Proceedings on 9th National Veterinary Conference of Nepal Veterinary Association

(VETCON'10)
22-24 April, 2010
Kathmandu, Nepal

Published by

NEPAL VETERINARY ASSOCIATION

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Tripureswor, Kathmandu
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Courtesy:
Heifer International Nepal, Hattiban

EDITORIAL

The Ninth National Veterinary Conference of Nepal Veterinary Association was held in Kathmandu during April 22-24, 2010 with the theme of "Veterinarians for Food Security, Public Health and Animal Welfare". More than 450 veterinarians from within the country and abroad participated in the conference. About 60 papers on various disciplines of veterinary science were presented.

Veterinarians in Nepal have been playing a pivotal role in the production and management of food animals and poultry, safeguarding the health of domestic livestocks, companion animals and wildlives. Food and nutrition security can be attained only with the enhanced animal production and productivity from the healthy livestocks and poultry. One cannot imagine how big is the magnitude of the economic consequences arisen from the heavy losses of animals and poultry from some dreadful diseases like PPR, Rinderpest, FMD, Bluetongue, highly pathogenic avian influenza, classical swine fever and Newcastle disease. The net effect of such dreadful diseases on the livelihood especially that of rural folks is very devastating. The most effective "Farm to Fork" approach can only be realized with efficient veterinary services in the country. With more than 70% of human diseases linked with animal diseases, roles of veterinarians in safeguarding public health through their timely detection, treatment, prevention and control has become more important.

Apart from veterinarians role in safeguarding animal health and ensuring food safety, prevention of cruelty against animals, ethical treatment of animals and complying with animal welfare issues are other obligations as per the standards set by OIE.

The present conference has contributed significantly to create awareness on animal welfarve, food safety, impacts of climate change and global warming on livestock production and health, and other innovations in the field of veterinary science along with the challenges and potential opportunities ahead. Furthermore, this conference has provided a common forum to interact and discuss on various issues related to the profession and livestock development and recommended the approaches to Government of Nepal for the overall development of livestock sector and veterinary profession.

This Proceeding includes majority of the papers presented in the conference, the remaining papers will be published in the forthcoming issue of Nepalese Veterinary Journal. I sincerely acknowledge all members of the editorial team for their tireless effort reviewing and editing the manuscripts. The assistance extended by Ms. Pramina Shrestha in computer correction is commendable. The publication team is looking forward to receiving the cordial help and continuous support from all colleagues to improve the quality of Nepal Veterinary Journal in future.

Editor-in-Chief

WORD OF GRATITUDE

Nepal Veterinary Association expresses its gratitude to all the Institutions & Personalities for their help to make the conference a grand success.

Special thanks go to the following institutions/organizations for their generous contribution

1. Probiotech Industries Pvt. Ltd., Sinamangal, Kathmandu
2. Heifer International Nepal, Hattiban, Lalitpur
3. Virbac, India
4. Avian Influenza Control Project (AICP), Budhanilkantha, Kathmandu
5. Indian Herbs Research and Supply Co. Ltd
6. Medivet Pharmaceuticals Lab, Kathmandu, Nepal
7. Bird Conservation Nepal
8. Intas Pharmaceuticals, Ahmadabad, India
9. Vetoquinol India Animal Health Pvt. Ltd, Mumbai (Wockhardt)

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Short communication

Program Schedule
IX National Veterinary Conference (April 22-24, 2010)

Day I: *April 22, 2010*

Venue: Nepal Academy Hall, Kamaladi

INAUGURAL SESSION

08.00 - 09.00 am	*Registration of Participants*
09.00 - 09.10 am	Chairing and Batch distribution to the Chief Guest and Guests
09.10 - 09.15 am	Welcome address highlighting the objective of the Conference
09.15 - 09.20 am	Inauguration of the Conference by lighting the lamp by the Chief Guest
09.20 - 09.25 am	World Veterinary Day 2009 award handover to NVA
09.25 - 09.30 am	NVA Life Time Achievement and NVA National Awards Distribution
09.30 - 09.35 am	Message from President of World Veterinary Association
09.35 - 09.40 am	Award Presentation: Industrialists and Entrepreneurs
09.40 - 09.45 am	Awards Presentation: Narendra Memorial Trust Awards, Pasudhan Kaushal Award, Pashudhan Vikram Award and NVA-Rotary-Shrijana Awards
09.45 - 09.50 am	Award Presentation : NVA Service Awards
09.50 - 10.35 am	Inaugural speech, Few Words, and Key note speeches by Guests and Chief Guest
10.35 - 10.40 am	Vote of thanks
10.40 - 10.50 am	Chairman's Remarks and Closing of Inaugural Ceremony
10.50 - 11.50 am	*Lunch*

TECHNICAL SESSION

I. THEME PAPERS

Chairperson: Dr NPS Karki; Rapporteurs: Dr UM Singh and Dr Rajani Pradhan

12.00-12.15	Zoonosis and human health: D. D. Joshi
12.15-12.30	Food safety and global concern: D. Sedhain and B. Parajuli
12.30-12.45	One World, One Health: More cooperation between veterinarians and physicians : S. Singh and S K Shrestha
12.45-13.00	Animal welfare: Natasha Lee
13.00-13.15	National wildlife health management policy: K. Gaire and S. Paudel
13.15-13.30	Impacts of climate change on livestock and vice versa: D. R. Khanal, S. P. Shrestha and A. Pradhan
13.30-13.45	National policy for the control of socio economically important animal diseases: R. K. Khatiwada, K.C.Thakuri, N. P. Ghimire and S.Karki
13.45- 2.15	Discussion and Chairperson's remarks
2.15-2.30	**Tea Break**

INDUSTRIAL/COMMERCIAL SESSION

Chairperson: Dr. P. Mainali, Joint Secretary, MOAC; Rapporteurs: Dr. J L Amatya and Dr K Premy

14.30-15.00	Probiotech Industries Pvt. Ltd.
15.00-15.30	Mars India
15.30-16.00	Virbac India
16.00-16.30	Intas Pharmaceutical India
16.30-17.00	Medivet Pharmaceuticals
17.00-17.30	Nepal Dairy Association

POSTER PRESENTATION

S. N.	Name of paper and author
1	Changes In The Genetic And Biological Characteristics Of Recent AIV Subtype H9N2 Isolated In Korea : M. P. Acharya, Hyuk-Joon Kwon, Il-Hwan Kim and Jae-Hong Kim

Day II: *April 23, 2010*

II. Ruminant Production and Health (Hall:1)

Chairperson: Dr. SK Shrestha; Rapporteurs: Dr SP Gautam and Dr S Kafle

Time	Name of Paper and author
9.00-9.10	Genetic improvement of dairy animals to meet farmer expectation for food security : K. P. Paudel and A. Shah
9.10-9.20	The effect of medicated and non-medicated urea molasses multi-nutrient block (ummb) supplement against nematode infection, on milk production and its composition : I. N. Shah and J. L. Yadav
9.20-9.30	A clinical study on anoestrus buffaloes in southern Nepal : S. K. Sah and Toshihiko NAKAO
9.30-9.40	Utilization of ground bamboo in fattening cattle : R. Pradhan and H. Kumagai
9.40-9.50	Transhumance effect on husbandry practices and basic physiological vitals of chauri, the yak cattle f1 hybrid: D. K. Chetri, R. A. Sah, N. R. Devkota and D. B. Nepali Karki
9.50-10.00	**Discussion**
10.00-10.20	**Tea Break**
10.20-10.30	Comparative study on povidone iodine cream and povidone iodine solution against sub-clinical mastitis of dairy cattle under farmer's management in Nepal : S. Thapa and B. R. Joshi
10.30-10.40	Sub-clinical bacterial mastitis (scm) in cattle of eastern terai of Nepal : S. Yadav and S. N. Deo
10.40-10.50	Early detection of subclinical mastitis among the dairy Buffaloes: C.Dhakal and B. P. Upadhyaya
10.50-11.00	Study on the survival of Streptococcus isolated from arthritis cases of lambs under various environmental conditions : R. P. Thakur
11.10-11.20	Clinical-laboratory investigation of mouldy maize and fodder poisoning in male goats in Kathmandu valley Nepal : K. Karki, P. Manandhar, S. Manandhar and P. Koirala
11.20-11.30	Discussion
11.30-11.45	Chairperson's remark
11.45-13.00	**Lunch Break**

III. Poultry Production and Health (Hall: 1)

Chairperson: **Dr. Tej B Basnet**; Rapporteurs: Dr Sulochana Shrestha and Dr B. R. Acharya

13.00-13.10	Effect of forage peanut meal on the production performance of lohmann layers (Action Research Through System Learning Approach) : B. N. Adhikari, I. P. Dhakal, N. R. Devkota and M. Sapkota
13.10-13.20	Biosecurity assessment of commercial poultry farms of Chitwan District : S.C. Chaudhary and S. Singh
13.20-13.30	Isolation and identification of salmonella species from the postmortem samples of poultry at Regional Veterinary Laboratory, Pokhara : B. K. Shrestha, P. R. Shrestha and S. P. Devekota
13.30-13.40	Seroprevalence of Newcastle Disease (ND) in commercial layers around vicinity of IAAS, Rampur : B. Shrestha and H. B. Basnet
13.40-13.50	Molecular characterization of a qtl associated with pulmonary hypertension syndrome in chicken : P. B. Kandel
13.50-14.00	**Discussion**
14.00-14.10	Surveillance of Avian Influenza In Nepal : L. Shrestha, P. Manandhar, S. Manandhar, S. Manandhar and P. Koirala
14.10-14.20	Effect of *Aloe vera* on immunomodulation, liver function, blood glucose and performance of broiler chickens : D. K. Yadav, D. K. Singh and M. C. Lee
14.20-14.30	*Enterococcus faecalis* and *Enterococcus faecium* the major cause of weakness and mortality in day old chick : H. B. Basnet, H. J Kwon, S. H. Cho And S.J. Kim
14.30-14.40	A case report on erysipelas in a broiler chicken flock : H. B. Basnet, H. J. Kwon, S. H. Cho, and S. J. Kim
14.40-14.50	Studies on counteractive effect of two phyto ascorbic acid products in aflatoxicated broilers : D. Sapcota and M. R. Wade
14.50-15.00	**Discussion**
15.00-15.15	Chairperson's remarks

15.15-15.30	**Tea Break**

IV. Companion Animals and Wildlife Management (Hall:1)	
Chairperson: **Dr. MN Shrestha**; Rapporteurs: Dr. B R Thapa and Dr Deuti Gurung	
15.30-15.40	Equine population, breeding, health Status and its future in Nepal : S. S. Sharma
15.40-15.50	A study of tuberculosis in rhesus macaques: a journey into zoonosis : S. P. Shrestha, S. Thapa, G. B. Girel and B. C. Kunwar
15.50-16.00	Effect of atropine sulphate, xylazine and ketamine combination protocol on clincaal, haematological and sero biochemical properties in dog : P. Phuyal And M. K. Shah
16.00-16.10	The relationship between blood parameter and mycobacterium culture status in captive elephants of Nepal : K. Giri, G. E. Kaufman And I. P. Dhakal
16.10-16.20	Incidence of Venereal Granuloma and its treatment in stray Dogs of Pokhara City, Nepal : H. R. Awasthi
16.20-16.30	Feasibility study on using non-surgical sterilization as a means of street dog population control in Kathmandu, Nepal: M. Bhandari, M. Aziz, B. P. Upadhyaya, J. Lindenmayer, A. Rauch, and G. Kaufman.
16.30-16.45	Discussion
16.45-17.00	Chairperson's remarks
17.00- 20.00	Cocktail Dinner

V. Zoonoses, food safety, food security, herbal medicines, animal welfare and others (HALL- 2)

Chairperson: **Dr. K P Dhakal**; Rapporteurs: Dr. P. Manandhar and Dr Dr. B Sapokota	
9.00-9.10	Sero-prevalence of porcine cysticercosis and human taeniasis/cysticercosis in Nepal: D.D. Joshi, A. Oommen, P. Dorny, P. R. Bista, V. Rajshekhar, J. Vercruysse
9.10-9.20	*E. coli 0157:H7* infection an emerging threat to public health: its epidemiology, prevention and control: A review : S. R. Aryal
9.20-9.30	Prevalence of *Taenia solium* Cysticercosis in Swine in Kathmandu Valley and Its Impact on Public Health : A. K. Karna and D. D. Joshi
9.30-9.40	Epidemiological situation of animal rabies and its control strategy in Nepal : S. Karki and K. C. Thakuri
9.40-9.50	Identification of two Rabies Virus lineages of Nepal : G. R. Pant
9.50-10.00	Epidemiology of Foot and Mouth Disease in Nepal: R. Giri, P. Parshin and V Makarov
10.00-10.10	**Discussion**
10.10-10.30	**Tea Break**
Chairperson: **Dr. DD Joshi**; Rapporteurs: Dr. S Aryal and Dr Dr. R Ghimire	
10.30-10.40	Prevalence of Salmonella in retail meat shops in Kathmandu, Nepal : M. Upadhayaya
10.40-10.50	A retrospective study of case flow pattern at HICAST Veterinary Teaching Hospital : P. Sharma
10.50-11.00	Evaluation of hepatoprotective activity of Cuscuta reflexa Roxb. (Aakashbeli) stem : P. Alam, M. P. Gupta
11.10-11.20	Effect of stinging nettle on productivity and immune status of laying hens: N. Poudel and D. R. Khanal
11.20-11.30	Effect of *Nyctanthes arbortristis* on dextran suflate sodium-induced colitis : S. Bhattarai and T. C. Bhattarai
11.30-11.40	Traditional veterinary medicine practice adopted by the people of Nepalese origin in north eastern India : K. Kaphle and D. Sapcota
11.40-11.50	**Discussion**
11.50-12.00	**Chairperson's remarks**
12.00-13.00	**Lunch Break**
Chairperson: **Dr. B. N. Thakur**; Rapporteurs: Dr. K Sharma and Dr. G. Panta	
13.00-13.10	Study on Effect of different Fat Levels on the Quality of Chhurpi prepared from Cow and Buffalo milk : D. N. Sah
13.10-13.20	Climate change: threats and opportunities for livestock development in Nepal : K. Pandey
13.20-13.30	Practice of animal welfare education (awe) in Nepal : B. S. Sapkota, S. S. Singh
13.30-13.40	Knowledge and attitude of Nepalese people towards the welfare of farmed animals

	and assessment of animal welfare during transportation : J. Pandeya
13.40-13.50	Health status of equines employed at Lalitpur brick factories: S. Koirala, S.Basyal and P.Thapa
13.50-14.00	Community based animal health care for rural livestock development: P S Kushwaha and S. N. Mahato
14.00-14.10	Prevalence of helminth parasites of piglet in peri-urban areas of Kathmandu valley: N Baskota and R P Thakur
14.50-15.00	Discussion
15.00-15.15	Chairperson's remarks
15.15-15.30	**Tea Break**

Day III: *April 24, 2010*

Venue: World Trade Centre

08.00-0.9.00	Breakfast
09.00-09.30	Constitution Amendment
09.30-11.00	Tribute to late Veterinarians and General Assembly Meeting
11.00-11.20	Tea Break
11.20-11.30	Group Formation for recommendation
11.30-12.30	Group Discussion
12.30-01.00	Group Presentation
01.00-02.00	Lunch Break
02.00-05.00	Election
05.00-06.00	Closing Ceremony

Closing Ceremony

05:00 pm	Arrival of Chief guest: Prof. Subodh Narayan Jha
	Chairing of the closing session by president
	welcome of chief guest with Bouquette presentation by president
	Award presentation by Chief guest:

1. Dr. Sunder Mohan Foundation Award
Dr. Mukul Upadhayaya

2. Election officers
Dr. Padma Nath Sharma

3. Evaluator of the paper

Cordinator	Dr. Nara Bahadur Rajwar
Member	Dr. Doj Raj Khanal
Member	Dr. D. K. Singh
Member	Dr. Neena Gorkhali
Member	Dr. Upendra Man Singh

4. The coordinator of the blood donation programme
Dr. Karuna Sharma

	Recommendation by General Secretary
	A few words by chief guest
DINNER	

9th NATIONAL VETERINARY CONFERENCE
(VETCON'10)

NEPAL VETERINARY ASSOCIATION
नेपाल भेटेरिनरी एशोसिएसन

From the Desk of General Secretary

Dr. Subir Singh
General Secretary

Nepal Veterinary Association (NVA) is a solely scientific and professional organization of entire Nepalese veterinarians. NVA was established on 11th Ashad 2024 (BS) and legally registered on 13th Falgun 2025(BS). The Association was affiliated as national member of World Veterinary Association (WVA) on 1986 and Federation of Asian Veterinary Association (FAVA) on 1998. In the year, 2009, NVA has received "World Veterinary Day Award" from OIE-WVA for global outstanding performance in celebration of WRD 09.

Until now 560 veterinarians including 45 foreign nationals have been registered as member of this association. The association has members with wide range of expertise in animal health and production, public health and natural resource management enabling the association fully capable for providing competent veterinary service in Nepal.

Objectives of NVA:

The objectives of the association are:

- **to emphasize the necessity of the unity among the members of the profession.**
- **to promote the spirit of harmony, cooperation among each other and the staff working in this profession.**
- **to make aware of new discoveries and development in the field of veterinary and allied sciences to livestock wealth of the profession.**
- **to work for the development of livestock wealth of the country.**
- **to encourage production and quality of livestock products.**
- **to improve animal disease diagnosis in the country.**
- **to provide consultancy service.**
- **to modernize veterinary medical treatment and surgery.**
- **to improve access of veterinary service.**
- **to help prevention, control and eradication of animal disease.**
- **to safeguard public health from diseases communicable from animals to man and vice versa (=zoonotic diseases).**
- **to keep up the honor and dignity of the profession in the society and to safeguard its interests.**
- **to publish scientific periodicals and journals to disseminate the information.**
- **to arrange seminars, conference and discussion forum to address national issues.**

Conference organization is one of the most significant affairs of NVA. The technical conferences, involving scientists, planners, policy makers, industrialists and farmers involved in various sector of livestock resource management at home and abroad, comprise incredibility to exchange technical information and experience in order to address scientific and filed level setbacks. In true words, this kind of conferences has played school for professional continue education. The association has so far organized eight such national veterinary conferences with the participation of distinguished personalities from national and international arena. A brief flash back of the conference is as follows:

National Veterinary Conferences of Nepal Veterinary Association

	Venue	*Period*	*Chief Guest*	*Chairperson*
First	**APROSC building, Singhadurbar**	**Aug 10-13, 1983**	**Mr. Lokendra Bahadur Chand, Rt. Hon. Prime Minister**	**Mr. Hem B. Malla Hon. Minister for Agri. & Land Reform**
Second	**Hotel Blue Star Thapathali**	**Feb. 23-25, 1987**	**Mr. Hari Narayan Rajauriya, Hon. Minister for Law & Justice**	**Dr. Ratna SJB Rana, VC, RONAST**
Third	**APROSC building, Singhadurbar**	**Aug. 10-12, 1990**	**Mr. Krishna Prasad Bhattrai, Rt. Hon. Prime Minister**	**Mr. Jhala Nath Khanal, Hon. Minister for Agriculture & Forest**
Fourth	**Hotel Blue Star Thapathali**	**Nov. 16-19, 1992**	**Mr. Ram Chandra Poudel, Hon. Minister for Agricuture**	**Dr. Ram Prakash Yadav, Hon. Member, NPC**
Fifth	**International Convention Center, Baneswor**	**Sept. 11-13, 1996**	**Mr. Sher Bahadur Deuwa, Rt. Hon. Prime Minister**	**Mr. Prem Bahadur Bhandari, Hon. Minister of State for Agriculture**
Sixth	**International Convention Center, Baneswor**	**Feb. 22-24, 1999**	**Mr. Kul Bahadur Gurung, Hon. Minister for Education (On behalf of Rt. Hon. Prime Minister Mr. Girija Prasad Koirala)**	**Mr. Prithbiraj Ligal, Hon. Vice President, National Planning Commission**
Seventh	**Hotel Radisson, Lazimpat, Kathmandu**	**Nov. 5-7, 2003**	**Dr. Prakash Chandra Lohani, Hon. Minister for Agri & Cooperative, and Finance**	**Dr. Neel Prakash Singh Karki, President, NVA**
Eighth	**Birendra International Convention Center, Baneswor & United World Trade Centre, Kathmandu**	**May 8-10, 2008**	**Rt. Honorable Sepaker, House of Representative Mr. Subash Chandra Nemwang**	**Dr. Braja Kishor Pd. Shaha, President, NVA**

<u>The Ninth National Conference (VETCON '10)</u>

The Ninth National Conference (VETCON'10) to be held with the theme of *"Veterinarians for Food Security, Public Health and Animal Welfare"* at Kathmandu on Baisakh 9-11, 2067 (April 22-24, 2010) which will be participated by members and scientists from home and abroad.

