 1991, similar poor hygiene condition of slaughter slabs were reported (Joshi, 1991). None of the butchers in Chitwan used water sanitizer (Ghimire, 2013) which can prevent the contamination of meat with zoonotic pathogens like *Campylobactor* (Diegaardt et. al., 2004).

Meat produced under unhygienic conditions as being practiced in Nepal, quickly gets deteriorated due to the bacterial contamination and could cause food poisoning. The meat quality is further affected adversely by careless handling under unsanitary conditions in the meat markets and meat shops (Parajuli, 2007). Though there are improvements compared to past but still not to the level should be.

MEAT BORNE ZOONOTIC DISEASE- a short overview

Salmonellosis

Salmonella is one of the most widespread food borne pathogen and a growing public health problem both in developed as well as developing countries like Nepal (Pedro & Boris, 1981). A cross sectional study of raw meat samples from local meat market of Kathmandu metropolitan city showed *Salmonella* spp in 11.4% (14/123) meat samples. The prevalence was 14.5% (8/55) in chicken meat, 13.5% (5/37) in buffalo meat and 3.3% in goat meat (1/31). More than 80% meat samples indicated coliform contamination. (Maharjan *et. al*., 2006). In another study, 40.2% of retail chicken meat shops of Kathmandu were tested positive for salmonella. Salmonella were isolated from chopping board samples, knife samples as well as from the table samples (Upadhyaya *et. al*., 2012).

Cysticercosis

Infection of *Taenia solium* is worldwide and it is becoming an increasing problem in Nepal (Joshi *et. al.,* 2004). Human acquire taeniasis infection by eating measly pork. Infection is common in low socio-economic and poor sanitary areas of the world (Schantz *et. al*., 1992).

With the increase in pig production and consumption in the country the likeliness of increment in zoonotic parasitic diseases is also increasing. The prevalence of cysticercosis in lingual and carcass examination were 0.63% and 0.94% respectively in Chitwan and Kathmandu during one study where 320 pigs were tested (Joshi *et. al.,* 2008). In another study in Kathmandu where 200 pigs were examined by ELISA, prevalence rate was 35.5% (Karna & Joshi, 2009). When 100 pig samples were tested from Chitwan, 30% were found sero-positive through ELISA test. When 20 slaughterhouse personnel's were tested, alarmingly 70% sero-positivity was found (Sadaula *et.al.,* 2012). Prevalence of human taeniasis was reported to be 43% in Syangja district and 18% in Tanahu district (Joshi *et. al*., 2004).

Echinococosis/ hydatidosis

Echinococcosis is caused by the larval stages (hydatid cysts) of *Echinococcus granulosus* and is known as one of the most important parasitic infection in livestock in the world (Capuano et. al., 2006). The definitive hosts are almost invariably canid carnivores like dogs, wolves and jackals. Intermediate hosts can be the domestic ungulates including sheep, goats, cattle, swine, buffalo, horses, and camels (Rausch, 1986). It can establish itself in many different hosts, including humans and is one of the most widespread zoonoses (Craig et. al., 2007).

Using an ELISA coproantigen test, the highest prevalence of *E. granulosus* (5/88=5.7%) was seen in domestic dogs from an area of Kathmandu city. The carcasses of animals were examined and hydatid cysts were found in water buffalo 5% (153/3065), goat 3% (55/1783), sheep8% (12/150) and pig 7% (10/143) in 17 different abattoirs in Kathmandu. Human serum samples tested by ELISA method showed 14.1% (113) screened positive (Joshi et. al., 1997). Rajbhandari (2010) reported about 20 pulmonary hydatid cysts cases from Birenda Military Hospital. All the histopathological report was positive for *Echinococcus granulosus* and all the cysts were unilocular in Nepal.

Other meat borne zoonotic diseases

There are many meat borne zoonotic diseases that can have serious public health hazards. These include *Escherichia coli* infection, anthrax, tuberculosis, campylobacteriosis, trichinellosis, etc.

Way forward

As the human health is deteriorating day by day because of the low standards of meat safety and hygiene, due consideration should be given towards promotion and promulgation of food

and meat safety rules. In this regard, Animal Slaughterhouse and Meat Inspection Act was launched in 1999 in Nepal. The main aim of the act was to make meat inspection scientific and to ensure the production of safe and hygienic meat for safeguarding the public health. Further the Animal Slaughterhouse and Meat Inspection Regulation-2001 has compulsory provisions of ante and post mortem examinations. These act and regulations highlighted the need and made legislative basis for licensing of meat shops; terms and conditions for meat seller; functions, duties and power of meat inspector and meat supervisor; standard means for meat transport; procedures and methods of disinfections of slaughter house and equipments; marking or stamping of carcass after inspection and other relevant activities (Parajuli, 2007). However, implementation of the act is still not presumed strongly in the nation. It is because of unpreparedness in fundamental requirements such as slaughter places, standard meat shops and importantly lack of awareness among different level of stakeholders from producer to consumer about meat hygiene. So, the consumers are compelled to consume the unhygienic meat and other meat products (Joshi, 2011). Considering the alarming risk existing on public health sector there is instant need of strict enforcement of Animal Slaughterhouse and Meat Inspection Act (1999) and Regulations (2001). Besides that, activities like (i) training to the officer level regarding meat inspection, meat processing and livestock market management (ii) training to the technician level regarding animal husbandry and hygienic meat production and (iii) training to farmers/entrepreneur regarding hygienic meat production and commercial farming system are must be carried out from all governmental and non-governmental sectors having concern over public health (Ratala, 2007). Only through infrastructure development; human resource development, awareness generation and strict implementation of the legislation control over meat borne zoonotic diseases can be controlled.

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E EFFECT OF DIFFERENT FARM MANAGEMENT CONDITION ON PRODUCTION PERFORMANCE OF CROSS BREEDS COW THROUGH ARTIFICIAL INSEMINATION IN NEPAL

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ABSTRACTS

Artificial Insemination service is a tool to spreading genetic merit among dairy animals for breed improvement. The study was carried out from August 2011 to April 2012 of five districts in Morang, Saptari, Chitwan, Rupandehi and Kathamandu which are providing the artificial insemination service for more than 5 years. The average milk yield of Local Female x Jersey male, Local Female x Holstein Friesian male, Local Female x Jersey male, Holstein Friesian female x Holstein Friesian male, Holstein Friesian female x Jersey male, Jersey female x Holstein Friesian male were observed 2304.90±480, 2836.50±772.71, 3076.0±358, 4114.50±572.53, 3503.0±52.27and 3503.0±202.0 liters,respectively per lactation. The total production of milk in F1 generation was significantly higher (p□ 0.05) and there was no any significant differences between total milk production performance of F1 generation Holstein Friesian cross female and Pure Jersey Male (3503.0±52.27) and similarly Jersey Cross Female and Pure Holstein Friesian Male (3503.0±202.0). The average milk production was 9.45 liter per day of cow high with given supplements than that of supply green fodder to the milking cow. The different farm activities of livestock production and management practices were jointly conducted by men and women in this study.The conception rate of this study was found 61.17% in cows and 49.75% on buffaloes. Therefore, the result of this study was focused on to estimate the production traits of different breeds of cattle, measure the milk production performance and conception rate of cow and buffaloes under different management practices of farm.

Keywords: Artificial Insemination, genetic groups, Milk production, different traits, conception rate

INTRODUCTION

Agriculture is the backbone of Nepalese economy which contributes 25.68 %of National Gross Domestic Products (GDP). Agriculture provides 66 % of employment for its active population and supports livelihood of 79 % of farm households. Livestock is an integral part

of the agricultural production system of Nepal. Livestock plays a vital role to the Nepalese rural economy and contributes about 15% to the total national GDP and about 27% to national Agricultural Gross Domestic Products (AGDP). Numerous people are involved in the production, slaughtering, processing, and trading of livestock and livestock products. Over 20 laks households own cattle and in which 9 lakhs milking cow and 12 lakhs milking buffaloes of which 43% are cattle and 57% are buffaloes, and produce about 15,00,000 Mt. milk annually. There is all together annual milk production of the country 29 % come from cattle and rest 71% from buffaloes.

Dairy sub-sector has a potential to grow up to 7% per annum (APP, 1995) but the current rate of growth is about 3% per annum. There is a need of realizing these benefits and thereby contributing to transformation of the national economy. Per-capita availability of milk (51.49 kg/year) in Nepal is far below WHO recommended 91.25 kg (NLBC, 2008/09) and FAO recommends consumption of 250 gm of milk/day, while at present consumption is only 144 gm daily. Milk production thus needs to be increased through increasing cow and buffalo productivity. Artificial Insemination (AI) being one of the most important tools of animal breeding for genetic improvement, is in top priority of the District Livestock service (DLS) and National Livestock Breeding Center (NLBC) which has been shouldering the responsibility of supporting AI program throughout the country. Productivity of domestic animals will be increased to meet domestic demand, and top priority will be given to milk and meat production. As slaughtering of cattle is prohibited in the country, buffalo will be the choice of animal for milk and meat production. Therefore, buffalo will be promoted and propagated in the country. However, buffalo alone cannot meet the national demand for milk and draft power, upgrading of nondescript cattle with exotic blood level up to 75% will be continued (NLBC, 2011).

In Nepal, the demand for milk and milk products is increasing. So, to meet this demand there is increasing demand of high yielding breeds of dairy animals. This demand is partially fulfilled by the introduction of AI. The national program of cross breeding between the exotic dairy breeds and native zebu breed of cattle has been implemented in Nepal since last four decades. The AI services as a tool for spreading genetic merit among dairy animals now covers 55 Districts through DLSOs from 249 AI centers including 69 Private AI centers. AI service to the farmers is provided by 414 inseminators including 92 private inseminators. In spite of widely accepted technology the coverage of AI is very poor. In India, Bangladesh and Sri Lanka the coverage of this service in milking dairy animals are 15-30% while it is only 11% in Nepal (NLBC, 2012), the lowest in the region. Therefore, it is of vital importance to improve the production potential of our indigenous stock to increase demand

of milk and milk products for ever increasing human population of our country. A Jersey cattle is high potential milk breed and is well adapted in rain-fed as well hilly areas. Because of its relatively small size the breed is suitable for hilly areas of the country. The productive performance of indigenous cattle is generally low in comparison to exotic breed. Therefore, the present study has been focused on this estimation the production traits of different breeds of cattle, measurement of the milk production performance and conception rate of cow and buffaloes under different management practices.

MATERIALS AND METHODS

The study was carried out from August 2011 to April 2012 in five districts of Eastern, Central and western Development Region of Nepal. Data was collected from milk shed areas where most of the farmers had milking cows and Artificial insemination techniques were adopted as Morang, Saptari, Chitwan, Rupandehi, Kathamandu districts are providing the artificial insemination services for more than 5 years. Data were collected using predetermined questionnaire from 400 household by expert consultant.

The questionnaire was developed specifically for the defined problem. During the questionnaire formulation, utmost care was taken to make the language. An attempt was made to make the questions closed ended. Questionnaire covers the areas like social-economical status of farmer, productivity of cattle, knowledge of farmer regarding the cattle management, living condition of cattle, problems faced by farmer for the marketing of milk etc.

Primary as well as secondary data from various sources had been used for doing analysis. These household surveys, focus group discussion, Non Governments Organizations (NGOs) interview and expert consultant were the approaches to collect the primary data. While the published reports of the department of livestock services, were the sources of the secondary data. Besides of published reports, some of the important data was provide by the AI centers.

The data obtained from the field survey were compiled in MS-Excel and was analyzed. Data collected included cow number, calving date, total milk yield, lactation length, daily milk yield, 305 day corrected milk yield, length of dry period, annual milk yield per cow were analyzed by using SPSS (version 16). The analysis of variance (ANOVA) was calculated to see the significance of difference.

RESULTS AND DISCUSSION

The reproductive performance of a dairy herd has a significant effect on the profitability of that herd. Common measures of reproductive performance are days to first service, days to conception, calving interval, services per conception, conception rate and estrus detection rate. The size of the calf crop is all important for herd replacement, and the production of milk depends heavily on reproductive activity. Possible genetic improvement in virtually all traits of economic importance is closely tied to reproductive rate.

Table 1: Means with their standard errors for reproductive and productive traits in different cross breed cattle

Traits	**local×Jer**	**local ×HF**	**Jer×jer**	**HF×HF**	**HF×Jer**	**Jer×HF**
A. Reproductive traits						
Birth weight, kg	30.87±7.03	35.79±9.62	25±2.51	38.76±4.76	31.82±2.51	31.37±2.00
Age at first service (months)	15.31±6.85	17.48±6.88	15.1±2.35	17.52±2.44	14.79±0.47	17.49±1.59
Age at first calving (months)	26.96±5.44	29.64±5.13	23.98±2.42	26.55±2.45	23.79±0.47	26.48±1.59
Post partum heat period (days)	65.93±27.83	79.24±33.5	61.05±9.64	74.16±12.64	63.75±2.54	70.07±5.762
Calving interval(months)	13.66±1.82	13.83±1.59	11.10±0.44	11.88±0.32	11.71±0.45	11.85±0.75
Dry period (days)	61.10±24.1	66.77±25.87	55.90±9.17	57.46±5.10	56.41±3.85	59.63±4.08
B. Production traits						
Av.Milk yield, lits/day	7.65±1.776	9.817±2.73	10.44±1.23	13.65±1.55	12.05±0.23	11.77±0.704
Milk yield (lits/ lactation)	2304.90±480	2836.50±772.71	3076.0±358	4114.50±572.53	3503.0±52.27	3503.0±202.0

Note: F_1 crosses: Local Female ×Jersey male, Local Female ×Holstein Frisian male, Locale Female ×Jersey male, Holstein Frisian female ×Holstein Frisian male, Holstein Frisian female ×Jersey male, Jersey female ×Holstein Frisian male.