The formal inauguration of the Ninth National Conference (VETCON'10) will be formally inaugurated on the first day at Nepal Pragya Pratisthan, Kamaldi, Kathmandu on 9th Baisakh, 2067 (April 22, 2010), the second day technical session will be organized at same venue and the last third day programs as the closing session will be organized at United World Trade Center, Tripureswor, Kathmandu. All the events and programs of the VETCON'10 are focused to the celebration of World Veterinary Day 2010 (April 24, 2010).

During the inaugural session of the conference, NVA will honour with NVA National Awards to some institutions and personalities who have significantly contributed in bringing effective veterinary services in the country. The NVA Service Awards will be provided to more than 08 members who have served the nation for more than 25 years. The Narendra Memorial Trust Awards, Pasudahn Kaushal Award, Pashudhan Vikram Award and NVA-Rotary-Shrijana Awards will also be presented to selected individuals during the inaugural ceremony. In addition, 3 industrial papers and more than 4 thematic papers will be presented and discussed on the second half of the first day.

On the second day of the conference, a total of 59 scientific papers will be presented orally and discussed at the conference. This day is hoped to serve the continue education motive of members. On the last third day, a procession will be carried out in Kathmandu valley to celebrate WVD '10 and closing session will run on the same day of the conference to deal/discuss NVA affairs, plan future activities and constitue the new executive committee for next two year tenure.

MAJOR ACTIVITIES OF 2008 – 2010 TENURE

After the formation of new executive committee with 8th National Veterinay Conference on 10th May, 2008 (Baisak 26, 2065), NVA took several steps to accelerate its activities. Apart from its routine and professional activities, NVA has carried out the following activities:

1. **NVA received a computer set from Avian Influenza Control Project (AICP) to support and strengthen NVA activities.**
2. **NVA office strengthening was conducted with furnishing the meeting hall. Records keeping of the members were established by maintaining complete personal database system.**
3. **Central Committee of NVA worked in the feeling to boost up and active all its regional chapters and sister organizations.**
4. **Publication of Proceeding of VETCON'08, Veterinarian's Directory, Veterinary Chaumasik, Souvenir of AGM 09 and Nepalese Veterinary Journal.**
5. **NVA put a thrust on communication network among the members and build up a regular e-mail contact group to circular the activities and message among them. Also, website of the association was prepared functional.**
6. **Advocacy and lobbing for the establishment of proposed "Ministry of Animal Husbandry and Fisheries", "Poultry Development Board" and "Agriculture, Veterinary Science and Animal Husbandry University". The team under NVA worked for logical documentation and coordination between allied professional organizations in this regard.**
7. **NVA made request to DLS to participate field/private vets at Meat Inspection training. Also, NVA made an effort to post/employ meat inspectors, wildlife officers and field vets to work under govt. service system.**
8. **The Nepal Veterinary Association and Directorate of Livestock Market Promotion jointly organized a national workshop on "*Status and Challenges of Meat Hygiene and Marketing in Nepal*" on the premises of the DLS meeting hall, Hariharbhawan, Lalitpur on July 6 to mark World Zoonosis Day. The purpose of celebrating the Zoonosis Day was to create awareness and seek the responsibility of govt. as well as line agencies to control the zoonotic diseases in Nepal. There were altogether 117 participants in the workshop, representating govt. high level officials, university faculties, scientists form NARC, vet. clinicians, retired vet. professionals, vet. students, paravets, representatives from meat animal production and marketing forum, butcher and meat sellers forum, representatives form Kathmandu metropolitan city as well as from Lalitpur sub metropolitan city, animal welfare forum and media houses/ news reporters.**
9. **Animal welfare allied group meeting: NVA organized a meeting with NGOs and INGOs of Nepal working for animal welfare, at NVA office, Tripureshwor, Kathmandu on 2065/04/25 (Aug. 9, 2008). Representatives from SPCA Nepal, SAWM Nepal, KAT Centre, HEIFER International/ Nepal, Animal Welfare lovers and NVA members attended the meeting.**
10. **Animal health and vaccination camp Koshi Flood Relief, Saptari: NVA conducted an "Emergency animal health and vaccination camp" at Bhardah, Saptari for the care of livestock and birds victimized by Saptakoshi flood. Altogether 45 thousand animal's populations are directly affected and 9,321 animals has been reported death due to flood. A volunteer veterinary team (Dr. Subir Singh, Dr. Manoj Kumar Shah, Dr.**

Pushpendra Sah and Dr. Susil Poudel) and IAAS Rampur's students (Mr. Anjay Sah, Mr. Gangaram Yadav, Mr. Indra Narayan Sah, Mr. Subash Chaudhary, Mr. Bhup Narayan Mandal and Mr. Rabindra Mandal) worked with the weekly camp at the site.

NVA also received enormous supports from pharmaceutical and feed companies with free drug and feed supply to conduct the camp. NVA was supported through Heifer International/Nepal, Pro- Bio Tech Industries Pvt. Ltd. (NIMBUS) and several pahrmacuticals companies like Verbac, Vetcare, Alembic, Wockhardt, TTK, Medvet, VetPharm, etc. provided more than 32 cartoons of medicines which worth about 3.5 lakh. Directorate of Animal Health facilitated to provide H.S. & B.Q. and PPR vaccines. The team worked in coordination with DLSO/Saptari and formulated four mobile and one stable camp at the site. Altogether, about 6 thousands cattles and buffaloes received H.S./ B.Q. vaccine while the team extended its major service to attended, treat and manage 355 sick animals affected by flood. The animals are suffering from Ulcer, Wounds, Stomatitis, Conjunctivitis, fractures, dehydration, emaciations, foot rot, degnela, dermatitis and poor performances. Many animals are dying due to lack of feed and forage. There was also threat situation due to outbreak of FMD, HS and BQ disease.

11. **Organisation of World Veterinary Day and Winner of the award: NVA organised World Veterinary Day on last Saturday of April every year from 2004-2009. In the year 2009, NVA celebrated World Veterinary Day 2009 (WVD '09) as weeklong profound celebrations with concluding ceremony on April 25 to conclude the festival. As the theme, "Veterinarians and Livestock farmers, a winning partnership", NVA too decided to conduct and celebrate the day with veterinary professionals, paraprofessionals, farmers, livestock industrialist, consumers, veterinary students and community people.**

The final event for the closing ceremony of WVD was organized at Rampur and Bharatpur of Chitwan, Nepal. NVA celebrated more than 12 remarkable events throughout the country on the occasion of World Veterinary Day 2009 with full endeavor and potentiality, which ultimately result to win the WVD'09 award. Technical seminar, Animal health camps, Vaccination camps, Castration camp, Press meet, Annual General Meeting of NVA, National march pass, Horse cart rally, etc. were the winning events of weeklong activities.

The 2009 Joint WVA/OIE World Veterinary Day Award was decided on April 30, 2009 and presented on May 29, 2009 to Nepal Veterinary Association. The declaration of WVD '09 award has lead NVA at international veterinary professional arena. The presentation was made during the 77th Annual General Assembly of the World Animal Health Organisation (OIE) in Paris. Nepal Veterinary Association won the $1,000 award for best promoting the theme "Veterinarians and livestock farmers, a winning partnership."

12. **Similarly, NVA implemented successful national plan and activites in celebration of World Rabies Day (Sept. 28) and World Animal Day (Oct. 04).**
13. **NVA supported for successful organization of Third general meeting of Nepal Veterinary Council was organized on 24 Mansir, 2065 at World Trade Centre, Tripureshwor, Kathmandu. About 210 registered NVC members participated the meeting which was chaired by Dr. Bhabananda Thakur, Chairman of NVC. The program was inaugurated by chief guest Hon'ble Minister Ganesh Sah, Minister of Environment, Science and Technology.**
14. **Nepal Veterinary Association organized a Welcome meeting of NVC executives at its office's meeting hall, Tripureshwor, Kathmandu, on 24th Mansir 2065.**
15. **NVA Conducted 6 animal health camps at several regions of the country.**
16. **Birdflu and zoonotic disease awareness campaign was organized at Jhapa, Chitwan, Dolkha, Kathmandu and Nepaljung.**

17. **NVA actively participated at three days NVC strategic planning workshop from 13 to 15 Falgun, 2065 (24 to 26 Feb. 2009) at Heifer Office, Hattiban, Lalitpur. About 24 delegates from all vet. sectors, participated the workshop and formulated the vision, objectives, strategy and indicators for the coming 5 years plan of NVC.**
18. **NVA supported and contributed human resources and technical advice to DLS and Govt. of Nepal in the control of birdflu in Nepal. In this regard, NVA developed the distribution of Veterinary health certificate format to facilitate the vets in carrying the inspection and certification works at ease.**
19. **NVA prepared the draft of "Participant Manual: Training on Biosecurity for poultry farms and markets Nepal" for STOP AI/USAID in Dec. 2009.**
20. **NVA received the responsibility to draft first amendment draft of "Animal Health and Livestock Service Act, 2055" from Animal Health Directorate, Kahtmandu and has successfully submitted to it.**
21. **NVA organized Talk Programme and Training on:**

Date & Venue	Title	Key Speakers
October 21, 2008, DLS Hall, Kathmandu	**Commonly Used Injectable Anesthetics For Companion And Large Animals Under Field Conditions**	**Dr. Pedro Boscan Colorado State University, US**
May 24, 2009, DLS, Kathmandu	**Training on Biosecurity and diagnosis on AI**	**Team of German scientists**
May 27, 2009, IAAS, Rampur	**Transboundary Animal Diseases**	**Prof. Dr. Anthony P. Knight Colorado State Universit, USA**
June 5, 2009, CVL, Kathmandu	**Biodefense and Transboundary Animal Diseases**	**Prof. Dr. Anthony P. Knight Colorado State Universit, USA**
July17, 2009, CVL, Kathmandu	**Cysticercosis and Taeniasis : Recent Advances of Serological and Molecular Diagnosis**	Prof. Dr. Hiroshi Yamasaki **Japan**
Oct. 16, 2009, CVL, Kathmandu	**An Epidemiologist's journey through one world one health**	Dr. Drona P. Rasali Epidemiologist, Canada
Jan. 04, 2010, CVL, Kathmandu	**Clinical Experience on Small Animal Practice**	Dr. Balram Aryal Clinician, Maryland, USA
Jan. 04, 2010, CVL, Kathmandu	**Zoonosis Control in South-East Asia Region : Opportunities and Challenges**	Dr. G. N. Gangol WHO Regional Office, India

Obituary

Nepal Veterinary Association expresses its deep sorrow for the untimely demise of its following members and prays to God for the eternal peace of the departed soul

1. **Dr. Bir Mardan Basnyat (69 years) on 19th Shrawan 2065. He was born on 1997 Asoj 01. He was associated with Ultra Pharmaceuticals Pvt. Ltd.**
2. **Dr. Dharmendra Chaudhary, who was posted at DLSO Mugu. He was permanent residence of Amawa-3, Bara, Nepal.**

Conclusive Remarks:

NVA heartily thanks to all its members, sister organizations, WVA, line agencies and well-wishers for providing their supports and encouragement to promote and perform professional activities.

A report on award winning "WORLD VETERINARY DAY 2009"

Dr. Subir Singh

General Secretary, Nepal Veterinary Association

In the year 2009, NVA planned to celebrate World Veterinary Day 2009 (WVD '09) as weeklong profound celebrations with concluding ceremony on April 25 to conclude the festival.

As the theme, "*Veterinarians and Livestock farmers, a winning partnership*", NVA too decided to conduct and celebrate the day with veterinary professionals, paraprofessionals, farmers, livestock industrialist, consumers, veterinary students and community people.

Following are the celebrations and events conducted on the eve of World Veterinary Day 2009.

1) Seminar on "Current outbreak Newcastle Disease and its control strategy"

   On the request of Nepal Egg Producers' Association and Nepal Poultry Entrepreneur's Forum, NVA-Chitwan Chapter organized a "Technical Seminar" in the beginning of the WVD week. Dr. Subir Singh, Dr. Dinesh Yadav and Dr. Khagendra Sapkota presented papers on current outbreak of Newcastle disease, its control strategy and biosecurity measures in the hall of the Narayangadh Chamber of Commerce and Industries. The focus of that talk was on the short term and long term strategy to control ND the disease causing huge economic loss to poultry farmers of Chitwan. Altogether 6o poultry farmers, entrepreneurs and businessman participated the seminar, while more than 25 vets working in the poultry field were present at the session.

2) Free Animal Health Camp at Lekhnath, Kaski:

   A wonderful animal health camp was organized at Lekhnath -9, Lamaswara, Kaski, on the occasion of World Veterinary Day 2009. The camp was organized in coordination with NVA-Pokhara chapter with leading support of Animal Health Training and Consultancy Service (AHTCS), Pokhara, on 18th April 2009, Saturday.

   The total animals treated in the camp were Cattle/Buffaloes: 109, Goat: 51 and Dogs: 17

   Service provided in the camp: Vaccination against HS, BQ and FMD; Castration, Dehorning, Pregnancy diagnosis, Internal and external parasitic drug administration, Anorexia, Infertility and other common ailments.

   Human Resource involved in the camp were Dr Shiva Devkota - Regional Lab, Pokhara/ Regoinal Coordinator-NVA, Dr. Kedar Raj Pandey-DLSO, Kaski/Regional Member-NVA, Veterinarians and Paravets from AHTCS and Village animal Health Worker (VAHW) trainees. Altogether 11 vets and 22 paravet professionals participated the camp.

3) Free Dog Sterilization and Anti-rabies Vaccination Camp at Sauraha, Chitwan:

   On April 17, a Dog Sterilization and Anti-rabies Vaccination Camp was organized at Sauraha, Chitwan. Celebrating WVD '09, the NVA-Chitwan Chapter and Chitwan National Park with joint support of Institute of Agriculture and Animal Science (IAAS), Rampur Campus, Mirgakunja Consumers' Society, Central region livestock service committee, Tarai Bhuparidhi Program, National Trust for Nature Conservation and Hotel Association Nepal/Sauraha organized the program at the buffer zone of Chitwan National Park. Altogether, 23 male dogs were castrated at the site and 48 dogs and 1 cat were vaccinated with ARV.

   Surgeons of IAAS, namely Dr. Subir Singh and Dr. Manoj Kumar Shah worked for surgery and the supporting team was made among the internship students and final year students of IAAS. Dr. Bhuminandan Devkota, Dr. Kamal Gairae, Dr. Jivan Thapa and Dr. Sarad Poudel helped to smooth organizing of the camp at local level. A team of 6 veterinarians and 22 veterinary under-

graduate students were involved for successful completion of the camp. Spay Clinic of IAAS (running with joint support of Tufts University and Tribhuvan University) provided all the surgical logistics while Anti-Rabies vaccine was supported form Merial.

The site of the camp, on one hand is densely located with tourist hotels and visitors while on other hand it is the buffer zone of national park, so the dog sterilization and vaccination camp was organized with an objective to control dog population and save the tourist society and animals with rabies.

4) Free Animal Health Camp at Madi, Chitwan:

As per the scheduled week long celebrations of the World Veterinary Day, a "One Day Animal Health & Infertility Camp" was jointly organized at Basantapur of Bagauda V.D.C. Madi, Chitwan, by Nepal Veterinary Association Chitwan Chapter (NVA-CC), District Livestock Service Office (DLSO), Bharatpur, and Veterinary Teaching Hospital (VTH), Rampur.

Madi, a valley within Chitwan National Park, is regarded to be a remote area within Chitwan district and lies to the southern part of the park. The very rough road to Madi is functional only in the dry season. Almost 13 kms of the road is within the dense tropical forest of the Chitwan National Park.

Senior veterinarians Dr. Bihsnu Kumar Shrestha (DLSO, Bharatpur), Dr Tez Bdr. Rijal (DLSO, Bharatpur), Dr. Bhuminand Devkota (President, NVA-CC), Dr. Bishnu Bahadur Adhikari (NVA members), Dr. Krishna Kaphle (GS, NVA-CC) were present at the occasion. Extensive treatment of animals including 150 medical cases, 80 gynecological cases and 40 medial patellar desmotomy operations were performed at the camp. Large animal especially the working buffalo bull was the most common patients. Goat was the most common case in case of small ruminants. The camp was mainly targeted to the working bull.

Other vets of NVA-CC, Dr. Laxman Dhakal, D. Rebanta Kr. Bhattarai, Dr. Narayan Paudyal, Dr. Suniti Jha and Dr. Prazila Shrestha along with the internship students of IAAS, Rampur also actively participated at the camp. Various medicines and feed supplements were donated by pharmaceutical companies.

5) Organization of Press Meet:

On April 22, 2009, at Narayangadh, Chitwan, Nepal Veterinary Association organized a "Press Meet" to brief about the celebration, objectives and events of World Veterinary Day. The meet was chaired by Dr. Adarsha Pradhan, President of NVA and the program was conducted by Dr. Subir Singh, General Secretary of NVA. The meeting was organized at Kitchen Café, Narayangadh, Chitwan. Dr. IP Dhakal (Ex-VP of NVA), Dr. B.N. Devkota (President of NVA-Chitwan Chapter), Dr. Sarada Bhattarai (Vice Chirman of NVC), Dr. Krishan Kaphle (GS of NVA-Chitwan Chapter) and Dr. Khagendra Sapkota (EC member of NVA-Chitwan Chapter) participated the meet on behalf of NVA.

Altogether 21 news reporters, media personals and journalist from different national and local media houses participated the meeting. The brief press release, in Nepalese language, published by NVA and distributed among the participants. The release described about WVD and its celebration in Nepal and was put to the meeting as the theme paper to the participants. The meeting was organized with an objective to create awareness about WVD among the people.

6) PPR Vaccination Camp at Chitwan:

Nepal Veterinary Association organized "PPR vaccination camp" at Chitwan on 22nd of April with joint effort of Nepal Veterinary Students' Association (NVSA) and District Livestock Service Office (DLSO) Chitwan. Two mobile units consisting 20 veterinary under-graduate students in each unit, conducted the vaccination in 420 goats at Dairy Chowk, Beluwa, Chauki, Bangai, Shardanagar Town and Dharmodaya Chowk of Shardanagar VDC, Chitwan. 135 farmer households were provided with the PPR vaccination facility and they were interacted about the

event and WVD celebration. The PPR vaccine was obtained from DLSO, NVSA provided human resource and NVA facilitated with all the logistic supports to organize the event on the eve of WVD 2009.