A. Reproductive traits of different cross breed of cow

Birth weights of female calves:

The average birth weights of L×J, L×HF, J×J, HF×HF, HF×J and J×HF calves were 30.87, 35.79, 25, 38.76, 31.82 and 31.37 kg, respectively (table 1), with HF×HF and HF×Jersey and Jer×jer crosses calves being significantly($p \square 0.05$) heavier than others. The observed birth weights of Jersey × Local and Holestein ×Local calves are also consistent with the observations of Hussain and Routledge(1982).

The age at first service:

The age at first service is between 14.79 to 17.52 months for all crosses breeds of cow. This may be attributed to the differences in environment and nutritional status during the growing period. The effects of nutrition on cattle reproduction are covered extensively here because most cattle in the tropics are poorly fed and improving their feeding can immediately increase their reproductive performance. All heifers attained puberty at about the same bodyweight (279-295 kg) but at different ages (Topps, 1977).

Age at first calving:

The mean age at first calving for L×HF was 29.64 months L×HF and it is 23.74 months for HF× J. The analysis shown in Table 1 indicates that breed group, year of birth and season of birth interactions significantly affect age at first calving. Mahadevan (1966) observed that irrespective of whether cattle were of Indian, African, European or crossbred origin, their mean age at first calving under a under tropical environment was essentially the same and ranged from 3 to 4 years. The present study seems to indicate that in the tropical and subtropical environment, given reasonably good management, age at first calving can be reduced to between 23 and 30 months.

Post partum heat period:

Themeanpost partum heat period of L×J, L×HF, J×J, HF×HF, HF×J and J×HF were found 65.93±27.83, 79.24±33.5, 61.05±9.64, 74.16±12.64, 63.75±2.54 and 70.07±5.762 days which were not significantly ($p>0.05$) different. In this study it is found appropriate post partum heat period and this might be due to good management and nutrition in the herd. It could be helps to the cow uterus undergoes involution in normal preparation of next pregnancy. High level of feeding before calving reduced the postpartum anoestrous period in taurine cows (Bellows and Short, 1978). In addition, more cows exhibited oestrus before the breeding season and subsequent pregnancy rates were increased. King (1968) estimated that a 1% change in body weight resulted in 1% change in first service conception rate.

Calving interval:

The calving interval is the period between two consecutive parturitions, and ideally should 12 to 13 months. The calving interval is thus closely matched to a yearly production cycle and influences the amount of milk a cow is likely to produce in a given period. This study found the calving interval between 11.10±0.44 and 13.83±1.59 months respectively. Reinhardt (1978) told that that inseminating Local cows with semen from Jersey or Holstein-Friesian bulls produced F_1 cows which produced more milk and had a shorter post-partum heat period, calving interval, dry period, age at first calving and age at fist heat than crossbred cows form Local cows inseminated with Sahiwal or Sindhi semen.

Calving interval can be influenced by the sex of the calf (Plasse et al, 1968). In a study of zebu cows in Kenya, Reinhardt (1978) observed that cows with male calves had a longer calving interval than those with female calves (430 vs. 383 days. Montoni *et al* (1981) noted that cows of with male calves had a calving interval 19.1 days longer than that of cows with female calves.

B. Production traits of different cross breed of cattle under F_1 generation

Milk yield:

The mean per day milk yield of L×J, L×HF, J×J, HF×HF, HF×J and J×HF were found 7.65±1.776, 9.817±2.73, 10.44±1.23, 13.65±1.55, 12.05±0.23 and 11.77±0.704 liter respectively. The average milk yield of Holstein Friesian crosses cow were found highest in this study. The yield of H×L and J×L cows were closer to those observed by Hossain and Routledge (1982). The average milk yield of L×J, L×HF, J×J, HF×HF, HF×J and J×HF were observed 2304.90±480, 2836.50±772.71, 3076.0±358, 4114.50±572.53, 3503.0±52.27, 3503.0±202.0 liters per lactation respectively. The total production of milk in F1 generation was significantly different (p□ 0.05) and there was no any significant differences between total milk production performance of F1 generation Holstein Frisian cross female and Pure Jersey Male (3503.0±52.27) and similarly Jersey Cross Female and Pure Holstein Frisian Male (3503.0±202.0).

The F1 (Local Female × Jersey Male) Produces 1631 liter of milk on an average (NLBC, 2008/2009) that was somewhat lower than the result of this study. The average milk production of Local, Jersey and Holstein Friesian cow were found 931, 2728 and 3283 liters of milk, respectively under DCIP herds of Nepal (2010/11).

C. Management conditions

Variation of milk production with the type of fodder

Milk production was observed high in the cow of farmers who give feed supplements to their cattle. Their average milk production is 9.45 liter per day. The farmers who provide green fodder to the milking cow as source of food also give a good quantity of milk that is 7.25 liter per day on average.

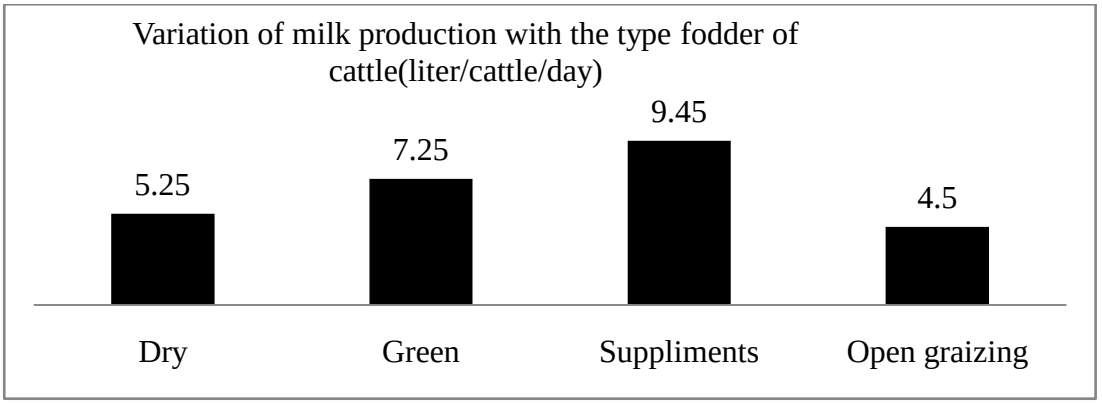

Fig.1 Variation of milk production with the type fodder of cattle (liter/cattle/day)

Variation of milk production with the accommodation and living conditions of cattle

Milk production was high of the cattle in the proper type of accommodation provided by the farmers. Proper accommodation means a well ventilated, airy, clean and a place where sunlight can reach, on the other hand improper accommodation means dirty, no ventilated and crowded place.