Since last 10 years, PPR disease in goat is causing major economic loss in this area. The camp is hopeful to reduce the farmers' loss due to this disease.

7) Free Anti-Rabies vaccination camp organized at Bhaktapur District:

Nepal Veterinary Association organized Free ARV Vaccination Camp on 23rd and 24th of April, on the eve of World Veterinary Day 2009 with joint effort of Himalaya College of Agriculture Science and Technology (HICAST) and vaccinated 39 community dogs. The veterinary students of HICAST actively participated the program.

The advertise of the camp was disseminated by broadcasting in local FM radio and using loudhailers in differently densely populated areas and pasting pamphlets on the walls, since three days before the program. On the first day, the camp was organized at HICAST Veterinary Hospital and on following day, the vaccination was carried at various wards of Bhaktapur Municipality.

8) Annual General Meeting (AGM) of NVA:

On the week end of the WVD celebration, NVA organized its "Annual General Meeting" at Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan. The meeting was organized at April 24, 2009 from 10 am to 7 pm.

Due to some political disturbance and strike at the highway, only 100 NVA members were able to actively participate the meeting. The leading farmers and chairpersons/representatives of several farmer associations (dairy, poultry, fish, swine, bee, etc.) participated the inaugural session of the meeting. Dr. Yadav Sharma Bajagai (secretary of NVA) conducted the meeting.

Dr. Adarhsa Pradha chaired the inaugural session of the meeting and Dr. Neel Prakash Singh Karki (Ex-President of NVA, Ex-Director General of DLS and Ex-Chairman of NVC) chaired the session as chief guest. Special guests and guests were Prof. Dr. Sundar Man Shrestha (Dean of IAAS), Mr. Arun Shanker Ranjit (Deputy Director General, Dept. of Livestock Services), Dr. Baikuntha Parajuli (Program Director, Animal Health Directorate), Mr. Mitra Raj Dawadi (Chairman, Narayangadh Chamber of Commerce and Industries) and Dr. I P Dhakal (Chief, Rampur Campus) chaired the dash. Dr. NP Ghimire, VP of NVA, formally welcomed all the guests and delegates. The chief guest inaugurated the meeting by lightening ceremonial lamp.
The papers were presented in this inaugural session:

1. Strengthening veterinary education to cope with challenges of livestock development in Nepal: Dr. IP Dhakal and Dr. Subir Singh
2. Role of veterinarians in the development of poultry industry of Nepal: Dr. Til Chandra Bhattarai
3. Veterinarian's role in vulture conservation of Nepal: Dr. Surya Poudel

The chief guest and guest delivered the Wishing Speech for the successful completion of the general meeting and they were also honored by token of love by the chairman on behalf of NVA. The chairman of the inaugural session gave vote of thanks to all the participants, guests and delegates with his closing remarks.

After a lunch break, the close session (second session) was held to discuss mainly with the progress report and financial report of NVA. The session also discussed to build up next year strategy planning and finally discussed to conclude major recommendations to the Govt. of Nepal. The session also discussed about the activities of NVA done in the favor of WVD and planned the next day ceremony.

After the completion of a day long session, the delegates enjoyed with magnificent cultural program conducted by the veterinary students of IAAS. Finally, the reception cocktail dinner was organized for all the delegates and guests.

ON THE DAY OF APRIL 25, SATURDAY

9) WVD National March Pass at Bharatpur, Chitwan:

On the eve of WVD, NVA organized "National WVD March Pass" at Bharatpur, Chitwan on April 25, at early 7 am to 9:30 am. The event was participated by more than 700 participants involving more than 100 vets, para-vets, farmers, students, staffs of govt., Nepal army, NGOs and INGOs staffs, entrepreneurs, vet. drug sellers and industrialists, etc.

For the first time in Nepal, such a big march pass of veterinary professionals was organized. The events highlighted with two leading elephants (from wildlife department) and four horses (from Nepalese army). Several veterinary related organizations and farmers associations participated with their banners and play cards. The play cards were decorated with several awareness quotations. The rally was successful to draw the public attention and create awareness about veterinary profession.

At last, Dr. Adarsha Pradhan, president of NVA, addressed the mass and congratulated all the professionals on the eve of WVD.

10) WVD Rally at Bhaktapur:

Under the organization of NVA, on 25th April, about 125 students and teachers from HICAST build a rally with placards which was started from HICAST, Gatthaghar, Bhaktapur and moved to different areas of Bhaktapur Municipality. The awareness pamphlets and broachers, published by NVA, were distributed to the passerby, shopkeepers and local peoples to create awareness of WVD '09.

The rally moved through Gatthaghar, Kausaltar, Sanothimi and finally ended at HICAST. The rally also relayed the information to the general public about the theme and objective of WVD celebration, using loudhailers.

11) Tanga (horse cart) rally at Birgunj, Parsa :

For the first time in Nepal, to mark the World Veterinary Day, NVA and AHTCS rallied mules, horses and donkey (with their owners and farmers) on the streets of Birgunj Sub-metropolis, Parsa on April 25, Saturday. The animals participating in the rally were sporting a tiring of slogans but the message was loud and clear. The animals were asking for love, care and respect. The owners and masters were holding placards and banners that vividly described the plight of their animals.

About 200 tanga (cart with horse) participated the rally which was about 2 km. long way street. The rally organizers also provided "special lunch" to the horses and donkeys along with their masters for their participation in the rally. The objective of the rally was to deliver animal welfare concept to the owners and farmers, by celebrating WVD. Dr. Grishma Neupane, Dr. Dinesh Tiwary, several paravets and hundreds of farmers and horse owners participated the rally. The vets in the rally emphasized the role of animal welfare and owner's/ farmers' responsibility.

CLOSING CEREMONY of WORLD VETERINARY DAY 2009

The final event for the closing ceremony of WVD was organized by NVA at Bharatpur, Chitwan. Just after the rally, the mass participated the formal closing celebration of WVD. The program started from 11 am and end at 4 pm. Altogether, 450 vets, paravets, students, farmers, political leaders, govt. officials, livestock industrialist, media persons, etc. participated the event.

President of NVA Dr. Adarsha Pradhan chaired the formal session. Minister of Science and Technology Hon'ble Ganesh Shah participated the ceremony as the chief guest while the special guests of the session were Hon'ble Ratneshwor Lal Kaystha, member of National Planning commission and Dr. Shubh Narayan Mahato, Chairman of Nepal Veterinary Council. Other guests were Dr. Neel Prakash Singh Karki, Ex-president of NVA, Mr. Arun Shankar Ranjit, Dy. Director General of Dept. of Livestock Service, Dr. Baikuntha Parajuli, Director, Animal Health Directorate, Dr. IP Dhakal, Campus Chief, Rampur Campus, Rampur.

General Secretary of NVA Dr. Subir Singh delivered the welcome speech to the delegates and guest and also explained the events and aims of WVD. Hon'ble minister lightened the ceremonial candle and also opened the SOUVENIR among all.

The session turn up to wonderful paper presentation session by Dr. S.N. Mahato on "Veterinary Statutory Body of Nepal" and Dr. Baikuntha Parajuli on "Veterinarians and Livestock Farmers - a winning partnership : a road map of success !".

Afterward, the session glows and cheered up to FARMER AWARD session. In this session the leading farmers from all over Nepal were recognized and awarded by "NVA-WVD Farmer Award 2009". The awards were distributed by the chief guest. The name of awarded farmers were:

a. Mr. Sanjeeb Kumar Rai, Kulung Swine Farm, Topgachi, Jhapa : For outstanding performance in swine production.
b. Barahi Integrated Agriculture Farm, Sonapur, Sunsari : For outstanding performance in commercialization of dairy cattle farming.
c. Paudar Nagi Joint Chauri Farming Committee, Paudar, Magyadi : For outstanding performance in production and diversification of Chauri milk.
d. Mr. Jeet Bahadur Rajwar, Telpara, Doti : For outstanding performance in goat farming in hilly remote area.
e. Miss Laxmi Rana, Bharatpur, Chitwan : For outstanding performance in commercialization of poultry farming.
f. Mr. Lekh Nath Dhakal, Rupa Tal fish farming cooperative, Rupakot, Kaski : For outstanding performance in establishing fish farming as national farming commodity and conservation of natural resources.

Similarly, the Hon'ble Minsiter also presented "SRIJANA ROTARY-NVA Award to Sangeet Lamichane, Jagdish Pandeya and Aruna Shrestha for their outstanding academic performance at 6th semester of B.V.Sc. & A.H. program of IAAS, Rampur.

The prize distribution session end with the distribution of TOKEN OF LOVE from NVA to all the chief guests and guests participating the session. Also all the paper presenters were granted with TOKEN of LOVE from NVA. The financial contributors like BCN, Heifer Nepal, AICP, Kantipur Vet, Nibus International were also thanked and recognized with TOKEN of LOVE. Similarly, DLS, NVC, IAAS, AHTCS, NVSA, etc were the major supporting organizations of the events.

At the mid of this session undergraduate students of IAAS, Rampur, played a drama showing the importance of livestock farming in rural community to reduce poverty, unemployment and malnutrition problems.

The representatives from the award winning farmer, representative from women farmers' cooperatives and poultry industrialist spoke their views on the occasion of WVD. On wards, the guests, special guests and chief guest spoke to the session. Dr. S N Mahato, chairman of NVC, wished and congratulated all the professionals on the occasion of WVD while Hon'ble Ratneshwor Lal Kaystha, member of National Planning Commission appreciated the works of vets done to the country and assured to make an effective implementation of livestock development plan of Nepal. The chief guest Hon'ble minister congratulated all the vets, para-professionals, farmers and students on the eve of WVD and thanks the organizing committee for invitation. He also remarked that the themes of WVD will certainly buildup winning partnership between veterinarians and livestock farmers, the effort of which will lead the livestock development in the country.

Finally, Dr. B N Devkota, president of NVA-Chitwan Chapter delivered vote of thanks and Dr. A. Pradhan, president of NVA and Chairman of the session stated his closing remarks. The gathering turned to reception lunch to all the participants after completion of formal program.

Declaration of the WVD Award

The 2009 Joint WVA/OIE World Veterinary Day Award was decided on April 30, 2009 and presented on May 29, 2009 to Nepal Veterinary Association. The declaration of WVD '09 award lead NVA at international veterinary professional arena. The presentation was made during the 77th Annual General Assembly General Assembly of the World Animal Health Organisation (OIE) in Paris. OIE deligates of Nepal Dr. Prabhakar Pathak (Director General of DLS/Nepal) received the WVD 2009 award on behalf of NVA at Paris Assembly. Nepal Veterinary Association won the $1,000 award for best promoting the theme "Veterinarians and livestock farmers, a winning partnership."

Around 600 participants, representing OIE Members and intergovernmental (FAO, WHO, World Bank, OMC, etc.), regional and national organisations, took part in the event. High-ranking authorities, including a numerous Ministers of OIE Members, honoured the Assembly with their presence.

Conclusion

Nepal Veterinary Association celebrated more than 12 remarkable events on the occasion of World Veterinary Day 2009 with full endeavor and potentiality, which ultimately result to win the WVD'09 award. All the above events and celebrations were published at national newspapers, aired by Kalika FM, Synergy FM, Hamro FM, Chitraban FM, Tribeni FM and Kantipur National TV and Avenews Channels broadcasted a special report and news coverage on WVD. We, the Nepalese vets, are proud to compete this global competition and awarded with international token. NVA heartily thanks all its members for all the hard work performed to make the history of such a great magnitude.

Special Events of VETCON'10

New Executive Committee

President:	**Dr. Adarsha Pradhan**
Vice President:	**Dr. Bimal Kumar Nirmal**
General Secretary:	**Dr. Subir Singh**
Secretary:	**Dr. Sital Kaji Shrestha**
Treasurer:	**Dr. Rajani Pradhan**
Editor in-Chief:	**Dr. Doj Raj Khanal**
Central members:	**Dr. Pradip Chandra Bhattarai**
	Dr. Hari Prasad Suwal

Regional Members:

Dr. Udaya Pratap Singh	***Eastern***
Dr. Chandra Dhakal	***Central***
Dr. Grishma Neupane	***Western***
Dr. Dirgha Nath Dungana	***Mid Western***
Dr. Mahesh Raj Bista	***Far Western***

Special Events of VETCON'08

NVA National Service Award

1.	Dr. Narayan Basel
2.	Dr. Narayan Prasad Ghimire
3.	Dr. Bishnu Kumar Shrestha
4.	Dr. Bhim Nath Chaulagai
5.	Dr. Bhagelu Yadav
6.	Dr. Mahant Yadav
7.	Dr. Munilal Chaudhary
8.	Dr. Misiri Mandal

LIFE TIME ACHIEVEMENT AWARD

1. Dr. Sundar Lal Shrestha for Animal Breeding
2. Dr. Khadga Prasad Dhakal for Bacteriological investigation

NVA NATIONAL AWARD

1. Dr. Dhan Bahadur Singh for equine medicine
2. Dr. Narayan P. Ghimire for control and containment of Bird flu

NVA EXCELLENCE AWARD

1. Dr. Govind Tandon, Chairman, Society of prevention of cruelty to animal (SPCA) for inspring work on animal welfare
2. Mr. Jyoti Bania, General Secretary, Forum for protection of Cosumer's Rights Nepal for Dedicated legal service for the implementation of slaughter house and meat inspection act

NVA ENTERPRENEUR AWARD

1. Dr. Guna Chandra Bista, Former Chairman, Nepal Hatchery Associtin for Pioneer work on hatchery industry development in Nepal
2. Mrs. Gita Devkota, Chitwan for Promotional work on Bee-Keeping
3. Mr. Sumit Kedia, Chairman, Sita Ram Gokul Milk Pvt. Ltd for Promotional work on Dairy industry
4. Mr. Bhim Bahadur Tiliza, Shikha Pauda, Myagdi for Initiative in dairy animal farming
5. Mr. Bishnu Bahadur Thapa for Promotional work in aquaculture
6. Mr. Upendra Rijal for Promotional work in feed industries

NARENDRA MEMORIAL TRUST AWARD (Vegetarian)

Dr. N. P. Singh Karki for Eradication of foot rot in Nepal

NARENDRA MEMORIAL TRUST AWARD (For veterinary students)

B.V.Sc & A.H.Topper

1. Dr. Sangeet Lamichhne, 2007 IAAS
2. Dr. Jagadish Pandeya, 2008 IAAS
3. Dr. Sundar Thapa, 2007 HICAST
4. Dr. Sheeva Bhattarai, 2008 HICAST

NVA-ROTARY-SHRIJANA AWARD

1. Sangeet Lamichane, Jagadish Pandeya and Aruna Shrestha

PASUDHAN KAUSAL AWARD

1. Dr. Kamal Prasad Gairhe for his recognition by International Scientific Society in naming crocodile nematode ***Proctocaecum gairehi***, after his name.

PASUDHAN BIKRAM AWARD

1. Central Biological Production Laboratory, Tripureshwor for Production of New Castle (ND-I2) Disease heat resistant vaccine for village chicken.

DR. SUNDAR MOHAN ACADEMY AWARD

1. **Dr. Mukul Upadhyaya**

Congratulations to all of you

Policy Papers

STATUS AND STRATEGIES FOR THE CONTROL OF MAJOR INFECTIOUS DISEASES OF LIVESTOCK IN NEPAL

Khatiwada. R. K[1], Ghimire. N. P[1], Thakuri. K. C[2] and Karki. S.[2]

[1]Directorate of Animal Health
[2]Veterinary Epidemiology Center

ABSTRACT

Infectious diseases have been considered as the major threat of livestock industry in Nepal. The epidemiological status and trend of major infectious diseases of livestock has been analyzed using secondary data from year 2000 to 2009, obtained at Veterinary Epidemiology Center from all 75 District Livestock Services Offices.

Foot and Mouth Disease (FMD), Peste des petits ruminants(PPP), Classical Swine fever, Haemorrhaghic Septicaemia and Black Quarter(BQ) were found to be most frequently occurring five infectious diseases among the top ten reported animal diseases. Emerging epidemics like highly pathogenic avian influenza (HPAI) with serious consequences to food security, food safety, public health and trade owing to huge socio-economic impact had been observed as constant threat in the recent years.

Appropriate control strategies including the need for public private partnership, regional cooperation, harmonization and capacity building of National Veterinary Services as per OIE guidelines has been recommended for prevention and control of infectious diseases of livestock in Nepal.

Key Words: Infectious diseases, Food Safety, National Veterinary Services.

INTRODUCTION

Nepal is a mountainous country, which shares border with Tibet of China on North and India on East, West, and the South. The total land area of the country is 147,181 square kilometers. There are 25.88 millions human populations in the country with 2.25% growth rate per annum. About 65.6% people in the country are engaged in agricultural farming. The natural forest in the country constitutes about 39%. Physically, country is divided into following three regions:

- **Mountainous region:** Mountainous region is located in the north part of the country, covering 35% (51817 Sq. Km.) of total area. The altitude of the region varies from 4800 m and above sea level (asl). Yak/Nak, sheep, alpine goats (Chyangra) and mule rearing form the way of life of people in this region.

- **Hilly region:** This region extends from east to west which is located in the middle part of the country, covering about 42% (61345 Sq. Km.) area. Hilly region ranges from 300 to 4800 m asl. People of divergent ethnic groups, caste and cultures share their common way of living. Agro-based livestock industries and horticultural production in the region are the main source of income of the people.

- **Terai region:** Terai region is located in the southern part of the country, covering about 23% (34019) Sq. Km. of the total area. This region serves as a main source of food supply to other region of the country.

Importance of Livestock

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 32.0% to Agricultural Gross Domestic Products (AGDP). It has been envisaged that the contribution of livestock to AGDP be reached from 32% to 45% by the end of 2014/15 (APP, 1995).

Livestock Population

Although, subsistence level of livestock farming is predominant in the rural areas of the country, commercialization has however, been rapidly coming up in the peri urban areas with the sharp increase in the number of cross bred livestock population. A detailed livestock population is summarized as follows:

Animal population of Nepal

Species	**Population**	**Growth rate**
	(millions) 2007/08	
Cattle	7.09	0.57
Buffalo	4.49	3.80
Goats	8.13	5.70
Sheep	0.81	-3.10
Pig	1.01	2.90
Poultry	24.67	3.02
Ducks	0.39	0.51

Source: Agri-Business Promotion and Statistics Divion 2007/08, MOAC, Nepal

Overview of Animal Health Situation

Prevalence of a number of infectious and parasitic diseases in livestock population is the major constraints affecting livestock production and productivity resulting into substantial economic loss in livestock industry of Nepal. National veterinary services of Nepal with its limited resources have continuously been putting its efforts for prevention and control of livestock and poultry diseases right from its establishment. There is a network of national veterinary services at central, regional and district levels. Under the Department of Livestock Services (DLS), Directorate of Animal Health is assisted by its different units at central level. Veterinary services at regional and district levels are being delivered through five Regional Directorates of Livestock Services, five Regional Veterinary Laboratories and one National Avian Disease investigation Laboratory, eight Animal Quarantine Offices with 24 Animal Quarantine Check posts, 5 Regional Livestock Training Centers and 75 District Livestock Services Offices. Moreover, there are 359 Livestock Service Centers and 640 Livestock Sub-Service Centers to provide veterinary services at the sub-district level. They deliver animal health, breeding, nutrition, training and extension services to the livestock farmers.

Nepal joined World Animal Health Organization (OIE) in 1996. Nepal became 147th member of WTO in 2004. The importance of national veterinary services has been well recognized in the process of getting WTO membership. The national veterinary services have to fulfill a series of obligation in the context of OIE/WTO membership.

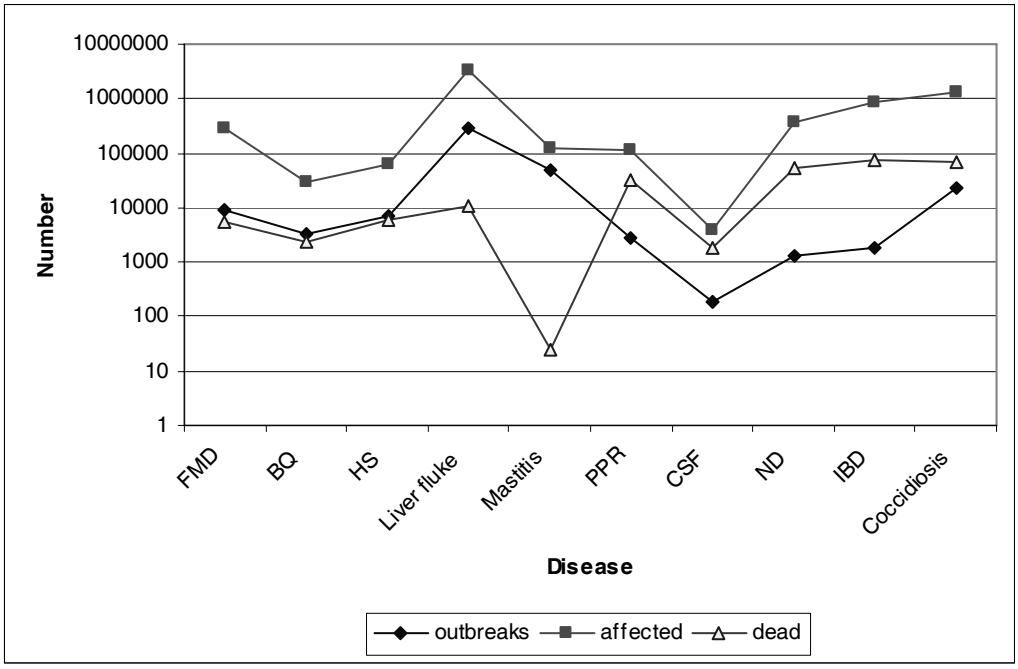
MATERIAL AND METHODS

The monthly epidemiological reports received at Veterinary Epidemiology Center from all the District Livestock Services Offices from 2000 to 2009 A.D were analyzed to have an overview of important animal diseases trend in Nepal from the following perspective.

1. Year-wise trend of cases of important animal diseases.
2. Month wise distribution of cases of important animal diseases.
3. Eco-zone wise distribution of cases of important animal diseases.

Control methods have been proposed based on epidemiological findings and literature review.

RESULTS AND DISCUSSION



Animal Health Situation Compiled (Number Affected and Dead from Important Livestock Diseases) (2000-2009)

Table 1: Status of Important Livestock Diseases in the Hills (2000-2009)

S.No	Disease	Outbreaks	Affected	Death	Case Fatality
1	FMD	4122	150174	2688	1.79
2	HS	2584	23843	2168	9.1
3	BQ	970	14606	1189	8.1
4	Liver fluke	143829	1854587	5696	0.3
5	PPR	797	29140	5794	19.9
6	Mastitis	25503	65639	8	0.01
7	CSF	122	813	422	51.9
8	IBD	1234	429697	41512	9.7
9	Coccidiosis	11022	559973	36619	6.5
10	ND	475	80477	21100	26.2

Table 2: Status of Important Livestock Diseases in the Mountain (2000-2009)

S.No	Disease	Outbreaks	Affected	Death	Case Fatality
1	FMD	1897	57604	1032	1.8
2	HS	955	11275	444	3.9
3	BQ	747	8555	346	4.0
4	Liver fluke	47112	559495	2795	0.49
5	PPR	279	28631	13972	48.8
6	Mastitis	5460	14569	3	0.02
7	CSF	0	0	0	0
8	IBD	94	4104	542	13.2
9	Coccidiosis	4689	82885	4154	5.01
10	ND	305	17771	5638	31.7

Table 3: Status of Important Livestock Diseases in the Terai (2000-2009)

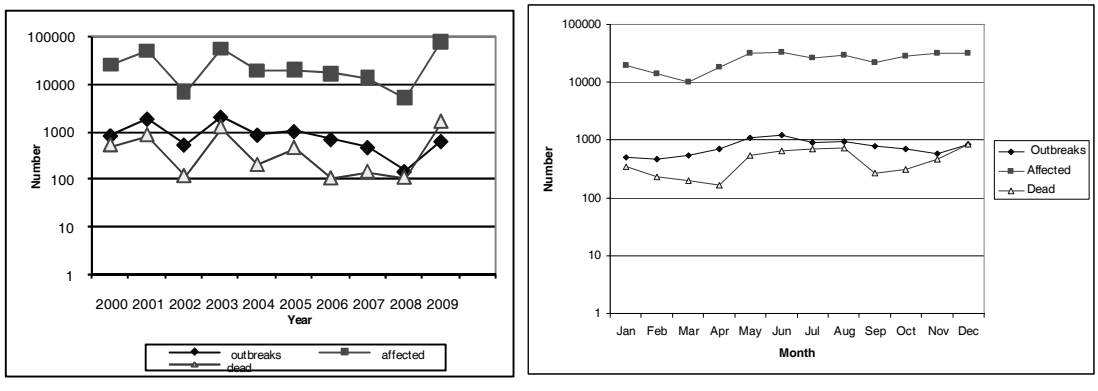
S.No	Disease	Outbreaks	Affected	Death	Case Fatality
1	FMD	3262	89485	1717	1.91
2	HS	3621	26334	3574	13.57
3	BQ	1526	7016	733	10.44
4	Liver fluke	97668	942597	2075	0.22
5	PPR	1828	56991	13273	23.28
6	Mastitis	19131	48361	13	0.02
7	CSF	61	3046	1399	45.92
8	IBD	482	421105	32299	7.67
9	Coccidiosis	6834	646563	27516	4.25
10	ND	539	260878	25507	9.77

Table 4: Ecozone-wise Case Fatality Rate due to important livestock diseases (2000-2009)

S.No	Disease	Case Fatality		
		Hills	Mountain	Terai
1	FMD	1.79	1.8	1.91
2	HS	9.1	3.9	13.57
3	BQ	8.1	4.0	10.44
4	Liver fluke	0.3	0.49	0.22
5	PPR	19.9	48.8	23.28
6	Mastitis	0.01	0.02	0.02
7	CSF	51.9	0	45.92
8	IBD	9.7	13.2	7.67
9	Coccidiosis	6.5	5.01	4.25
10	ND	26.2	31.7	9.77

In all three eco-zones, FMD, HS and BQ were the major infectious diseases affecting large ruminants while PPR and CSF are the major infectious diseases affecting small ruminants and swine respectively. In Poultry, ND, IBD and Coccidiosis are major problem.

1. Foot and Mouth Disease



(a) (b)

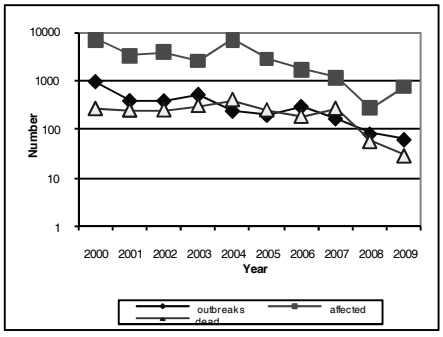
Fig. 2: Year-wise (a) and Month-wise (b) prevalence of Important Livestock Diseases (2000-2009)

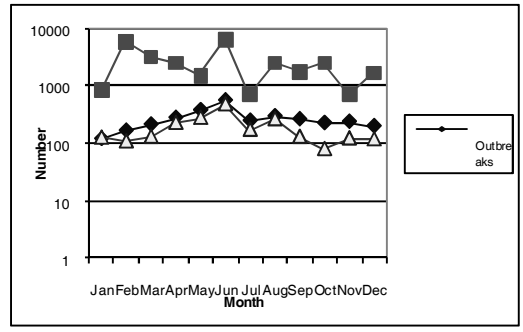
Year-wise trend of FMD shows that the highest number of outbreaks was recorded in the year 2003 (fig 2.a) with more than 2000 outbreaks throughout the country followed by around 1900 outbreaks in

2001. But in terms of number of animal affected, year 2009 was highest with more than 80,000 animals affected with only 600 plus outbreaks compared to around 57000 in 2003 and around 51000 in 2001. The number of death due to FMD was also highest in 2009. The emergence of Panasia 2 strain might be the cause for higher number of cases and subsequent death in 2009.

Month-wise pattern of FMD shows (fig 2.b) that this disease is prevalent throughout the year. However, there are some more incidences in the month of May and June and later on November and December. The greater animal movement owing to the agricultural activities during those months might be the reason for the spread of disease.

Fig 3. 2: Black Quarter



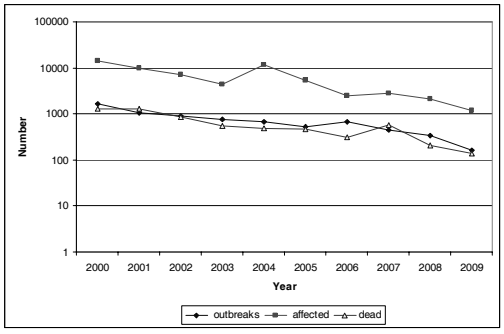


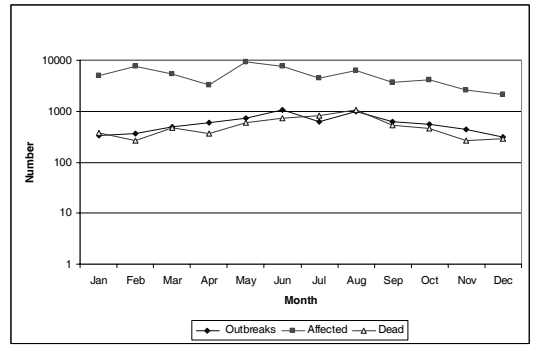
(a) (b)

Year-wise trend of BQ shows (fig 3.a) that the highest number of outbreaks was recorded in the year 2000 with more than 900 outbreaks followed by around 500 outbreaks in 2003. Since then the disease is in decreasing trend.

Month-wise pattern of BQ shows (fig 3.b) that this disease is prevalent throughout the year. However, there are more outbreaks in the month June compared to other months.

Fig 4. 3: Hemorrhagic Septicemia

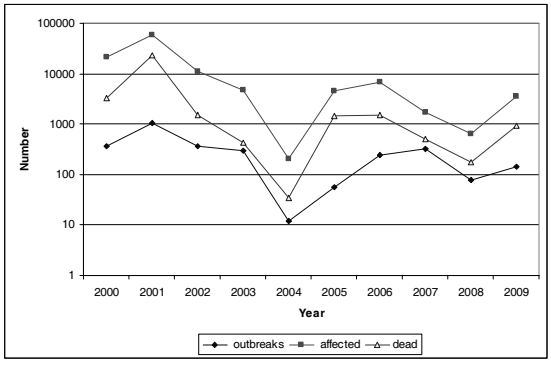




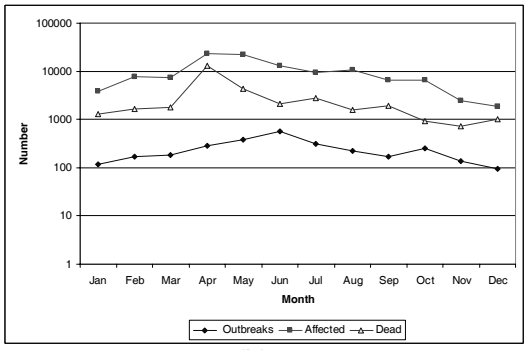
(a) (b)

Year-wise trend of HS shows (fig 4.a) that the highest number of outbreaks was recorded in the year 2000 with more than 1600 outbreaks throughout the country affecting more than 14000 animals and more than 1300 deaths followed by around 1000 outbreaks in 2001. The disease seems in decreasing trend but nevertheless on an average hundreds of outbreaks are still recorded annually.
Month-wise pattern of HS shows (fig 4.b) that this disease is prevalent mostly during the rainy months i.e. May to July however disease incidence has been recorded throughout the year.

Fig 5. 4: PPR



(a)

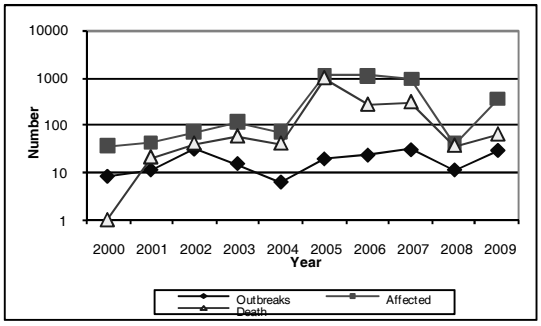


(b)

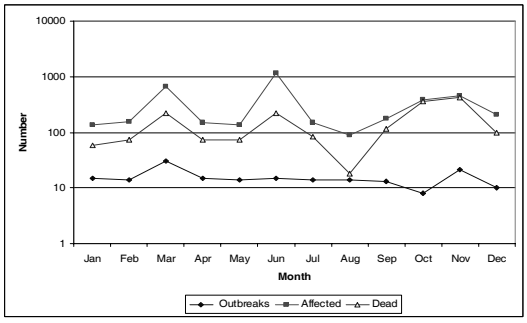
Year-wise trend of PPR shows (fig 5.a) that the highest number of outbreaks was recorded in the year 2001 with more than 1000 outbreaks affecting around 60000 sheep goats and more than 23000 deaths (i.e 38.81% of the affected). The number of outbreaks rapidly declined after 2002 until 2004 with only about 100 outbreaks annually and concentrated in only few districts. Since then the disease has shown somewhat erratic trend but the number of outbreaks and case fatality rate is far less compared to before 2001 situation. Intervention by National PPR Control Program might have reduced the incidence of PPR.

Month-wise pattern of PPR shows (fig 5.b) that this disease is prevalent during the months of May and June. However the outbreaks are recorded throughout the year.

Fig 6. 5: Classical Swine Fever



(a)



(b)

Year-wise trend of Classical swine fever shows (fig 6.a) that the highest number of outbreaks was recorded during the 2006 to 2008 and around 1000 pigs are affected annually. Case fatality rate for this disease is high i.e around 48%.

Month-wise pattern of CSF shows (fig 6.b) that this disease is prevalent during the months of March and June. However the outbreaks are recorded throughout the year.

ECONOMIC ANALYSIS

Detailed economic analysis needs to be undertaken to know the exact magnitude of the loss caused due to livestock diseases. However, looking at the huge number of animals dying and being sick from number of infectious and non-infectious diseases, one can easily infer that the economic loss is enormous and needs immediate attention. For some diseases, the loss caused due to that particular disease might be low. It might not be a big deal for the country but for those individual farmers, that might worth a lot. In addition to the economic loss, its social implication is also very high.

RECOMMENDATIONS

From the above review, following recommendations can be made:

1. National Veterinary Services as per OIE guidelines: The structural limitations of the Department of Livestock Services are one of the hurdles for combating livestock diseases. So re-structuring of the national veterinary services as per OIE guideline is necessary to obtain positive results.

2. Public private partnership approach: This approach is necessary to effectively control the livestock diseases. A guideline should be developed to implement this approach.

3. Legislation and capacity building: Inadequate legislation and capacity has impeded the effective control of the livestock diseases. So necessary legislation need to be formulated with improvement in the capacity of veterinary services.

4. Risk based disease control approach: Risk analysis should be done to know the possible threats due to particular disease. This will also enable the veterinary services to know where the major thrust should be given. Detailed economic analysis of the livestock diseases also should be done by multi-disciplinary team.

5. International co-operation: Trans-boundary diseases cannot be controlled without regional and international co-operation. So, necessary regional and international co-operation should be done to effectively control the TADs.

6. One Health Concept: There are number of diseases that are shared between humans and animals. Accepting this fact, world is moving forward with the concept of one health. This concept should be materialized in Nepal also.

CONCLUSION

The analysis of the epidemiological status and trend of major infectious diseases of livestock of Nepal shows that infectious diseases have been the major threat of livestock industry in Nepal.

Foot and Mouth Disease, Peste des petits ruminants, Classical Swine fever, Haemorrhaghic Septicaemia and Black Quarter were found to be most frequently occurring infectious diseases which have huge socio-economic impact.

Appropriate control strategies including the need for public private partnership, regional cooperation, harmonization and capacity building of National Veterinary Services as per OIE guidelines have been recommended for prevention and control of infectious diseases of livestock in Nepal.

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FOOD SAFETY AND GLOBAL CONCERN

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ABSTRACT

Urbanization, globalization and terrorism have brought the need for a stronger, larger, more diverse, and more competent public health workforce to the forefront of public planning. The present health security challenges include zoonotic diseases, food or waterborne illnesses and bioterrorism.

The OIE and its Member States believe that the concept of veterinarians as professionals who are only concerned with animal diseases should be broadened to include areas of activity that focus on public health outcomes, the control of risks along with the food chain, as well as the welfare of animals.

The OIE considers Veterinary Services to be a Global Public Good and their bringing into line with international standards (in terms of legislation, structure, organization, resources, capacities, the role of the private sector and paraprofessionals) as a public investment priority. The official agreement signed by the OIE and the World Bank in 2001 supports this view.

There has been a renewed focus on the important relationship between public health and veterinary medicine for improving human health, animal health and food safety. Because veterinarians work at the interface of human, animal and environmental health, they are uniquely positioned to view health through the lens of public health impact. Changes in land use, creation and operation of large terrestrial and marine food production units and microbial and chemical pollution of land and water sources, have created new threats to the health of both animals and humans (Zinsstag, Schelling, Wyss, & Mahamat, 2005).

In Nepal, very scanty information is available on the incidence which is being reported either as gastro-enteritis or typhoid and others in a vague term. The study done by Thakur, (2009) in this sector indicated that 22% of poultry meat samples were found positive for Salmonella. Among them 11% was S.enteritidis; 5% S. pullorum; 4% S.gallinarum and 2% S. typhi (Thakur, 2009).

Antibiotic residues are equally alarming in food chain. A study conducted in milk samples in Kathmandu Valley has indicated that 10.66% were positive for antibiotics (Maharjan, 2009).

INTRODUCTION

Approaches to food safety have evolved in recent decades from traditional controls based on good practices via more targeted food safety systems based on hazard analysis and critical control points (HACCP) to risk-based approaches using food safety risk analysis. Food safety and quality are best assured by an integrated, multidisciplinary approach, considering the whole of the food chain. Food borne illness affects 6-80 million people per year and causes 9,000 deaths per year. The cost involved in the treatment, prevention and control of these diseases is estimated about $5 billion per year.

According to the World Health Organization (WHO) about 70% of the 2 million deaths per year from diarrhea in developing countries are related to contaminated food. More than 200 known diseases are transmitted through food include viruses, bacteria, parasites, toxins, metals, and prions. Symptoms of illness range from gastroenteritis to neuralgic, hepatic and renal syndromes depending upon the nature of the organism.