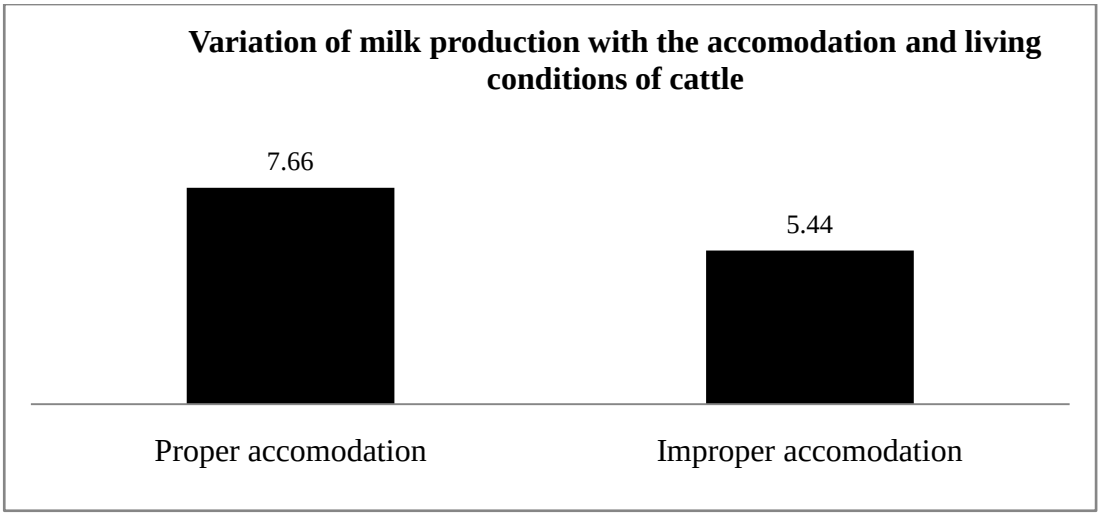

Fig.2 Variation of milk production (Liters) with types of accommodation

It was found that the cattle that were living in proper accommodation condition giving more milk as compare to cattle that were living in improper accommodation. Average milk production of per cattle under living in proper accommodation was 7.66 liter while for cattle living in improper accommodation was 5.44 liters.

Gender Role in Livestock Management

Farmers' roles, gender-wise, in different livestock activities as responded by the key-informants had been presented in figure 3. It showed that women were work in most of the hard tasks of farm like feeding and shed cleaning the gutter, grazing the cows in study site equally and whereas men were involved relatively in easier and smart household tasks of the livestock activities such as medication and selling and buying of animal and milk.

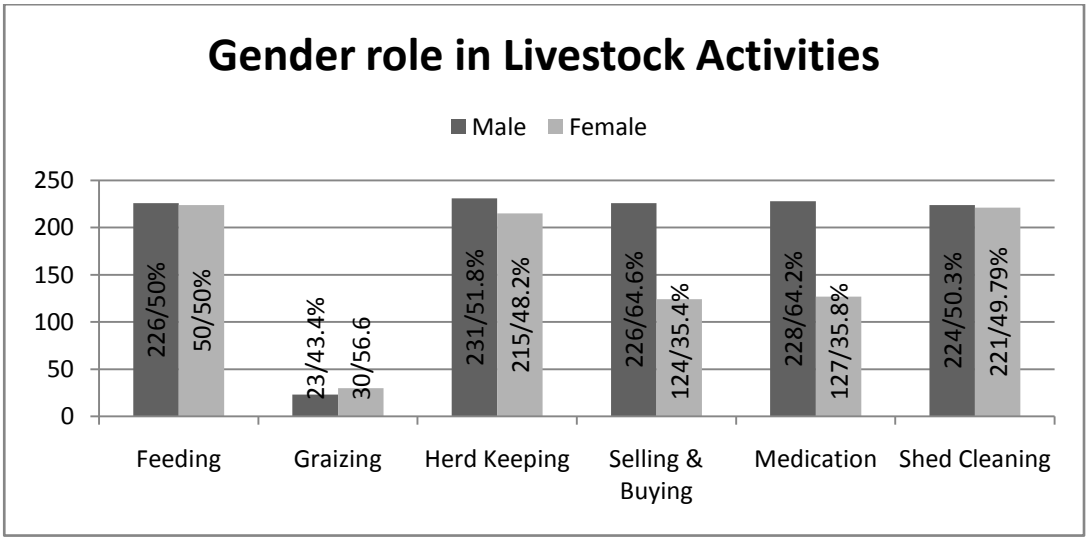

Fig.3 Gender participation in major livestock activities

Women are producers of food, manages natural resources, care children and provide a proper nutritional balance for households (Brown et al 2001). The farm activities of livestock production and management are jointly conducted by men and women in this study. Gender roles in agriculture became an important subject of inquiry after Boserup (1970) questioned if women and men benefited equally from development programs. The livestock sector offers advantages to women over other agriculture sectors because of the reason that almost all household members of most societies have more access to livestock than to land (Bravo-Baumann 2000).

D. Conception rate

The conception rate in this study was found 61.17% in cows and 49.75% in buffaloes. The number of services per conception was found 1.63. The number of services per conception depends largely on the breeding system used. It is higher under uncontrolled natural breeding practices in rural areas and low where artificial insemination is used. The number of services per conception (NSC) values greater than 2.0 should be regarded to be poor (Grusenmeyer, D. 1983).

Choudhuri *et al* (1984) estimated the repeatability of number of services per conception to be 19% from 2152 records for Haryana cattle. The number of services per conception was 2.81 ±0.03 and was significantly affected by herd, season, placenta expulsion time, lactation length and milk yield.

The achievement of Artificial Insemination depends upon many factors like healthy animals, quality of frozen semen, farmers know how on obvious reproductive physiology of animals and most important is the well-trained inseminators (Shresta, 1996). AI business needs conception rate in order to properly evaluate inseminators, bulls and semen handling process (Salisbury, 1985). Time of insemination during estrus is one of the most important factors influencing conception rate.

The Conception rate of cows markedly reduced when a higher temperature prevails for two days before insemination to 4-6 days after insemination (Gwazdauskas *et al*., 1975). Conception rate is generally lower in older cows. In a Virginia study conception rate remained constant (50%) during the first 3 lactations. Conception rates were 10% higher in virgin heifers and 10% lower in 4th lactation and older cows. Higher temperature and relative humidity (Zakari *et al*., 1981) and poor management affect on fertility of cattle. It had been reported that cows grazing on improved pasture and supplementation with legume fodder had a conception rate of up to 64.4%, compared with 6.3% for cows grazing only on unimproved pasture (Kleinhesterkamp *et al*, 1981).