Common causes of Food borne Infections

Many types of organisms are identified to cause food borne infections. Among them the most concerned organisms are Salmonella spp., *Campylobacter jejuni, Listeria,* Hepatitis A virus. But some

unusual issues of HPAI, BSE/ TSE are also coming up in these days. The source and common symptoms of diseases caused by these organisms are briefed as below:

Campylobacter jejuni :

Sources: raw poultry, unpasteurized milk
Symptoms: Diarrhea, nausea, vomiting and may last for 7-10 days

Listeria

Sources: raw milk, raw seafood, soft cheeses
Symptoms: 7-30 day onset; miscarriage, sepsis, meningitis

Salmonella

Sources: raw or undercooked poultry, eggs…
Symptoms: 6-48 hr onset; fever, chills, vomiting, abdominal cramps, diarrhea.

Hepatitis A virus

Sources: Undercooked or raw shellfish, human contact
Symptoms: 15-20 day onset; hepatitis, tiredness, nausea and vomiting

Highly Pathogenic Avian Influenza (HPAI)

HPAI Virus is considered to be circulating in Southeast Asia since 2003. In the globe, it has killed 292 humans. Due to its outbreak more than 260 million birds were killed or forced to culling and caused economic loss of about $20 billion and devastated livelihoods at the family-farm level

Situation of food borne diseases in Nepal

In Nepal, very scanty information is available on the incidence of food borne disease which is being reported either as gastro-enteritis or typhoid and others in a vague form. Antibiotic residues are equally alarming in food chain. Adverse immediate effect of such antibiotics may be anaphylactic reaction and /or allergy, whereas delayed effect may be drug resistance, treatment failure or impart on immune system in the humans. Maximum residue limit (MRL) provided by CAC (2006) recommends that the level for tetracycline, sulphonamides and aminoglycosides group for muscle should be 200 µg/kg, 100 µg/kg and 50 µg/kg respectively. But VSDAO (2006) found 86.88% positive samples for tetracycline residue ranging from 1 mg/kg to 6.4 mg/kg and 64% for aminoglycoside and macrolid residue ranging from 0.25 mg/kg to 32 mg/kg in the poultry samples during study.

DFTQC (2007) in Kathmandu conducted a test on 26 samples of poultry meat for tetracycline residue .The residue detected in the different organs were 46 %,12%,53%,46%, and 15% in muscles, skin, gizzard, liver and heart respectively. Out of 50 milk sampled screened in Kathmandu valley, Sedhain (2008) found that 5(10%) were positive for residue of sulphonamide group and 1(2%) for tetracycline group. Yet in another study conducted in 150 milk samples from Kathmandu valley, 12% samples were found to be positive for penicillin and 5.3% for sulfonamides (Thapaliya, 2008). Similarly, a study conducted in milk samples in Kathmandu Valley has indicated that 10.66% were positive for antibiotics (Gautam, 2008). Likewise, out of 75 meat samples for tetracycline residue (Group A) and aminoglycoside, macrolid and sulphonamide residue (Group B), 16% samples were found positive for antibiotic residue (Dhakal, 2008). Thakur (2009) also indicated that 22% of poultry meat samples were positive for *Salmonella spp* of which 11% were *S. enteritidis*; 5% *S. pullorum;* 4% *S. gallinarum*; 2% *S. typhi.* Furthermore, Maharjan (2009) found that 11.9% poultry meat samples were infected with *Salmonell* spp.

Zoonotic diseases prevalent in Nepal

According to the epidemiological report prepared by Veterinary Epidemiology Center, Kathmandu, many types of animal diseases are identified in Nepal. In relation to the food borne disease followings are the important ones:

- Anthrax
- Avian influenza
- Brucellosis
- Hydatidosis
- Japanese Encephalitis
- Neurocysticercosis

- Rabies
- Swine flu
- Toxoplasmosis
- Viral influenza

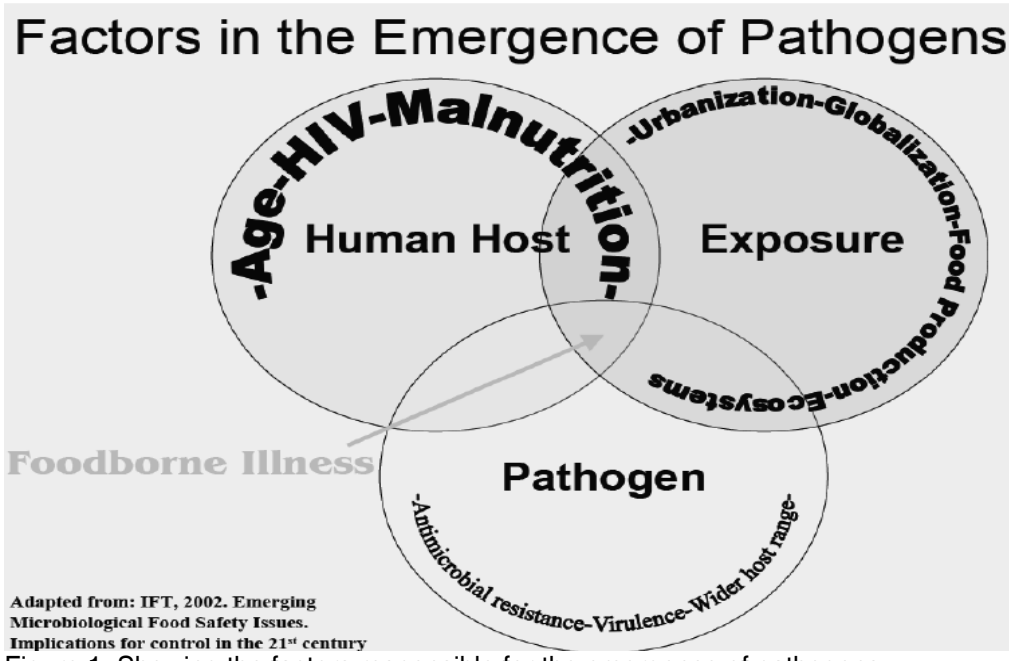

Figure 1: Showing the factors responsible for the emergence of pathogens.

Food Safety in Developing Countries

Recognition of importance of food safety is very recent in the developing countries involved for the food safety is listed below. Some of the major factors listed below:

- Consumer awareness is very little
- Fragmented industry
- Majority of food processing units are in small and unorganized sector
- Food handlers are not well trained
- Diversity of food products / cuisines and food habits
- Inadequate laboratory and testing infrastructure
- Traditional practices of street food, carrying and storage etc.
- Enhanced food safety in the country should provide opportunities for additional value-added agri-food exports
- Food safety is no longer a national issue. It will become an increasingly important global issue.
- Nepal can play an important role by enhancing its food safety capacities which in turn will bring additional benefits.
- The food safety programs offer opportunities for human capital formation through food safety education and training.

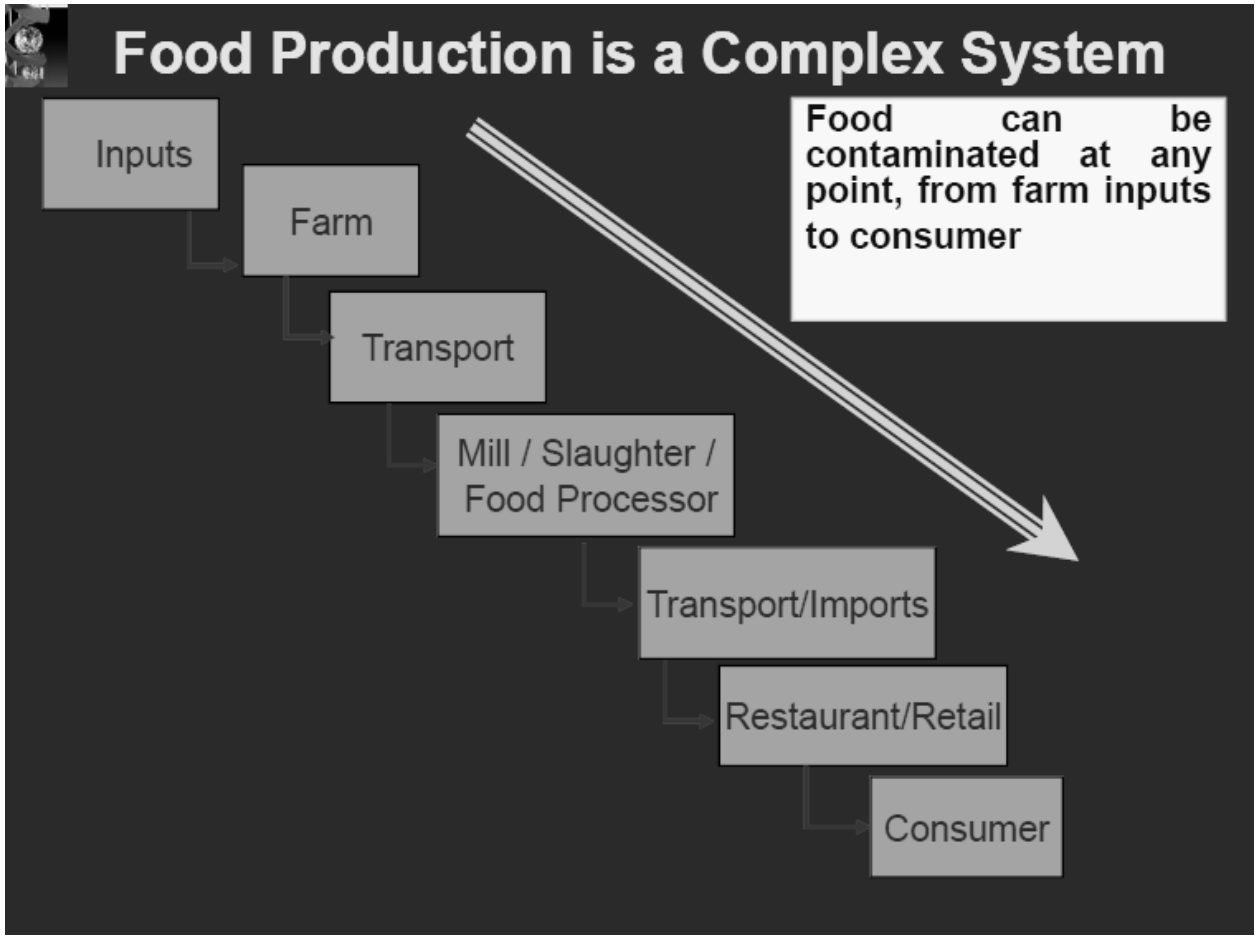

Figure 2: Showing a typical food chain

Regulatory Framework in Nepal for food safety

There are some legal frame works in place. They are in scattered as well as in impractical forms due to very old versions and are not addressing the concerns raised by WTO-SPS agreements, OIE guidelines. Some important animal production policies and Veterinary drug act, Animal welfare acts, etc. are not in place. Prevailing acts / regulations are waiting for amendment as there are plenty of lacunas in them in the present context. Veterinary paraprofessionals are not covered within the Veterinary Statutory Body. Some acts related to food safety and quality and implemented by the departments are as below:

Department of Food Technology and quality control:
Food Act, 1966 and regulation, 1970
Feed Act

Department of Drug Administration:
Drug Act, 1978 (Both for Human and Vet Drugs) and Regulation, 1979

Department of livestock services:
Animal Health and livestock services Act 1998 and Regulation, 1999
Slaughterhouse and Meat Inspection Act, 1998 and Regulation, 2001
Nepal Veterinary Council Act, 1998 and Rules, 2000

By Others:
Consumer Protection Act

THE ROLE OF VETERINARY SERVICES IN THE FOOD CHAIN

Veterinary service can play significant roles in the entire food chain. Among them some of such roles can be listed as below:

1. Application of WTO-SPS-TBT measures
   e.g. control and eradication of animal, bird, fish disease on line of OIE Guidelines, application of related laws/vet inspection and quarantine and disseminate the information to stakeholders.
2. Care in the primary production of livestock and poultry.
3. Food animal transportation/ Animal welfare
4. Application of code of hygienic practice in animal feed and meat industry including meat inspection and supervision. (As per Codex Alimentarius Vol. 10, FAO series 160)
5. Veterinary drug control/ antimicrobiol drug residue control to protect human health (As per WHO TRS 939, 2008)
6. Effective implementation of quarantine control (i.e. cross border trade of animals or foods from animal origin.
7. Promote organic farming 8. Application of disease traceability system (On the line of FAO Animal Health and Production manual, 2008), farm registration, identification of animals, GVP, GLP, etc.
9. Investigation of foodborne outbreaks all the way back to the farm and in formulating and implementing remedial measures once the source of the outbreak has been identified.
10. *Veterinarians* are well equipped to assume important roles in ensuring food safety in other parts of the food chain, for example through the application of HACCP based controls and other quality assurance systems during food processing and distribution. In the Prevention and Management of Food borne Hazards, veterinarian has following roles:
    - The basic training of Veterinarians covers nearly all aspects of food hygiene, food processing, pharmaceuticals and pathogenic agents which may be present in foods of animal origin.
    - The presence of a Veterinarian on the farm is the key to an integrated approach and will ensure that animals and animal products sent to slaughter-houses or dairies are free from disease.
    - The movement of animals causes stress to them, which consequently affects the safety and the quality of the meat.
    - The Veterinarian will also supervise the implementation of Goos Hygienic Practices (GHP), GMP, the HACCP, samplings for the residues control, the microbiological testing of carcasses and the general sanitation of the slaughter house.

Relevance of Veterinary Input

During the last few decades, countries with surveillance on food borne diseases have reported significant increases in the incidence of diseases due to biological hazards in food of animal origin (figure 1). The factors which contribute to the increase of biological hazards in foods are related to:

- Changes in farm practices
- Intensive rearing of animals
- Increase in consumption of meat
- Globalization of food trade
- Urbanization
- Changes in lifestyles
- International travel
- Preference for fresh and undercooked fast foods
- Eating of food prepared outside the home
- Demographic changes with increased proportion of old and immunosuppressed people
- Environmental pollution from unsafe disposal of animal manure, inedible animal products etc.

Veterinarians, due to their ability to link the health of the animal and human populations, are in an ideal position to address the concerns. They possess the broadest combination of knowledge and skills in the interdisciplinary '**stable to table"** or "*GOTHA DEKHI OTHA SAMMA"* public health team.

THE RISK BASED APPROACH:

Food safety issue is very complex and for guarantee of safe food science based methodologies needs to be applied. There are four generic pillars of risk analysis viz: **Hazard**, **Risk Assessment, Risk Management and Risk Communication.**

Risk Assessment: Risk assessment can be a qualitative or quantitative estimation of a risk to a group of people. It is based on a four-step procedure. Namely,

- ***hazard identification,***
- ***hazard characterization*** **(dose/response),**
- ***exposure assessment,***
- ***risk characterization***

and estimation of an overall probability of consumption and the severity of health effects in a given population of consumers

Risk Management
Risk management is strongly linked into the food safety policy and alternative policy to shoot-out the fore-coming risk and the definition of the appropriate level of consumer protection. It involves the development of strategies and the selection and implementation of appropriate control actions necessary to ***prevent, reduce or eliminate*** the risk to ensure the decided level of health protection.

Risk Communication
This is interactive communication among all stakeholders to eliminate or reduce the risk.

VETERINARY SERVICE AND ITS FUNCTION

To guarantee a high level protection of consumers from food borne diseases there is an urgent need to integrate the ***feed production, farming, transport of animals and slaughtering, primary and secondary processing, storage, distribution, sale, cooking and serving of foods*** in a Quality Assurance System, which will link the entire chain of food production from animal breeding and feeding until the time the food is placed on the table of the consumer. For this

- Veterinary Services and veterinarians, have a leading role in the safety of foods of animal origin.
- The adoption of the integrated approach from the ***गोठदेखि ओठसम्म***, and the introduction of quality assurance systems through the entire production process which is a new challenge for the Veterinary Services and the veterinary profession in general (figure 2). Veterinarians should seek and accept responsibilities for developing a new quality-oriented procedure. The process must be designed to deliver the highest levels of food safety guarantee for animal production, the food industry and the consumer.

Functions of Veterinary Services
The Veterinary Services should achieve its objectives through the direct performance of some veterinary tasks and the auditing of animal and public health activities conducted by other government agencies, private sector *veterinarians* and other stakeholders. Where veterinary or other professional tasks are delegated to individuals or enterprises outside the *Veterinary Authority*, clear information on regulatory requirements and a system of checks should be established to monitor and verify performance of the delegated activities. The *Veterinary Authority should* retain the final responsibility for satisfactory performance of delegated activities.

The *Veterinary Services* should

- Play a key role in ensuring that animals are kept under hygienic conditions and in the early detection, surveillance and treatment of animal diseases, including conditions of public health significance.
- Provide Technical support to both private *veterinarians* and employees of the *Veterinary Authority.*
- Play a central role in ensuring the responsible and prudent use of biological products and veterinary drugs, including antimicrobials, in animal husbandry

- The *Veterinary Services* also play an important role in raising the awareness of food producers, processors and other stakeholders to adopt measures required to assure food safety.
- There should be a clear and well documented assignment of responsibilities and chain of command within the *Veterinary Services*.
- The national *Competent Authority* should provide an appropriate institutional environment to allow the *Veterinary Services* to develop and implement the necessary policies and standards and adequate resources for them to carry out their tasks in a sustainable manner.
- In developing and implementing policies and program for food safety the *Veterinary Authority* should collaborate with other responsible agencies to ensure that food safety risks are addressed in a coordinated manner.

Provisions in Codex Alimentarius

Followings are the provision made in Codex Alimentarius Vol. 10, FAO /WHO for the food safety and quality:

- "Veterinary science and the sciences of meat hygiene should be applied throughout the food chain, starting at the farm of origin, so that fresh meat produced from slaughtered animals is safe and wholesome.
- This code, together with the code for ante- mortem and post- mortem inspection of slaughter animals and for ante- mortem and post- mortem judgment of slaughter animals and meat, describes requirements necessary to achieve that goal."
- Section I of the code explain that "This code of hygiene practice applies to fresh meat, other than commodities covered by other codes, namely poultry, fish and game, intended for human consumption, whether sold directly to the consumer in that form or after further processing. It contains minimum requirements of meat hygiene up to and including the transport of meat. This code should be read in conjunction with the code for ante mortem and post-mortem inspection of slaughter animals and for ante mortem and post- mortem judgment of slaughter animals and meat".
- The Section III part 6 has explained that "the controlling authority means the official charged by the government with the control of meat hygiene, including meat inspection". Similarly, Section–III Part 14 has defined the fresh meat as "meat that has not yet been treated in any way other than by modified atmosphere packaging or vacuum packaging to ensure its prevention, except that if it has been subjected only to refrigeration, it continues to be considered fresh for the purpose of this code".
- Section III Part 16 says that "The supervision of meat hygiene, including the inspection of meat, should be the responsibility of a veterinary inspector". Likewise, Section III Part 23 says that "safe and wholesome refers to meat that has been passed as fit for human consumption using the criteria that it: (b) does not contain residues in excess of established Codex limits".
- Section-VI says, "All animals should be inspected ante-mortem; A veterinary inspector should have the final responsibility as to fitness for, and any conditions applying to, slaughter of animals for the production of fresh meat".
- Section–IX says, “All aspects of meat hygiene should be supervised by an official veterinarian**.** Each abattoir or establishment and its supervising Veterinarian should have access to laboratory facilities and analytical procedures to support hygienic practices and process control program". Section IX, article 117 further clarifies the roles of veterinarian as *“All meat hygiene requirements in this code should be supervised by an official veterinarian”.*

Meat Inspection

- Slaughter house inspection of live animals (*ante-mortem*) and the carcass (*post-mortem*) plays a key role in both the surveillance network for animal diseases and zoonoses and ensuring the safety and suitability of meat and by-products for their intended uses. Control and/or reduction of biological hazards of animal and public health importance by *ante-* and *post-mortem* meat inspection is a core responsibility of the *Veterinary Services* and they should have primary responsibility for the development of relevant inspection program.

Certification of animal products for international trade

Another important role of the *Veterinary Services* is to provide health certification to international trading partners attesting that exported products meet both animal health and food safety standards. Certification in relation to animal diseases, including zoonoses, and meat hygiene should be the responsibility of the *Veterinary Authority.*

What Needs to Be Addressed?

- Government Authority should recognize fully the necessity of direct contribution of veterinary service to public health policy development.
- The involvement of veterinary expertise at the policy level is necessary to ensure adequate planning, design, implementation and supervision of VPH programs and to ensure appropriate integration and collaboration with human health programs.
- Further, involvement of Veterinary Service is also necessary across all sectors of food safety regulation and public health.

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WILDLIFE HEALTH MANAGEMENT IN NEPAL

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ABSTRACT

Nepal is rich in faunal and floral diversity due to its geographical variation in altitude and climate. Wildlife disease management in free ranging populations is inherently technically difficult and is frequently a contentious ecological issue when dealing with endemic or indigenous diseases in native species. The effects of disease on wildlife populations have been recognized for years. Wildlife may be affected by infectious diseases of bacterial, parasitic, viral, rickettsial, chlamydial and mycoplasmal origin. Efficient control of diseases of wild animals and ensuring a particular animal health status require appropriate diagnostic measures and constant surveillance. Intermingling of wildlife and domestic animals facilitates transmission of pathogens among them that eventually jeopardize conservation efforts. Action plans for conservation of flagship species are implemented to establish their good population but consideration for monitoring disease dynamics and surveillance in wildlife is almost neglected. For safeguarding wildlife and human health, establishment of special wildlife health unit and addressing these issues in the wild animal conservation action plans enhances the conservation efforts of the parent organizations. This will eventually lead to the conservation of human and animal health and endangered wildlife species.

INTRODUCTION

Nepal is rich in faunal and floral diversity due to its geographical variation in altitude and climate and thus is a home to a number of endangered species of animals like Royal Bengal tiger, Greater one horned rhinoceros, Asian elephant, snow leopard, etc. Wildlife disease management in free ranging populations is inherently technically difficult and is frequently a contentious ecological issue when dealing with endemic or indigenous diseases in native species (Nandi and Chauhan, 2006). These diseases are often considered to be one of many selection pressures. During the current period of increasing globalization, the emergence of new diseases of wildlife or reemergence of old diseases have been reported. The world is undergoing rapid ecological change, populated by pathogenic organisms, their vectors and hosts which are capable of equally rapid change. Some of these pathogens may cause significant disease in wild species, but in other cases, the wild animals may serve as reservoirs for pathogens which do not induce overt illness in their wild hosts. Wildlife may be affected by several infectious diseases of bacterial, parasitic, viral, rickettsial, chlamydial and mycoplasmal origin. Efficient control of diseases in wild animals ensures their long term survival but require appropriate diagnostic measures and continuous surveillance.

The effects of disease on wildlife populations have been recognized for years. Tuberculosis has been reported as re-emerging disease of both captive and free-ranging wildlife. Other diseases such as anthrax, pasteurellosis, salmonellosis, and canine distemper have constantly affected wildlife all over the world. Spread of highly pathogenic avian influenza among domestic and wild aquatic birds has even further emphasized continuous surveillance need. The interaction of wildlife and livestock sharing common habitat is an important factor for contracting or transmitting animal diseases that will eventually affect conservation efforts. Viable conservation initiatives can only be designed by addressing the health issues of wildlife. The expertise of all relevant disciplines including health specialists may enhance better management of wildlife and ecosystems, and safeguard public health and related industries.

ACTS AND ACTION PLANS

More than a half dozens of action plans primarily for the conservation of flagship species have been prepared and launched to establish their good population. These are Snow leopard (2004), Tiger (2007), The Greater One-horned Rhinoceros, Vulture (2009), and Elephant Conservation Action plan (2009). These action plans are implemented with great efforts to maintain viable populations of the stated wildlife, minimize habitat fragmentation, resolve conflict with resident communities and thus,

maintain ecosystem integrity. They have also addressed particularly for tigers to preserve, recognize, and increase the effective land base that supports tigers in Nepal in order to maintain a viable tiger population. Vulture action plan emphasizes protection from extinction through better understanding of the ecological importance, ensuring re-introduction, safe food supply, maintenance of suitable habitats where as the greater one-horned rhinoceros conservation action plan emphasizes on *in-situ* conservation or what needs to be done to preserve the species in perpetuity and reinforce the continuing recovery of rhinoceros populations in Nepal. Similarly, elephant conservation action plan is implemented to save elephants in the wild from extinction, address the habitat loss and mitigate human elephant conflicts. All these action plans seems to be unaware about the role of diseases in population declines and interrelatedness of human and wildlife except on the case of vultures which has covered one aspect i.e. ban of Diclofenac and other non-tested NSAIDs, and promoted the use of alternative safe NSAIDs aiming at increased level of vulture conservation awareness among general public. The National Parks and Wildlife Conservation Act 1973 (amended in 1992) has primarily focused on the protection of wildlife and their habitats; however, there is very little consideration given to wildlife diseases though samples can be collected for the scientific research with due permission from the concerned authority (DNPWC, 1992).

This shows that very little attention is currently paid to the future consequences of diseases, except the recent tuberculosis control strategy in captive elephants as well as the surveillance of highly pathogenic avian influenza. The only action plan related to the control of diseases is The Nepal Elephant TB Control and Management Plan which is about to be endorsed by the Government of Nepal with the goal of eliminating TB in captive elephants and prevent transmission to wildlife species. A major controversy has already arisen on the sampling, surveillance and research of diseases on wildlife among Department of National Parks and Wildlife Conservation and the Department of Livestock Services with regard to the responsibility. Wildlife species need expensive tools and permits for essentials of disease monitoring such as sampling. The department of Livestock Services and Department of Public Health may have disastrous effect on human health and livestock industries if potential disease spread from the wildlife species. This interrelationship for conservation of species, protection of human and animal health and related enterprises must be understood well, taken into account and work together.

CONCLUSION

The abundant manpower and laboratory facilities available in the medical and veterinary sector of the country should be best utilized to investigate diseases of wildlife. Various events such as wildlife translocations can be utilized for sampling from wildlife reducing the vast expenses. However, laboratories must help parks and reserves by providing basic training and medical equipments for collection, preservation and transportation of samples. Otherwise, Department of National Parks and Wildlife conservation must establish its own wildlife health unit equipped with high quality manpower. This can envisage constantly on wildlife disease and can minimize threats for the conservation of endangered species. The cooperation among related agencies to achieve the healthy wildlife, domestic animals and peoples can never be overlooked.

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IMPACTS OF CLIMATE CHANGE ON LIVESTOCK AND VICE VERSA

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ABSTRACT

Of late, impact of global warming on animal agriculture has been a subject of greater concern. Rise in average temperature has profound effect not only on the life cycle of parasites but also on the sifting location of the migratory birds carrying influenza viruses. Very important trend that has been observed in migratory birds is that they need not go south during chilly winter and instead they are seen even in northern Nepal, Tibet and Japan which were otherwise cold places previously. This sifting distribution of carrier birds has led the outbreaks of avian influenza in previously unknown areas of the world. How progressively tick population in domestic animals is being substituted is discussed. Besides, the dynamics of emerging diseases in new geographic locations will be highlighted in this paper. An attempt will also be made on discussing how livestock and poultry industries are contributing to rise in the production of green house gases that ultimately contributes to global warming and climate change.

INTRODUCTION

Climate change is a global phenomenon, occurring at an alarming rate. It is not only the rise of temperature but also includes trends towards strong storm systems, increased frequency of heavy precipitation events and extended dry periods. These changes have implications for food production, food security and food safety. Most of the actions causing climate change originate from the developed world.

Visible effects of climate change in Nepal

Abundance of mosquitoes and reporting of mosquitoes borne diseases such as malaria, Japanese encephalitis and Dengue are on the rise in Kathmandu valley, which never existed earlier. Similarly, other visible changes observed in Nepal include ripening of Ka-fal (*Myrica nagi*) in Falgun (February-March) instead of late Chaitra to early Baisakh (April-May) and blooming of *Rhododendrons* in January-February instead of late March and early April. Late monsoon in October 2009 (after Dashain Festival in 2066 BS) left many people homeless with mudslides in many parts of Nepal. Aftermath of Koshi flood in 2008 left thousands of people and animals missing from Nepal and Bihar, damaging thousands of hectares of cultivated lands. Himalayas are visibly less capped with snows than in previous years.

Impact of climate change

Climate change is likely to result in an increase of diarrheal diseases with more risks in urban poor, elderly and children, traditional societies and subsistence farmers. Diseases associated with weather pattern such as El-Nino Southern Oscillation (ENSO) include diarrhea and cryptosporidiosis. Death toll of more than 300 lives in 2009 due to outbreaks of diarrhoeal diseases in mid western region of Nepal might have resulted from the effect of ENSO. Water availability and access to clean water, rainfall, runoff and temperature are all related to the incidence of diarrheal diseases. Prolonged drought followed by erratic and late monsoon in 2009 damaged millions of rupees. Glacial meltdown and water runoff are not synchronized with crop production which is expected to result in the reduction of number of crops from 3 to 2 crops in a year in much of the Gangetic plains (Leng, 2009).

Meteoclimatic fluctuations can affect the infective agent directly by modifying the life expectancy of the free living stages or indirectly through changes in the immune response, behavior, demography, resources availability of the host and vectors (De Leo, 2009). Climate change induced floods and droughts are thought to result in losses of 50 million cattle and buffaloes and 100 million sheep and goats per year from parasitic and infectious diseases besides morbidity due to inadequate feeds.

The effect of anthropogenic climate change on infectious diseases can be substantially altered by several cultural and socio-economic factors including sanitation and vaccination programs, development of drug resistance, intensive management of livestock, land use changes, etc.

Causes of Climate change

Burning of fossil fuel and land use change are the main factors to rise in global temperature since mid 20th century (IPCC, 2007). The rich 13% of the world's population were responsible for 48% of all CO_2 emissions in 2000. The consumption of fossil energy, forest burning, and intensive agriculture have produced many atmospheric trace gases, some of which are very volatile, while others have a very long lifespan in the atmosphere. For instance, CH4 has a mean residence time of 12 years and N_20 of 114 years. Deforestation, soil erosion and industrial animal farming are proved to have detrimental effects on the environment both in the short and the long term. Globally, farms animals produce 13 billion tonnes of waste per annum. Animals on industrial farms consume high-protein feeds and produce waste contributing 5-10% of the total green house gases in the world, accelerating climate change. Moreover, large amounts of water and fossil energy are required for growing, processing and transporting industrial farm animal feed and treat the animal waste.

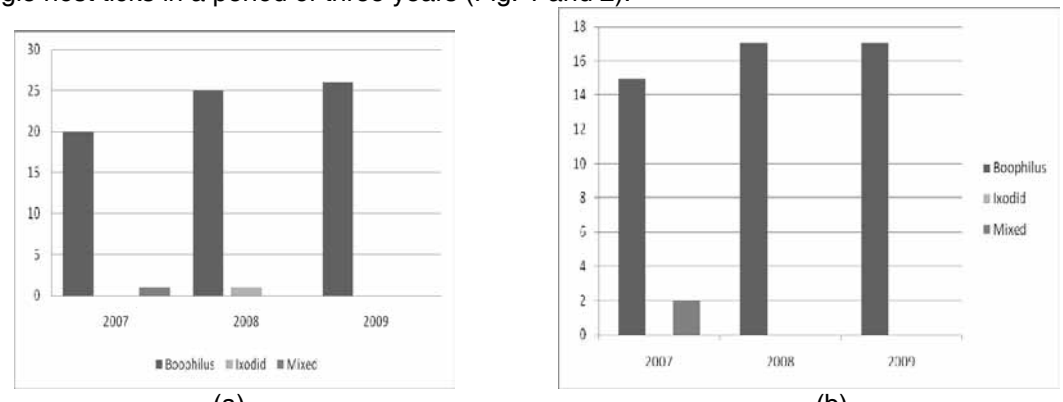
Applications of fertilizers has led to serious environmental problems: leaching of nitrate from the topsoil to the groundwater negatively affecting the quality of the drinking water and high levels of ammonia emission from the cows negatively affecting the quality of nature in the surroundings of the farm. The way cows are fed affects the quality of manure and the quality of manure affects the quality of the soil. The quality and fertility of the soil affects the quality of the pasture and fodder crops and hence the feed, in turn affects the health of the animals and the quality and quantity of the products. e.g., reducing the amount of protein and increasing the amount of roughage in the feed, the quality of manure becomes much better than the slurry produced by conventional methods.

Consequences of climate change

Unpredictable rains and dry spells have immediate impact on crop growth and productivity. Intergovernmental Panel on Climate Change (IPCC, 2007) reported that 0.76°C increase in the world's average temperature in the last century and is expecting temperatures to rise by 2°C by 2050 which will result in rising sea levels, the disappearance of glaciers, and to drastic changes in rainfall patterns, affecting the production potential of rural areas. Extreme weather is also associated with increased disease risk. Droughts lead to poor hygiene and malnutrition while floods result in contamination of drinking water. Changes in temperature, rainfalls as wells as an increase in extreme weather events are likely to change food production and food distribution systems or to change the purchasing power of, for example, flood victims.

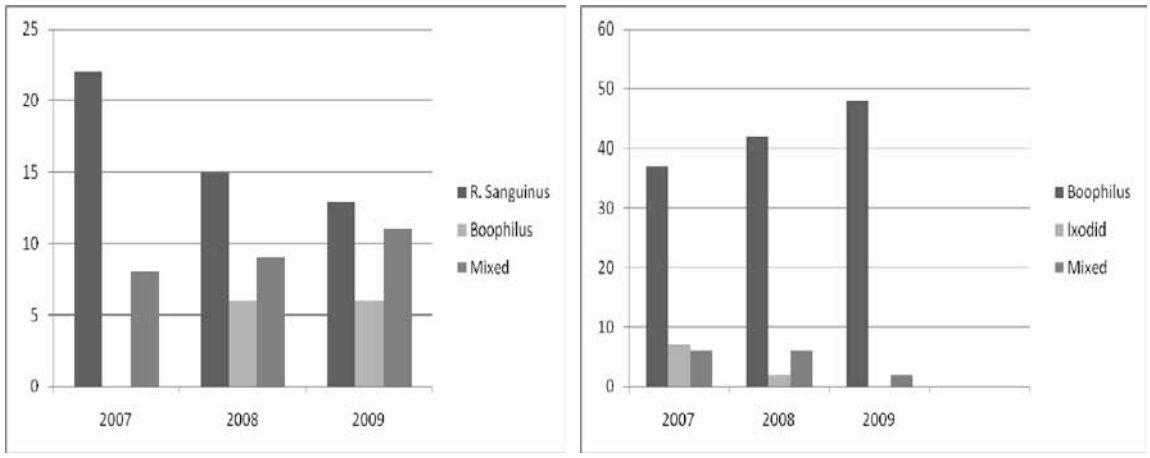
Climate change is altering the distribution, incidence and intensity of animal and plant pests and diseases such as bluetongue, a sheep disease that is moving north into more temperate zones. Temperate countries and regions will be more vulnerable to invasions by exotic arthropod-borne virus diseases and parasites. Change in climate resulting in changes in species composition will augment the emergence of unexpected events, including the emergence of new diseases and pests.

Shrestha (2010) reported that multi host ticks in cattle, buffaloes, dogs and goats have been replaced by single host ticks in a period of three years (Fig. 1 and 2).



(a) (b)

Fig. 1. Showing progressive displacement of multi host ticks by a single host tick population at Lele, Lalitpur in cattle (a) and buffaloes (b) over three years period (Courtesy: Shrestha, 2010)



(a) (b)

Fig. 2. Showing progressive displacement of multi host ticks by a single host tick population at Lele, Lalitpur in dog (a) and goats (b) over three years period (Shrestha, 2010).

Retrospective analysis of temperature data in Agricultural Research Station, Pakhribas over the last 10 years starting from 2001 to 2009 revealed that some decline in temperature during 2007 due to La Nina and started to rise since then due to El Nino (Fig. 3).

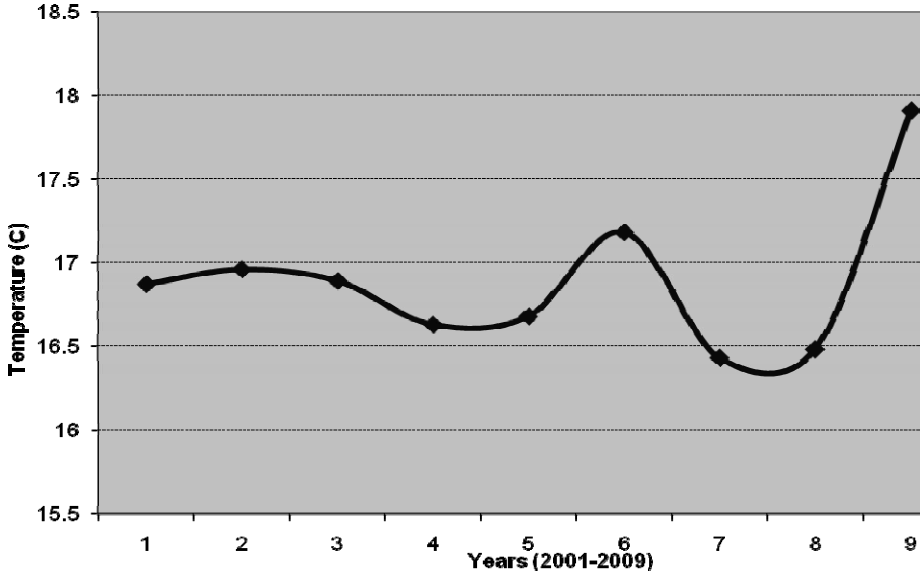


Fig. 3. **Overall average temperature (max and minimum) in ARS, Pakhribas**

Role of livestock in climate change

Livestock was never really mentioned in the climate change debate until 2007, when FAO reported that livestock keeping produces 18% of all green house gases (Zijpp A. van der, 2008). They account for 37% of emissions of methane, which has more than 20 times the global warming potential of CO_2, and 65% of emissions of nitrous oxide, another powerful greenhouse gas, most of which comes from manure. Another main source of the green house gases (GHG) related to livestock production is poor land use.

Climate change has important effects on parasitism and disease in fresh water and marine ecosystems with consequences for human health and socio-economics. Distribution of parasites and

pathogens will be directly affected by global warming and indirectly through effects on host range and abundance. El-Nino was implicated into numerous disease outbreaks in marine animals. The proliferation of zoonoses and other animal diseases may result in an increased use of veterinary drugs that could lead to increased and possibly unacceptable levels of veterinary drugs in foods (FAO, 2008). The growth of commercial farms, their proximity to congested cities in the developing world, and the globalized poultry trade are all culprits behind the spread of avian flu, while livestock wastes damage the climate at a rate that surpasses vehicular emissions.