CONCLUSIONS AND RECOMMENDATIONS

It is concluded that the collected data from field survey in artificial insemination launched program since five year of different districts for breed improvement the crossbred's cow of F_1 generation increasing the milk production. The average milk production is 9.45 liter per day of cow that has been supply feed supplements than the green fodder to the milking cow as source of feed that is 7.25 liter per day on average. It has found that the cattle which were living in proper accommodation condition are giving more milk as compare to cattle that were living in improper accommodation. The conception rate in this study has found 61.17% in cows and 49.75% on buffaloes. However, it must need further study in this field, there are following recommendations to the related field technicians and innovative farmers:

1. Targets should always be based on the number of outputs that is calving and not on the bases on inputs that is artificial insemination.
2. Issuing of the birth certificate for the cattle born through artificial insemination must help in proper monitoring

3.More training and orientation programs for farmers regarding the improved livestock farming methods
4. Promoting the green fodder through Forage Mission Program in AI Mission District to encourage the farmers for low cost milk production.
5. Teat dipping program should be compulsorily in a dairy farm to control mastitis through the farmers
6. Silage feeding technology in the form of small plastic bag is one of the important technologies for the regular adoption should be extended in all cattle farm.
7. Total Mixed Ration are mixed of forage, concentrate, other feed ingredients, vitamins and minerals which increase dry matter intake and milk yield so it could be expand and adopted in cattle farming as a major feed.
8. Upgrading the local breeds of cattle with improved jersey bull through artificial insemination in rural area to get improved cattle

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OHEAT SYNCHRONIZATION AND ARTIFICIAL INSEMINATION IN SHEEP OF LIVESTOCK FARM, POKHARA

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ABSTRACT

Artificial insemination was performed in matured Kage ewes of Livestock Development Farm, Lampatan, Pokhara during July 9-10, 2011, possibly for the first time in Nepal. Fifteen matured ewes were selected for synchronization of estrus on the basis of history of non pregnancy, cyclic estrum and dry period of lactation. All selected ewes were injected with 2 to 3 ml of Prostaglandin ($PGF_2\alpha$Lutalyse) deep intramuscularly. The heat was detected between 48 to 72 hours of post injection of hormone by applying the teaser ram and all the ewes (100%) showed estrussymptoms. Both imported frozen semen from New Zealand and warm liquid semen collected locally from Kage ram of the same farm were used for artificial insemination through cervical and trans-cervical methods. Among all 15 ewes, one was inseminated with frozen semen of Romany mars; two were inseminated with frozen semen of Coopworth after properly thawing whereas rest of twelve ewes were inseminated with freshly collected warm semen from three rams of the same flock. The volume of the semen used for insemination was 0.5 – 1.0 ml. Two ewes died due to accident or disease within one month of insemination. Out of 13 inseminated ewes, six (46.2%) were detected pregnant, and they lambed within the gestation period of 147±3 days. None of the ewes inseminated with frozen semen were found pregnant. However, 6 out of 10 ewes (60%), inseminated with warm semen were pregnant and lambed. The birth weight of Kage lambs was ranging from 1.7 to 2.0 kg with the mean value of 1.87kg. It is reported that this is the first officially done artificial insemination in Sheep in Nepal.

Key words: Kage sheep, Artificial insemination, Heat synchronization, Prostaglandin, warm semen, Frozen semen

INTRODUCTION:

The objective of this explorative experiment on AI in sheep is to introduce the technology and assess its success under Nepalese conditions aiming at adoption of AI in future programs for breed improvement of sheep.

Nepal has sheep population of about 0.9 million (MOAC 2010). Baruawal, Bhyanglung, Kage and Lampuchhre are the predominant sheep breeds for wool and meat. They utilize the sloppy mountain and hilly land for converting them for meat and wool. Baruwal are used for draught and can carry load of up to 13 kg on their back. Kage is smaller breed for meat and wool. Among introduced breeds, Polwarth is continually introduced breed that is being used

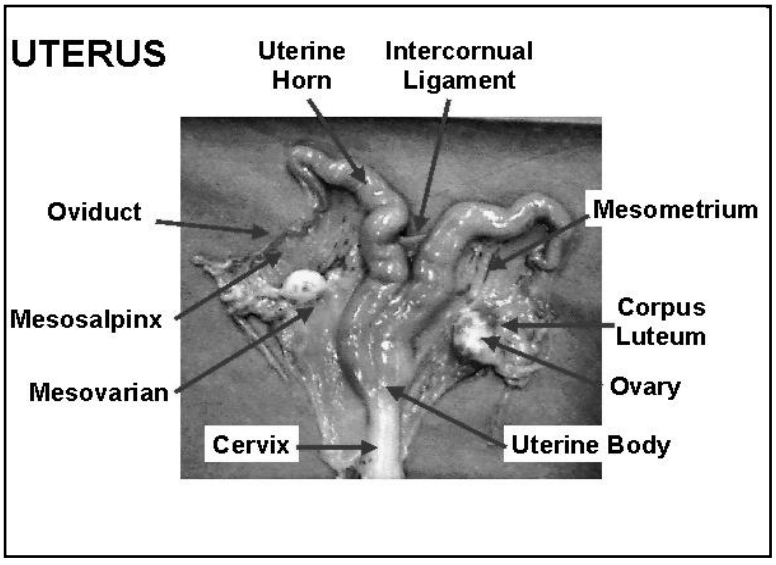
for improving wool production. Merino, Rambouillet, Border Leicester and Scottish black face are other introduced breeds (DLS, 2010).

ARTIFICIAL INSEMINATION (AI) IN SHEEP: A BRIEF REVIEW

Although the techniques for artificial insemination (AI) of sheep using fresh-extended semen have been available for some time, use of frozen semen has not been wide spread because of limited success with when frozen-thawed semen is deposited in the vagina or into the uterus via cervix. Laparoscopy has evolved as one of the least invasive techniques depositing frozen-thawed semen in the uterus of sheep.

Selective Breeding and Artificial insemination:

Artificial insemination (AI) is an important tool for modern livestock breeding and its impact on dairy animals is tremendous, however, has not grown to its potential in small ruminants including in sheep. The ovine cervix is relatively difficult to traverse with traditional AI tools, so techniques that work easily with cattle have been less successful with sheep. The anatomy of the uterus of the ewe is shown in the figure, 1. .Surgical AI has a higher success rate, but is prohibitively complicated and expensive. This method is also called laparoscopic method of A.I. The semen is usually deposited in horn of uterus (G.Lewis, 2009).