In the world of fisheries, migration of fish from one region to another in search of suitable condition leads to spatial distribution of fish stocks; phytoplankton growth, algal boom, etc.

Animal health problem

High-inputs farming system has led not only to environmental pollution but also to animal diseases. Very high protein in the rations is causing digestive problems and malfunctioning of the liver and high incidence of mastitis besides animals becoming susceptible to hoof diseases, and prolonged calving intervals due to fertility problems.

These health problems increase the veterinary costs and decrease the milk production. There is much evidence to suggest that parasite and disease transmission, and possibly virulence, will increase with global warming. Climate change would almost certainly alter bird migration, influence the AI virus transmission cycle and directly affect virus survival outside the host. The net effects of these changes are rather unpredictable, but it is likely that AI virus circulation in water bird populations will continue with endless adaptation and evolution. In domestic poultry, too little is known about the direct effect of environmental factors on HPAI transmission and persistence to allow inference about the possible effect of climate change.

At higher temperatures, numerous diseases display greater virulence as a result of reduced resistance of the host due to stress, increased virulence factor or increased transmission. Increased temperature may cause thermal stress in terrestrial and aquatic animals, leading to reduced growth, suboptimal behaviors and reduced immunocompetence. Any negative effects of climate change on biodiversity may affect disease transmission because biodiversity may act as a buffer against disease propagation (dilution effect). Anthropogenic changes that affect biodiversity will increase disease risk. Heat stress can have a direct and detrimental effect on health, growth and reproduction. Climate change may affect zoonoses by increasing the transmission cycle of many vectors, and the range and prevalence of vectors and animal reservoirs.

How to cope with climate change?

Since climate change is a global problem affecting both land and oceanic areas and having an impact in every country, a global cooperation is required to deal with it by keeping in mind the Kyoto initiative, “Think Globally Act Locally”. Global political decision is vital for coping with climate change with adoption of sustainable agricultural practices which help soils retain higher quantities of water thereby helping to withstand periods of droughts.

Ruminants produce 80 million tonnes of methane annually mostly by animals fed low quality feeds. Improving these feeds reduces methane production per unit of product and utilization of nitrate to replace urea in low protein diets can reduce methane production / animal to almost zero removing the downside of increasing enteric methane production, a major contributor to global warming, as production from ruminants increase. By reducing the percentage of crude protein in the diet (from 18% to 16-15%) and increasing the crude fibre content (roughage), methane production can be reduced. Besides, we will have to attempt to increase the C/N ratio of manure from 7 to around 10. Government should subsidize organic crop and animal farming and put restriction on industrial animal farming not meeting EPA guidelines.

To prevent global climate change by greenhouse gases, it is important to reduce methane emission by cattle (through speed up animal production per unit area and to reduce the slaughter age to get a lower ratio of kg methane/kg animal protein (meat). Use of grains for animal feed has to be reduced as well, giving priority to human consumption. One has to replace non productive animals. Uses of UMMB can also reduce methanogenesis. Use of *T. chebula* (24%) and *A. sativum* (11.9%) were found

to inhibit *in vivo* methanogenesis (Kamra, et al., 2009) and hence more works need to be done in this aspect.

The use of animal for draught power is to be encouraged instead of tractors which help in reducing emission and provides social benefits through employment generation. With live fencing, controlled grazing, water harvesting and manure use could double the production of grains and cattle with low inputs. Plants like sugar cane need to be planted extensively in the tropical areas and its all parts can be efficiently used: leaves for roof, bagase for bed and cooling and stem for sugar and fuel. We all have to stop burning crop residues in the agricultural lands as agriculture is responsible for 14% green house gas emissions.

Pasture land should be rested which is as good as irrigation. Research has shown that pastures with at least 50 shade trees/ha yielded 15-30% increase in milk production and around 20% meat due to the comfort that makes cattle produce well (Primavesi, A & Primavesi, O., 2002). Rotational grazing is far better than fire as demonstrated from the fact that eight consecutive years of burning with one fire per year was enough to decrease plant production to 25% of the initial due to loss of organic matter that nourishes the soil microorganism from burning out.

Covering soils with vegetation allows for better infiltration of rain water, improves the soil structure and thereby increases its air and water circulation and storage capacity necessary for plant metabolism and efficient plant nutrition. One has to adapt pasture to the soil and cattle to the pasture.

Plantation of bio-diesel plants like *Jatropha* in non-agricultural waste lands should be encouraged instead of promoting corn based bio-ethanol production. Corn based bio-ethanol production has already been attributed for the environmental degradation through the leaching effect of chemical fertilizers and pesticides massively applied in the corn fields for higher yields besides causing scarcity of corns for animal feeding.

Issues and concern

The introduction of diseases and pests will result in higher costs to the national industry in relation to inspection, treatment and compliance with obligations of the trading partners. Trade disputes in the WTO systems could become more frequent. Investments in early control and detection will be valuable to avoid higher costs of eradication and control.

Impact of climate change on food security

Transboundary plant pests, animal diseases and invasive alien aquatic species are a constraint to food security due to their impacts on food availability, food access, food safety and food stability as listed below:

Availability of food: Animal and plant pests and diseases and alien invasive aquatic species reduce the availability of quantities of food of appropriate quality.

Access to food: Animal and plant pests and diseases and alien invasive aquatic species reduce food access through reduction of income from animal production, reduction of yields of crops, reduction in forest productivity, changes in aquatic populations and increased cost of control.

Utilization of food: Climate change may result in food borne zoonoses and increased use of veterinary drugs, while redistribution of plant pests and changes in pest incidence and intensity may result in additional and inappropriate pesticide use leading to unacceptable level in food. Changes in rainfall, temperature and relative humidity may favour the growth of fungi that produce mycotoxins and thus make food unsuitable for human and animal consumption.

Stability of food: Individuals or populations must have access to adequate food at all times. Introduction of new pests and animal diseases may have substantial effects on the stability of food supply through direct losses as well as through the reduction of income.

Adaptation measures

The following measures are expected to help in the adaptation process of minimizing the harmful impacts of climate change on livestock keeping:

- Adjustment of technologies: improved breeds, shading, sprinkling, increasing air circulation, improved management practices (stocking rates, rotational grazing, improved pasture species) and improved resource efficiency use (land, water and feed)

- Risk management measures: weather based insurance schemes, climate information system (early warning), risk fund schemes and feed storage
- Infrastructure and services: provision of water points, appropriate shades, enhanced veterinary services, extension services, fodder banks and feed lots
- Changes of production systems: breed selection for greater tolerance and increased productivity, intensification of livestock production; substitution of livestock species (drought resistant, monogastric for ruminants; changes in land use choices (agro forestry, conversion of marginal grazing land to forests)

ACKNOWLEDGEMENT

The following persons namely Dr. G. A. De Leo of Univesita degli Studi di Parma, Italy, Prof. R. A. Leng of University of New England, NSW, Australia and Mr. Keshab P Ghimire, ARS Pakhribas deserve special thanks for providing necessary input materials for this paper.

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ZOONOSES AND HUMAN HEALTH

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ABSTRACT

Zoonotic diseases are those in which human beings are infected with pathogens carried by animals. They can be transmitted directly through animal-to-person contact, or indirectly through consumption of contaminated food. Livestock carry potential health hazards, so food animals are an integral part of public health protocols. Hence the myriad of food safety policy legislation found globally. Regarding pigs, classical swine influenza is documented as having been transmitted to people on occasions, as has Streptococcus suis, which could be considered an occupational health hazard for those working in the pork industry. The domestic pig is known to be susceptible to several other zoonotic diseases like rabies, leptospirosis, brucellosis, erysipelosis, tuberculosis, Japanese B encephalitis (JE), etc. Pig meat from infected pigs, when consumed raw or inadequately prepared, can transmit a number of pathogens, such as Trichinella spp., Cysticercus spp., Salmonella spp. and Listeria spp.; for Taeniasis/cysticercosis inadequate hygiene during meat processing or at home can also be a source of contamination to human beings.

In many circumstances, particularly for livestock production diseases, the environment in which livestock are kept determines the course and severity of disease expression; a highly contaminated environment for livestock with a weakened immune system often tips the balance and makes a disease clinical epidemiologically.

In Nepal, so far mostly diagnosed zoonotic diseases are brucellosis, cysticercosis, trichinellosis, tuberculosis, salmonellosis, echinococcosis/hydatidosis, Japanese encephalitis, rabies, streptococcus suis and listeriasis.

INTRODUCTION

Most Emerging Zoonotic Diseases are as follows:

- Viral diseases - Rabies, Japanese Encephalitis, Influenza, Ebola virus, BSE, Hanta Virus, Lassa fever, Hendra Virus, NIPHA Virus, Eastern Equine Encephalitis (EEE), Western Equine Encephalitis (WEE), Venezualan Equine Encephalitis (VEE)
- Bacterial diseases - Anthrax, Brucellosis, Campylobacter, Leptospirosis, Glanders, Plague, Psittacosis, Salmonellosis, Tetanus, Tuberculosis, E.coli infection, Lyme disease
- Parasitic disease - Cryptosporidiosis, Echinococcosis, Fascioliasis, Leishmaniasis, Scabies, Schistosomiasis, Taeniasis & Cysticercosis, Toxoplasmosis, Trichinellosis (Trichinosis), Filariasis
- Rickettsial disease - Q. Fever
- Poisoning – Snakebite
- Of the 1407 human pathogens→ 58% are known to be zoonotic
- 177 are categorized as emerging/ reemerging and zoonotic

Emerging and re-emerging zoonoses, (1996–2006) recorded are as follows:

A. <u>Parasites/Bacteria</u>

Cryptosporidiosis
Letopsirosis
Lyme Borreliosis
Brucellosis
E coli O157
Multidrug resistant *Salmonella*
Plague

B. Viruses/prions

Ebola and | **Crimean-Congo haemorrhagic fever** (**CCHF**)
Influenza H5N1
Lassa fever
Monkey pox
BSE
Rift Valley Fever
SARS CoV
VEE
West Nile
Hendra/Nipah
Rabies
Marburg

OBJECTIVE:

1. To assess the Risk Factors Associated to Zoonoses Occurrence like:

- Epidemiological factors;
- Environmental factors;
- Animal reservoir factors;
- Socio-economic factors;
- Community economical development factors.

2. To Project Livestock Production as per the Demand in Nepal like:

- In developing countries average consumption will increase from 26 (8 in 1970) to 42 kg per capita per year in 2050.
- The developing world is expected to produce 300% more metric tones of meat than today in 2050.
- Asia will almost double the volume of meat it produces by 2050 (Meslin, 2005).

3. To Predict zoonotic disease as public health importance

- "Predicting which zoonotic diseases may arise in the future is extremely difficult, due to the multifactorial and constantly evolving nature of the risk factors involved (WHO 2004)."
- Vector-transmitted (WNF, RVF…) infections may be the exception, as they are strongly influenced by environmental factors.

METHODOLOGY

There are number of emerging and reemerging zoonotic diseases to be understood which are going to have outbreak in Nepal like:

A. HIV Aids/TB Diseases

- HIV-1 through transmission from chimpanzees
- HIV-2 from Sooty Mangabey
- Chytridiomycosis- global decline in a variety of Amphibian species
- International trade a key role in the worldwide dissemination of diseases (Globalization)
- Global climate change responsible for regional climate alterations

→ affect physical and biologic systems

B. Hunting, Pets and Globalised Trade in Wildlife

- Facilitate rapid movement to distant sites and greater human-pathogen contact
- Surveys of live wildlife markets (Guangzhou- China)
  - Masked palm Civets
  - Ferret badgers
  - Barking deer
  - Wild boar
  - Hedgehogs
  - Foxes
  - Squirrels

- Bamboo rats
- Gerbils
- Snakes
- Leopard cats
- Rabbits
- Dogs and cats

- 2003 SARS outbreak 838,500 wild animals were reportedly confiscated
- North Sulawesi (Indonesia) in a single market- 90,000 mammals are sold per year
- Thailand, over 25 weekends 70,000 birds of 276 species were sold
- Asia alone, 10 million wildlife species were shipped.
- A multibillion dollar industry
- Bushmeat consumption in Centre Africa- 1 Billion kg per year
- Amazon Basin- (67 to 164 million kg/year)

C. Severe Acute Respiratory Syndrome (SARS)

- 2002- first in Guangdong Province, China
- Spread to Hong Kong and then to 5 continents and 25 countries via infected people
- 2003- a new coronavirus was the causative agent
- 2003- WHO reported 8,437 in human and 813 deaths
- Detected in farmed palm civets
- Culled more than 10,000 masked palm civets
- Later in raccoon, ferret, badger and domestic cats
- Bats - reservoir hosts for Lyssa, Nipah, Hedra and Ebola virus

D. Monkey Pox

- Central and Western Africa
- 1958 in laboratory primates
- 1970 first human case in Africa
- Cowpox in Nepal recorded in Karnali in 1968.

E. Ebola Hemorrhagic Fever (Ebola)

- Bushmeat (Chimpanzees and Gorillas)
- Movement of non human primates - for biomedical research- a source for the spread
- 1989- related simian hemorrhagic fever diagnosed in Virginia from cynomolgus monkey from the Philippines
- Later Ebola Reston- not zoonotic

F. Avian Influenza (Bird Flu)

- 1997- H5N1 in Hong Kong
- 2003-2004- throughout Asia
- 2005- WHO confirmed 142 human cases and 74 deaths. Out break of AI in Jhapa Nepal in 2008 but no human cases recorded so far. About 1,472 chickens and four ducks were culled on the first day by 10 Rapid Response Teams. No humans have contracted the disease so far. It has been issued countrywide alert but especially 20 districts in Terai bordering India. The team said it would cull over 9,000 chickens and other birds in the area in course of a week. The government had culled around 24,000 birds. The government has started killing poultry and poultry products in the bird flu affected area of Sharanamati VDC, Jhapa from 21 February. At least 232 chickens, one duck, 26 pigeons and 41 eggs were destroyed upto 12 March 2009 at Mohmuddin Tole in Pathamari VDC, Jhapa. In all three outbreaks of AI no human cases were detected and diagnosed for bird flu. (Joshi, 2009a and 2009b).

G. Swine Flu

Swine flu is a respiratory illness in humans and in pigs caused by a flu virus. The swine flu virus routinely causes outbreaks in pigs but doesn't usually kill many of them. There have been cases of the virus spreading from human to human, probably in the same way as seasonal flu, through coughing and sneezing by infected people. The symptoms are similar to those of regular flu-fever, cough, fatigue, lack of appetite.

During the year 1918, Swine influenza (SI) was first observed at the time of the pandemic in humans and since that time subtypes H1N1 and H3N2 have been widely reported in pigs, frequently associated with respiratory disease. FAO underlines the great value of the influenza veterinary laboratory network called OFFLU. The most notorious flu pandemic is thought to have killed at least 40 million people worldwide in 1918-19. Two other, less deadly flu pandemics struck in 1957 and 1968.

On June 29, 2009 three Nepalese family member back from USA were found positive to Influenza A H1N1 first time in Nepal. Since then the distribution of human cases of swine flu within the country confirmed, diagnosed, recorded and reported according to their home location. Out of 63 cases one case was from Australian citizen and one from Romanian citizen and rest are all Nepalese, of which 21 were from Kathmandu, 1 case was from Lalitpur, 1 from Dhanusha, 1 from Kailali, 1 from Kanchanpur, 1 from Chitwan and last 1 from Kaski districts of Nepal. So far, there is no mortality in human cases in Nepal (Joshi, 2009).

H. Arthropod Vector Borne Diseases

- Climate alterations may affect the distribution of vector species
- Changing their range due to altered conditions for breeding and feeding

I. Hantavirus

- A zoonotic virus of rodents and has emerged as a human pathogen
- As result of human induced landscape alterations and climatic changes influencing population of rodent reservoir hosts

J. West Nile Virus

- 1937-first isolated in the West Nile district of Uganda
- Thereafter isolated from wild mammals, horses, birds (284 spp) and mosquitoes
- Spread across North America, Tropical America and the Caribbean
- Now isolated from ticks
- Global climate change→ favor the increase in abundance and distribution of mosquito vectors
- This disease recorded in India but so far not recorded in Nepal

K. Dengue Fever

- Between people or between monkeys through mosquitoes
- Warming of 2°C will result in a net increase in the potential latitude and altitude range of dengue fever
- This disease has been recorded in India and also in Nepal.

L. Leptospirosis

- Re-emerging zoonotic disease of global importance
- Outbreak associated with increase in rodent population after heavy rainfall or during floods

RESULTS:

Outbreaks of different zoonotic diseases are being recorded and their importance are as follows:

1. Global Perspective

- Public health→ WHO
- Livestock health→ FAO, OIE
- Wildlife health→ No organization
- Until such a body is formed, wildlife will continue to be a footnote to humans and will continue to be the surprise critical variable in emerging disease spread
- WTO must start better regulation
- Nations must implement and enforce laws
- Trade and the consumption of wildlife have led to global health disasters

2. Role of Zoo and Wildlife Health Professionals

- Requires a multidisciplinary approach to problem solutions
- Surveillance for the emergence of new disease
- Participate in disease reporting
- Establish collaborative and communications links with public health and animal health agencies, diagnostic centers, human and veterinary medicine facilities and university based health research institute

3. Changing farm Management and Consumer habit

- With respect to the prevalence and socio-economic consequences of zoonotic diseases in Nepal the conditions have actually deteriorated in recent year which is due to:
- Farm management, changing land-use patterns, and animal industries have led to ecological developments without appropriate controls of their respective public health hazards.
- Changing consumer habits rapidly developing food industries by inadequate services of diseases surveillance and control.

4. International Health Regulations (IHR)

- IHR are the legally binding international legal instrument endorsed by the WHO in May 2005
- IHR aims to prevent, protect against, control and provide a public health response to the international spread of disease
- IHR come into force in June 2007 –voluntary application for HPAI since WHA 2006
- The IHR requires swift official notification of potential Public Health Emergency of International Concern (PHEIC)

- WHO has the right to use other sources to request information on the potential PHEIC and
- WHO DG has the final word when determining if an event is a PHEIC.
- IHR enters in force on 15 June 2007.
- IHR should increase transparency, speed up containment when a PHEIC is detected in one or more WHO Member States.

5. Global Early Warning and response System (GLEWS) for animal diseases including zoonoses

- GLEWS is a joint system that builds on the *added value* of *combining and coordinating* the alert and response mechanisms of OIE, FAO and WHO
- To assist in prediction, prevention and control of animal disease threats, including zoonoses.