Figure,1: Anatomy of uterus of ewe

Scientists have developed a rapid, economical alternative to existing surgical AI methods. The researchers used a spiral insemination catheter to traverse the ewe's cervix and deposit thawed semen directly into the uterus. Each sheep takes about 2 minutes to inseminate making it significantly faster and less expensive than laparoscopic surgical insemination. Early tests have had success rates of about 55 percent when using fresh semen and about 10 percent when using frozen semen (Salamon, 1987)

Evaluation of semen:
Semen is collected by artificial vagina from mature rams, and evaluated for volume, percent motility, sperm concentration and percent of normal cells. The maximum percentage of abnormal sperm should not exceed 10-15%. Cell loss during freezing-thawing may be 40-50 %, so number of cells per insemination dose must be doubled. Ejaculates can be diluted in extension rates (semen: diluent) ranging from 1:1 to 1:4. Rates higher than 1:4 (5 folds) are not recommended for ram semen (Rodriguez *et. al.,* 1988).

Estrous synchronization:
Efficient use of frozen semen requires that ewes in a flock be synchronized to estrus. The primary basis of synchronization is to mimic the hormone profile during the estrous cycle. Norgestomet, melengestrol acetate (MGA), controlled internal drug release dispensers (ClDRs) or medroxyprogesterone acetate (MAP) are options for providing a source of progestogen for the approximate lifespan of the corpora lutea (10-14 days). The progestogen is followed with a source of follicle stimulating hormone to stimulate follicular development which stimulates secretion of the remaining hormones and helps promote a close timing of estrus among ewes not added Ref . Prostaglandin F2a, the uterine luteolytic factor, has also been used for synchronizing in combination with 7-day progesterone or in a two-shot regimen given 9 days apart not added Ref. At Dubois mature ewes are synchronized with medroxyprogesterone acetate vaginal pessaries (MAP) for 12 days and injected intramuscular with 400 IU pregnant mare serum (PMSG) when the pessary is removed. Ewes are inseminated 52-56h after pessary removal. Ewes must be taken off feed and water for 24 h before AI. The controlled Internal Drug Release (CIDR) is generally applied intravaginally in the ewes and left for 12 days. It is principally said that the use of CIDR increases the progesterone level, however whenever the CIDR was removed, the progesterone level is dropped where as estrogen level is increased drastically and it evinces the onset of estrus.

Laparoscopic techniques:
Ewes are sedated with 1 mlof diazepam (5 mg/ml) given intravenously (I.V). The ewes are restrained on laparotomy cradles and the abdomens shorn and scrubbed with Providone l% iodine solution. The laparotomy cradle restrains the females and allows elevation of the sheep at approximately 30 degree angle. This reflects intestines away from the reproductive tract. A small incision (1/2 inch) in the skin is made with a surgical blade about 3 to 4 inches anterior to the udder on each side of midline (about 2.5 cm). Trocar and trocar sleeves are inserted into the skin incision and forced through the body wall into the peritoneal cavity. Once inserted into the peritoneal cavity, the trocar is removed leaving the trocar sleeves to allow insertion of a 0 degree 10mm rod lens laparoscope and a manipulating probe. Initially, the peritoneal cavity is viewed to see if the scope is in the greater omentum. The scope is maneuvered into the ewe until it is out of the omentum and then CO_2 is used to inflate the peritoneal cavity and allow the uterus to be observed. The manipulation probe is used to place the uterus on top of the intestines and omentum so the uterine horns are visible. The manipulation probe is then replaced with the insemination pipette. The insemination pipette holds a 0.25 cc straw with an AI sheath and a 0.25 inch long needle. A quick thrust of the

needle into body wall of the horn creates a feeling of "popping" unto the uterine lumen. Once in the lumen, another person injects half the thawed semen dose (50,000,000 to 75,000,000 sperm) into each horn. After the semen is deposited the AI pipette, laparoscope and trocar sleeves are removed and Furazolidone aerosol powder is sprayed on the incision sites if there is no superficial bleeding. If bleeding occurs the veins are tied off with Dexon "S" suture. Infection is reduced by application of Furazolidone powder sprayed on the incision sites. The ewes are removed from the laparotomy cradles and allowed to walk from the surgery room to a recovery pen. Sheep recover on feed and water for at least 48 h before transporting. Clean-up rams are not introduced until seven to ten days after AI. The surgical preparation and Al procedure requires about two minutes per ewe for a 6 person team consisting of two people assisting with restraining and preparation of sheep, two people preparing insemination pipettes with frozen semen and two persons inseminating. The laparoscopic method of artificial insemination in ewe has been shown in the figure 2.

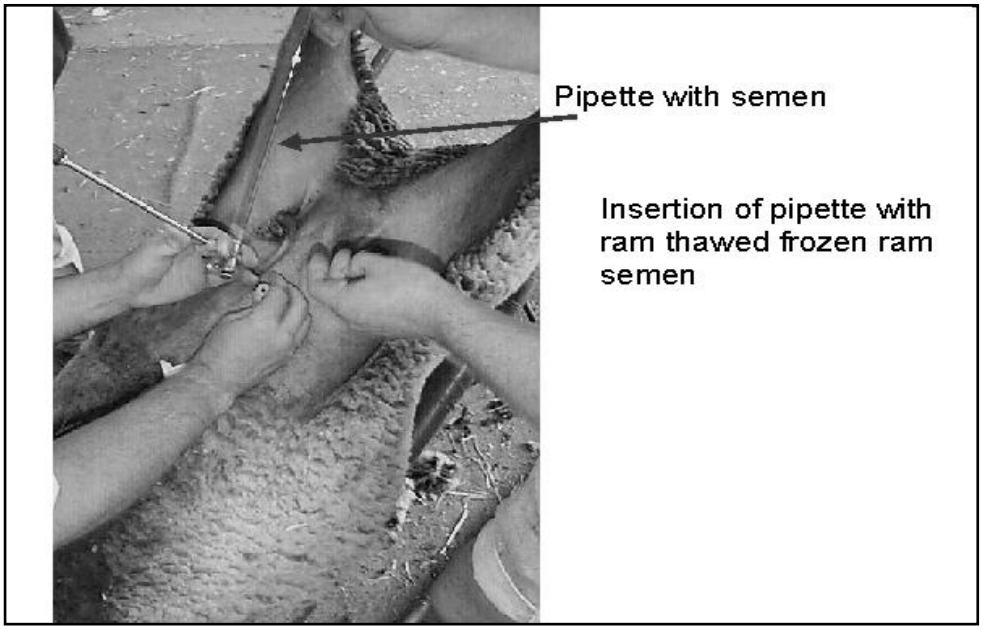


Figure 2: Laproscopic method of A.I. in ewe

METHODS AND MATERIALS:

Artificial insemination was performed in matured *Kage* ewes of Livestock development Farm, Lampatan, Pokhara during July 9-10, 2011. Total 15 matured ewes were selected for synchronization on the basis of history of non pregnancy, cyclic estrum and dry period. All selected ewes were injected with Prostaglandin ($PGF_2\alpha$*Lutalyse*) of single dose 2 to 3 ml deep intra muscular for heat synchronization. The teaser rams were applied to detect the heat of the synchronized ewes. The heat was detected between 48 to 72 hours of post injection of hormone and all the ewes (100%) showed the symptoms of heat. Both imported frozen semen from NewZealand and warm semen locally collected from *Kage* ram of the same farm were used for Artificial insemination. The insemination was done by cervical and trans-

cervical methods. Among all ewes, one was inseminated with frozen semen of *Romany mars*; two were inseminated with frozen semen of *Coop worth* after properly thawing, The frozen semen of Romany mars and Polworth was imported from Australia around 5 years back and on evaluation the motility of the spermatozoa was found to be more than 60% which is supposed to be good quality. The rest twelve ewes were inseminated with freshly collected warm semen using artificial vagina from three rams of the same flock. The volume of the semen used for insemination was 0.5–1.0 ml. The inseminated ewes were kept separately from the ram to avoid the natural breeding with supervision for the detection of pregnancy.

All eweused in this study were matured and were in good body condition and good general health. It was experienced that rams need to be previously trained to collect semen for artificial insemination. An industry recognized artificial vagina was used with appropriate lubrication and at the industry recommended temperature. All the equipments were disinfected or a new liner was used between each collection. Collection from a single ram was maximum of 10 times over a three day period, or a maximum 4 times per day with enough recovery times between each collection. To facilitate the procedure and promote the safety of both animals and operators, teaser ewes were experienced in the process of semen collection. They were appropriately stimulated to bring them into estrus for the purpose. Teaser ewes were appropriately restrained to prevent them moving excessively, to avoid injury to the ewe or ram during collection procedures. The floor of the collection area must be non-slippery with enough space in the pen for the restrained ewe, the ram and the operator to work easily, without risk of injury. The methods of collection of fresh semen and artificial insemination in ewes, done in the Livestock Farm, Pokhara has been shown in the figure 3. After the cervical insemination of the ewes, they were kept in the continuous supervision and without free grazing. They were kept separate from the ram, to prevent natural breeding.

Figure. 3. Cervical method of Artificial insemination and Artificial Vagina method of semen collection in Kage sheep.

RESULTS AND DISCUSSION:

Table no.1 describes the detail result of the cervical insemination in the ewes.

Table 1. Recording of results of heat synchronization and artificial insemination in ewes:

Ewe No.	Dose of Hormone Prostaglandin	Hours of heat detection	Kind of Semen used	Pregnancy status	Gestation Period	Kids	Birth weight kg	Lambing date
1	2 ml	72 hrs.	Romany Mars	Negative	-	-	-	-
2	2 ml	72 hrs.	Coop worth	Negative	-	-	-	-
3	2.5 ml	72 hrs.	Coop worth	Negative	-	-	-	
4	2.5 ml	72 hrs.	Kage (warm)	Not known	Dead	-	-	-
5	3.0 ml	48 hrs.	Kage	Pregnant	144 days	Male	1.94	4 Dec.
6	3.0 ml	48 hrs.	Kage	Negative	-	-	-	-
7	3.0 ml	48 hrs.	Kage	Pregnant	150 days	Fem.	1.88	10 Dec.
8	3.0 ml	48 hrs.	Kage	Pregnant	148 days	Fem.	1.70	9 Dec.
9	2.5 ml	72 hrs.	Kage	Negative	-	-	-	-
10	3.0 ml	48 hrs.	Kage	Pregnant	147 days	Male	1.80	8 Dec.
11	2.0 ml	72 hrs.	Kage	Negative	-	-	-	-
12	3.0 ml	48 hrs.	Kage	Pregnant	150 days	Male	2.00	10 Dec
13	3.0 ml	48 hrs.	Kage	Negative	-	-	-	-
14	2.0 ml	72 hrs.	Kage	Not known	Dead	-	-	-
15	2.5 ml	72 hrs.	Kage	Pregnant	148 days	Fem.	1.92	9 Dec

Two of the ewes died due to accident or disease within one month of insemination and the pregnancy status of those were not known as post mortem examinations was not performed. Out of 13 inseminated ewes, six (46.2%) were detected pregnant, and they lambed during 4-10 December, 2011 with the gestation period of 147±3 days (table 1). None of the ewes, inseminated with frozen semen were pregnant. It has been said that artificial insemination with cervical and transcervical has very less conception rate and even less than 10% conception has been reported in many cases.

However, 6 out of 10 ewes (60%), inseminated with warm semen were found pregnant and lambed during 4-10 December, 2011. The ratio of male and female lambs was 1:1(shown in the table 1). The birth weight of *Kage* lambs was ranging from 1.7 to 2.0 kg with the mean value of 1.87kg.

CONCLUSION AND IMPLICATION:

Artificial Insemination in sheep was introduced first time in Nepal. This was one of the best experiences achieved and on the basis of this; National Livestock Breeding center could initiate the program of artificial insemination in ewes by these methods. The cervical method was applied in the case of fresh semen, collected from elite ram for selective breeding

purpose or for kidding of future breeding rams which will be distributed in the flocks around the country. It will be introduced in Baruwal (80% of total sheep) for wool production. It is suggested to implement the laparoscopic method of insemination in the case of frozen semen as in the method done, the conception rate is nil or even less than 10% as described by different animal breeder.

Inbreeding is a serious problem in livestock especially in sheep breeding as there is no existing system of exchanging ram time to time. The same rams may remain in a folck for more than 4 years which in turn causes inbreeding, resulting reduced the productivity poor health disease resistance capacity. By adopting artificial insemination, inbreeding can be reduced in large extent. The major implication of artificial insemination in sheep is selection of good and productive ewes. Superior rams thus produced could be distributed throughout country. Since Nepal has become the member country of WTO and being the exporter country of carpet, it requires using 8% of wool from native production. Economic implication for wool production from Nepalese sheep is important. Providing a superior live breeding ram to each flock of sheep in mountainous regions create the multiplier flocks. The application of artificial insemination applies the collection of semen, processing and storing of frozen semen for sheep like Lampuchhre, Kage and Bhanglung which are on verge of extinction. The semen and embryo of these animals can be stored in Gene bank.

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A CASE REPORT ON SUBCLINICAL KETOSIS IN CATTLE

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1. Case description

Species: Bovine Breed: Jersey cross Sex: Female Age: 2nd lactation (parturated two months ago).

2. Patient history

The patient in Mangalpur, Chitwan was presented with a history of lethargy and loss of appetite. The cow was severely emaciated and it had turned completely cachectic. The cow was dull. There was reduction in milk yield from 7 liters to 2.5 liters per day.

3. Physical examination

The cow was giant in appearance. The vital signs included a body temperature of 102^0F, pulse of 68 beats per minute and respiratory rate of 54 breaths per minute. The rumen motility was reduced to 1 per 2 minutes and the conjunctival mucous membrane was pink. A positive skin test was found and her mucous membranes were tacky, suggesting dehydration. The patient was responsive but was lethargic. Severe emaciation and cachectic condition was well marked (Fig 1). Other detected abnormality included swollen foreleg knee joint inferring to infectious bursitis.

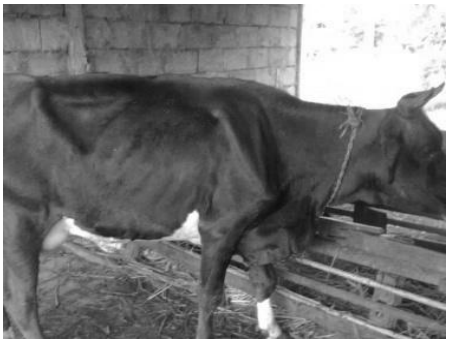

Fig 1: Physical appearance of the cow

4. Laboratory findings

Table 1: Hematological findings of the ketotic cow

Parameter	Patient Values	Units/Intervals	Reference
WBC	6859	*1000/µl	7.03
Fibrinogen	400	mg/dl	200-700
PCV	18	%	33-70

Table 2: Selected Biochemical testing of the ketotic cow

Parameter	**Patient values**	**Units/ intervals**	**Reference**
Total protein	10.2	g/dl	6.7-7.5
Albumin	2.0	g/dl	3-3.6
ALP	136	IU/L	90-130
SGPT	35	IU/L	14-38
SGOT	58	IU/L	78-32
Creatinine	1.1	mg/dl	1-2

Under hematology, PCV was found to decline (Table 1). Total protein and ALP increased while albumin level was found to decrease following serum analysis (Table 2).

Colorimeteric dip stick test for the analysis of urine was done. The change in color to blue revealed slight ketosis on the dip stick test (Fig 2). *Fasciola* eggs were found on faecal examination.

5. Differential diagnosis

a. Wasting form of abomasal displacement
b. Traumatic reticulitis
c. Primary indigestion
d. Cystitis and pyelonephritis
e. Diabetes mellitus

6. Diagnosis

From above findings and elimination of the possible other condition it was clinically diagnosed as ketosis.

7. Treatment

1. Inj. Dextrose-5% - 500ml

Sig: 4 bottles *IV* instantly followed by 2 bottles*IV* OD*3 days

2. Bol. Albendazole – 1000mg - 3

Sig: At haust P/O

3. Bol. Ecotas - 12 bol

Sig: 2 bol*bid*3 days

4. Advice: Feed molasses @ 3 kg per day for 1 month.

DISCUSSION

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation Period. It is characterized by increased levels of ketone bodies in the blood, urine, and milk. Subclinical ketosis is defined as abnormal concentrations of circulating ketone bodies in the absence of clinical signs of ketosis. Ketone bodies comprise beta-hydroxybutyrate (BHBA), acetoacetate (AcAc), and acetone (Ac), at 70, 28 and 2%, respectively.

Ketosis causes economic losses due to decreased milk production, impaired fertility, and increased risk of other infections. Subclinical ketosis has been associated with decreased milk production, impaired reproductive performance, displaced abomasums, metritis, mastitis, and clinical ketosis (Duffield, 2001).

Fig 2: Colorimetric dip stick test of urine

Ketosis is basically a problem related to negative energy balance. This is further aggravated by mobilization of non-sterified fatty acids from fatty tissues.

Blood Non esterified fatty acid (NEFA) ⟶ Oxidation ⟶ Ketone Bodies

Blood NEFA ⟶ Esterification ⟶ Triglycerides

Both of the above changes are possible in liver and depend on glucose availability. Glucose availability depends upon gluconeogenesis and availability of substrate for gluconeogenesis.

There are four types of this condition:

1. Subclinical: Presence of ketone bodies in blood and urine but no signs of disease
2. Digestive or wasting form: Digestive upset, decreased milk yield and depression
3. Nervous form: Nervous signs
4. Milk fever type: where hypocalcemia accompanies the hypoglycemia

Rothera test is the biochemical test that could be easily carried out in field. This reaction detects the presence of ketone bodies either in urine or in milk. The appearance of the pinkish colour is visible in positive reaction. Further, diagnosis is done as per history, clinical picture and biochemical evaluation. Maximization of the energy intake during pregnancy, parturition and lactation period could successfully control the condition.

Clinical Pathology

Table 3: Normal ranges and comparative ranges of various parameters in ketotic cattle

Particulars	**Normal level(cow)**	**Ketotic cow**
Blood sugar	40mg/100 ml	Reduced
Ketone bodies		
Acetoacetic acid	0.1 mg/100 ml	Upto 7mg/100ml
Beta hydroxy butyric acid.	8 mg/100 ml	Upto 30 mg/100 ml
Ketone bodies in milk	Nil	40 mg/100 ml
Ketone bodies in urine	Little or Nil	500-1000mg/ 100 ml
Serum calcium	9-11 mg/dl	Decreased
Serum magnesium	2-2.5 mg/dl	Reduced
Hematology	-------	Eosinophilia, Lymphocytosis, Neutropenia, SGOT increased.

(Source: Chakrabarti, 2004)

Hypoglycemia, ketonemia and ketonuria are characteristics of this condition (Table 3).

In the presented case, hypoalbuminea is likely to be because of the non- hepatic cause inferring to inadequate diet, protein loosing enteropathy and increased protein need i.e. in pregnancy and lactation. Also, stagnant level of fibrinogen indicating the non inflammatory condition supporting ketosis.

The clinical picture is usually too indefinite especially in cattle, to enable a diagnosis to be made solely on clinical ground (Radostits *et al*, 2006)). General consideration of history with particular reference to time of calving, the duration of pregnancy and feeding program and biochemical examination to detect the presence of hypoglycemia, ketonemia and ketonuria are necessary for accurate diagnosis. The increased γ globulins may refer for the infectious bursitis (response to the antigen) present in the fore leg. Starvation (from history) and parasitism seems to predispose the condition in this case. However, hormonal disturbance was not elicited.

Line of treatment

1) Glucose replacement therapy :
   500-800cc of a 40-50% solution of glucose (Dextrose) should be given intravenously at once.
   Oral hyperglycemic agents such as glycerol, propylene glycol, lactates and sodium propionates could be used.
2) Hormonal therapy:
   Betamethasone and Dexamethasone upto 30 mg could be used intramuscularly. Similarly Triamcinolone and Prednisolone is also used with success. Moreover, anabolic steroids such as Durabolin, Dinabolis also used effectively (60-120mg).
3) Vitamin B12 is also used. It acts as a cofactor in metabolism of propionate.
4) Biological precursors of COA such as Cysteamine (750mg IV, 3 doses, 1-3 days interval) and sodium fumarate has also been used.
5) Liver extract injections @ 5-10 ml IM in alternate days for 3 injections is used as supportive therapy.

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