6. What does Global Outbreak Alert and Response Network (GOARN) do?

- **Assists countries with disease control efforts** by ensuring appropriate technical support to affected populations rapidly
- **Investigates and characterizes events and assess risks** of rapidly emerging epidemic disease threats
- **Sustains national outbreak preparedness** by ensuring that responses contribute to sustained containment of epidemic threats

7. Transmission Pattern of Zoonotic Diseases in Nepal

S.N.	Type of zoonotic diseases	Reported/Studied in Nepal		year Reported	Control Plan envisaged	
A.	**Bacterial Zoonoses**	**Yes**	**No**		**Yes**	**No**
1.	Anthrax	√		1984		√
2.	Brucellosis	√		1972		√
3.	Tuberculosis	√		1985		√
4.	Salmonellosis	√		1986		√
5.	Escheria Coli	√		1983		√
6.	Streptococcosis	√		1983		√
7.	Staphylococcosis	√		1983		√

8.	Complybacteriosis	√		1983		√
9.	Glanders		√	1987		√
10.	Leptospirosis		√	1987		√
11.	Plague	√		1964/65		√
12.	Tetanus		√	1964/65		√
B.	**Viral Zoonoses**					
1.	Rabies	√		1960	√	
2.	Cow pox		√	1973		√
3.	Foot and Mouth Disease	√		1962	√	
4.	Goat pox		√	1973		√
5.	Monkey pox		√	1987		√
6.	Avian Influenza/Bird Flu	√		2006	√	
7.	Swine Influenza/Swine flu	√		2008	√	
8.	Japanese Encephalitis	√		1978		√
C.	**Parasitic Zoonoses**					
1.	Cutaneous Leishmaniasis	√		1983		√
2.	Visceral Leishmaniasis	√		1983		√
3.	Toxoplasmosis		√	1986		√
4.	Cryptosporidiosis		√	1988		√
5.	Echinococcosis/Hydatidosis	√		1971/72		√
6.	Fascioliasis/Liverfluke	√		1971/72		√
7.	Schistosomiasis		√	1992		√
8.	Taeniasis/Cysticercosis	√		1982/83		√
9.	Ascariasis	√		1982/83		√
10.	Cutaneous Larva Migrans		√	1990		√
11.	Trichinosis	√		2003/04		√
12.	Zoonotic filariasis (Brugia malay/Dirofilaria immitis		√	2008/09		√
13.	Zoonoses scabies /mange/*Scabiasis sarcopties*	√		2001/2002		√

CONCLUSION

- In conclusion Nepal government should have following activities urgently to control zoonotic diseases in Nepal: Legislation/Acts to Control Zoonoses
- Research Activities in Zoonoses;
- Surveillance Prevention and Control of Zoonoses through surveillance;
- outbreak investigation;
- disease reporting; and development of prevention and control strategies
- Strengthen inter-institutional global frameworks for early detection and containment
- Refined existing and develop new tools for control strategy selection
- Promote new concepts for zoonoses prevention
- Control of the zoonotic diseases should be integrated within existing health systems.
- Control packages for animal diseases addressing several disease/health problems should be developed (WHO, 2005).

ACKNOWLEDGEMENT:

I am most grateful to the organizing committee of Vet Con 2010 for inviting me to present this theme paper in this conference. I am also thankful to my staff of NZFHRC particularly Ms. Meena Dahal for preparing the paper for presentation.

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Technical Papers

GENETIC IMPROVEMENT OF DAIRY ANIMALS TO MEET FARMER EXPECTATION FOR FOOD SECURITY

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ABSTRACT

Genetic improvement with increased milk productivity of dairy animals is one of the permanent changes that farmer can achieve for ensuring food security. This paper reviews past efforts undertaken in Nepal for genetic improvement of dairy animals and analyzes the prevailing breed improvement programs. Adoption of breed improvement programs, based on scientific principles for three decades, could have adequately substantiated some genetic improvement in milk productivity. However, the annual growth of milk production of about 3.06% is attributed to increase in number of crossbred cattle and buffaloes per se. There is no evidence to support the fact that productivity of dairy animals has increased over years. Developed countries achieved exceptional increase in milk productivity through adoption of 1) permanent and unique identification of the animals, 2) parentage recording, 3) recording of milk yield and other traits of economic importance, 4) artificial insemination, and 5) statistically advanced genetic evaluation and selection based on the performance. Same scientific principles are applicable also in Nepal to achieve genetic improvement. From past efforts, some of the positive developments so far include creation of infrastructure for semen laboratory, acceptable level of improvement in conception rates from AI, execution of Dairy Cattle Improvement Project and increasing involvement of private sector in the delivery of services.

It is important to note that lack of clear policy on dairy animal breeding, use of breeding stock of inferior genetic merit, more focus on AI ignoring selection of sires, lack of breeding plans, and lack of resource centers have adversely influenced genetic improvement. Similarly, inefficient mechanism for distribution of semen and liquid nitrogen and ignorance of farmers' role in the management of the institution responsible for breeding management are also important issues. Based on farmers' preferences it is recommended that a major revamping is necessary to transform National Livestock Breeding Center into a financially sustainable autonomous corporate body with involvement of farmer representation in its organizational management.

1. BACKGROUND

Introduction of high yielding breeds of cattle in Nepal can be traced back as early as 150 years ago. The then Prime Minister Jung Bahadur Rana imported high yielding Jersey cattle in 1917 B.S from the United Kingdom. Then subsequent rulers also imported dairy cattle and buffaloes from India. With the establishment of Livestock Improvement Section in 1952 (2008 B.S.) more breeds namely Red Sindhi, Sahiwal, Jersey and Brown Swiss of cattle and Murrrah of buffalo were introduced and their bulls used for upgrading native animals. Artificial insemination (AI) program using liquid semen began in the year 1962 (2018 B.S.). With the implementation of an Artificial Insemination Project In 1969 (2025 B.S.), the AI service was further expanded in areas outside Kathmandu valley. Production of frozen semen began in 1980 (2038 B.S.) with the establishment of liquid nitrogen plant in Tripureshwor. The AI service as a tool for spreading genetic merit among dairy animals now covers 49 districts. Similarly, bulls of crossbred cattle and buffaloes (mostly of unknown genetic merit) have been used aiming at enhancing milk productivity through natural service. However, the genetic improvement is not substantial; rather, it is stagnated of even declining in major dairy pockets. In this context, this paper critically analyzes the prevailing breed improvement programs, farmers' preferences and recommends future strategies for the genetic improvement of dairy animals for desired outputs.

2. PRODUCTIVITY SCENARIO

Total milk production from an animal largely depends on its milk productivity and reproduction rate. There is wide variation among the performance of different categories of dairy animals. Milk yield and

its quality (volume as well as composition), age at first calving and calving interval are the most important traits of economic importance The performance of dairy animals in terms of milk productivity and reproduction rate is far less than their biological potential (Table 1). Milk yield per lactation is too low. Similarly, the calving interval is quite long, and first calving is at later age. The existing wide variation in productivity traits signifies opportunity for improvement through interventions in breeding and husbandry practices. Looking at the data trend from dairy cattle improvement project, it is quite clear that a wide variation exists for milk yield in crossbreds cows (ranging from 2 to 24 liters/day) with a yield of the highest yielding cow above 8,200litre/lactation. This opens the scope for enhancing the milk productivity largely by selecting high yielding cows inseminating them with proven imported semen allowing their progeny to multiply.

Table 1: Milk productivity and reproductive performance of different categories of dairy animals

Animal type	Milk yield (L/Lactation).	Calving Interval (months)	Age at first calving (month)	Remarks
Crossbred cattle	1800.0	18	26 35 (high mountains)	Farmers conditions
Native cattle	438.0	21	48 60 (high mountains)	Farmers conditions
Murrah buffalo	1872.0	20	44	From: LDF and RARST
Murrah crosses	1440.0	20	50	Farmers conditions
Native buffalo	852.0	22	52	farmers conditions

Adapted from ABPSD (2008), Paudel (1998), LDF (2007), and data from field observations

3. PRODUCTIVITY ISSUE

Farmers in localities with access to formal milk market keep Jersey and Holstein Frisian among cattle, and Murrah and its crosses among buffaloes. Farmers argue that the progenies of their cows produce less milk than their mothers did due to poor genetic quality of the semen supplied by DLS. Similarly, purchase of breeding bulls (mostly indiscriminately) for natural mating/semen production is based on phenotypic characteristics. Evaluation of sires for selection is non-existent. Annual growth of milk production of about 3.06% (ABPSD) is attributed to increase in number of crossbred cattle and buffaloes *per se* as there are no evidences to support the fact that milk productivity has increased. The focus must be to increase productivity to realize efficient use of limited feed and fodder available for dairy animals.

Developed countries achieved exceptional increase in milk productivity through adoption of five steps of genetic improvement program in addition to improved feeding, husbandry practices and healthcare. These are: 1) permanent and unique identification of the animals, 2) parentage recording, 3) recording of milk yield and other traits of economic importance, 4) artificial insemination, and 5) statistically advanced genetic evaluation and selection based on the performance. Same scientific principles are applicable also in Nepal to achieve genetic improvement. Implementation of breed improvement program in a country like Nepal with smallholder farming system is tedious, therefore, requires farmer friendly approach that is practical but without loosing the scientific principles. Past efforts failed to establish performance-recording system and create elite herds of cattle and buffaloes. As a result, farmers now are facing acute shortage of high yielding dairy animals within the country at the time the country is facing an acute shortage of liquid milk. Many factors including lack of priority and dedication in adoption of science-based activities in the execution of breeding programs are responsible for this

situation. No scientific approach was applied to produce/purchase breeding animals, semen and even embryos of superior genetic merit though some positive attempts were made in creating infrastructures and extension programs.

3.1 SOME POSITIVE DEVELOPMENTS

Infrastructure and laboratory facilities at NLBC

National Livestock Breeding Center (NLBC) is presently equipped with state-of-the-art facilities for semen collection, evaluation, processing and storage. The center in recent years standardized its semen processing protocol by changing from conventional citrate buffer based extenders to Tris based one. This change in dilution protocol has made tremendous improvement in the quality of frozen semen of buffalo (post thawing motility increased form 30% to 48%). The existing post thawing motility of the cattle frozen semen is around 52% (NLBC 2006, Adhikari 2008). The quality of frozen semen produced at NLBC Pokhara is satisfactory based on seminal parameters and meets the post thawing quality of frozen semen set by DLS for importation purpose (*i.e.* 50% motility for cattle and 40% for buffalo semen) (DLS, 2004). There are still rooms for further improvement with gradual standardization of the process and protocol for semen processing. Provided pedigreed proven bulls are kept for semen collection, it is possible to produce frozen semen of genetic merit with quality comparable to international standards (60 % post thaw motility for cattle and 50% for buffalo semen). New tests (HOSST, microbial culture) for evaluation of the semen have been standardized and frequently applied for the assessment of *in-vitro* fertility status of the semen produced. Semen samples subjected to bacteriological cultures revealed no growth of any kind of micro-organisms in nutrient agar incubated at 37ºC (Malla, 2005. Adhikari, 2008) signifying adoption of aseptic measures in semen processing.

The existing laboratory facility is adequate for the production of sufficient number of semen doses needed in the country. The center needs to keep more number of bulls for increasing the production and diversify genetic spectrum. Semen collection/bull distribution should be carried out after sire evaluation so that only pedigreed proven bulls are used for semen production for AI or bull distribution for natural service.

Improved conception rates at the field level

In recent years, NLBC and Livestock Breeding Offices (LBOs) have monitored conception rate post AI. The mean conception rate for cattle is 52.4% with an increment of about 10% in the last decade. The figure for buffalo was 28.8% in the year 1996/97 that has increased to 45.31 % in 2006/07 (Adhikari, 2008; NLBC 2007).

Dairy Cattle Improvement Project

This project under FAO Technical Cooperation Program is being implemented jointly by DLS and NARC to establish a pilot performance recording scheme and identify superior cows. Around 5600 milking cows have so far been included in the recording scheme The superior cows thus identified are/will be mated with imported semen to produce the next generation young bulls. The project aims at comparing (based on the performance records) different types of crossbreds, which are most suitable for the Nepalese conditions. Finally, it will also recommend strategies that will help the private sector to undertake dairy cattle performance recording and improved breeding practices fully or partly (farmers and AI centre). The project duly recognizes the potential role of farmer organizations for its long-term sustainability, and NLBC in collaboration with NARC has to play pivotal role for its sustainability and shifting the responsibility to private sector, which the private sector can manage.

This project if implemented wisely will help in providing quality bulls and cows of high genetic merit. These bulls if used rationally would improve milk productivity per animal and make dairy production more profitable and efficient. In order to do so, bulls with high genetic worth need to be produced every year meaning this project has to run on a long term and sustainable basis. The NARC has to evaluate sire very precisely and provide juvenile bulls to NLBC every year and NLBC has to produce semen from these tested bulls. These bulls have to be replaced every year, if not at least once in two years.

Increased private sector involvement as service providers

The AI service covers around 7.93% of the milking cattle and 1.5% of the milking buffaloes. To fulfill growing demand for liquid milk in the country, this situation demands for extensive involvement of private sector to increase the AI coverage to at least 35% of the milking cows and buffaloes within 6-7 years. Meeting this target is possible if coordinated efforts are made involving private sector in the lead role in delivering AI service. The contribution of private inseminators is illustrated in Table 2.

Table 2: Contribution from private sector to AI promotion.

Parameter	FY 2005/06			FY 2008/09		
	Govt.	Pvt.	Total	Govt.	Pvt.	Total
Inseminations (nos.)	65462	8305 (12.7%)	73,767	84,038	22,057 (22.4%)	106,095
Liquid nitrogen (liter)	38493	2284	39,077	24,258	3,895	27,153
N2 Consumption/ insemination (liter)	0.58	0.27		0.45	0.17	
Mean AI per / inseminator /annum (nos.)				239	291	

Source: Adapted from NLBC (2006) and NLBC (2009). Figures in parentheses indicate percentages of the total inseminations

Private inseminators are more efficient in delivering AI service than are their government counterparts. They have higher mean inseminations number per technician and lower quantity of liquid nitrogen consumption per insemination than those of their government counterparts (Table 2). It implies that private inseminators appear to be more efficient in delivering AI services and handling liquid nitrogen. The present contribution to total inseminations is still underestimated because private inseminators under report their performance for several reasons including use of imported semen. A crude estimate indicates that about 8000–12000 inseminations, are not reported through official channels. If this figure is also included in the calculations, the private practitioner contribution will exceed 24% to total annual inseminations.

3.2 MAJOR ISSUES

Lack of clear policy on dairy animal breeding

A technical team under DLS prepared a draft policy for livestock breeding. The draft document is comprehensive and provides policy guidelines to conceptualize, plan, develop and implement breed improvement activities at the community level. It also considers comparative advantages of species and breeds of farm animals at the local and regional level. If this draft policy is approved, it opens door for other breeds of cattle to enter into the breeding system based on their performance in the local environment. It also visualizes scientific approaches to management of superior germplasm at the local and national level. However, unfortunately, the policy is not yet officially endorsed by the GoN.

NLBC focuses on AI program only

Genetic improvement of dairy animals through natural service is more important than AI service as many remote districts and even many areas within accessible districts do not have access to AI service. Much wider coverage in genetic improvement can be achieved if the service is managed properly through production and distribution of pedigreed proven bulls for natural service. NLBC should actively participate at the follow up of DCIP in collaboration with NARC for this purpose. Generally, farmers and DLSOs look for an established resource centers to purchase breeding stock. Therefore, NLBC programs should focus more on backstopping farmers managed resource centers by supplying proven bulls for the production of breeding stock of either sex.

Promotion of mono-breed "Jersey"

Farmers express their concerns that they do not find the semen of the breed of their choice. Among high yielding cattle, crossbreds of Jersey, Holstein Frisian, and Brown Swiss and to a limited extent

Tarentise exist in the country. Similarly, Murrah crossbred predominates among buffaloes. Farmers, especially those engaged in commercial dairying, have appreciated the benefits of HF breed of cattle and demand for its promotion from the government sector. However, since last several years, the DLS is refraining from promoting it without relying much on dependable scientific research. Research recommends that under intensive management system with good feeding and husbandry practices HF is more beneficial for commercial dairying (BRP, 2005). Informal observations in limited commercial farms also indicate similar results. This could be the result that about 10, 000 doses of HF semen are imported informally from India. The genetic merit of such imported semen is not known. Even sex-differentiated frozen semen has been introduced. Use of sexed sperms for AI is an ethical issue and needs policy for it.

In areas where HF semen is not available, technicians inseminate HF cows with Jersey semen. Farmers report progenies from such mating are inferior to their mothers. It is true that many farmers have cows yielding more milk than probably that of the mothers of the bulls kept at NLBC. Use of semen of such bulls in mass results in reduction in the mean productivity of the future progenies thus posing a setback instead of genetic progress ahead.

Jersey appears more appropriate in smallholder farming in mid and high hill conditions and for upgrading native breeds. Presently, the AI service is confined to crossbreds. Native cows are rarely inseminated.

Lack of breeding plans for AI/Natural service

Research results recommend that an exotic blood level of 50 – 75 % should be maintained for dairy cattle. No such authentic documentation is available for buffaloes. NLBC has to develop and execute a sound breeding plan. It then should maintain pure and crossbred bulls of different exotic blood levels to provide semen of the desired level. For example, semen from a HF bull of 75% exotic blood level will be appropriate to inseminate a HF crossbred cow of 50% exotic level to achieve 62.5% progeny. Similarly, pure Jersey bull semen will be appropriate for upgrading native cattle so that 50% blood level is achieved in F_1 generation itself. Arrangements have to be made for crossbreeding of native cattle through natural services in areas where AI service is not accessible.

Lack of resource centers of elite animals

The existing government farms (both DLS and NARC) are not adequate to produce the genetic materials for distribution to the farmers. Moreover, these farms also lack plans for genetic improvement through use of modern technologies (AI and embryo transfer). DLS however is attempting to establish resource centers of cattle and buffalo at farmer level. The effort is made through implementation of *Brihattar Gai or buffalo Bikas Karyakram'* the only program implemented so far for developing a resource center. However, there is no operational recording system. Performance recording is yet to be initiated in villages where these programs are implemented. The program needs strong technical backstopping on record keeping; breed selection and artificial insemination so that at the end the successful sites could be developed into reliable resource centers for cattle and buffalo.

Newly implemented Dairy Cattle Improvement Project by NARC and DLS with support from FAO through its Technical Cooperation Program will help in producing bulls and cows with defined genetic potential. It will strengthen DLS endeavor to produce the dairy animals of superior genetic merits. However, to achieve desirable results, much needs to be carried out to materialize breeding plan for continuation of the programs after the FAO funding terminates. Both NARC and DLS have expressed their commitment to its continuation. They have to work together to delineate specific roles and responsibilities for its smooth implementation.

Indiscriminate purchase/import of breeding stock

National Livestock Breeding Center purchases bulls for semen collection mostly from farmers based on phenotypic evaluation. The genetic merit of such bulls is not predictable. Similarly, farmers have to rely on random purchase, as there are no established resource centers of cattle and buffaloes based on evaluation. Moreover, farmers tend not to grow calves of either sex in majority of the instances resulting in inadequate supply of dairy animals within the country. In the recent years, enhanced demand for milk has encouraged farmers to purchase dairy animals (both cattle and buffalo) from adjoining border areas of India. Field observation revealed that the genetic merit of such animals is

questionable. Moreover, animals with past record of infertility, mastitis and other infections are imported. For example, incidence of hemoprotozoan diseases is spreading in new areas. This situation has contributed to emergence of new diseases that the technicians are not capable to address. This has discouraged farmers who have chosen dairying as a major vocation.

Inefficient semen and liquid nitrogen distribution

Interruption in supply of semen and nitrogen for AI services has been a consistent problem in Nepal. Public sector management model appears to be most inefficient in managing production and distribution of liquid nitrogen to DLSOs and private insemination centers. Moreover, private inseminators are discouraged through discriminating policy. For example, Government AI centers receive liquid nitrogen for free and semen for NRs 25.0 a straw. In contrast, private inseminators are charged for both semen and liquid nitrogen. Many private practitioners are affiliated with MPCs, CBOs, local NGOs, private dairies and Technical Schools. NLBC should consider developing a system of AI input delivery based on a business principle where the supplier (NLBC) and customer (inseminators) consider themselves as partners and build on mutual respect. Private inseminators struggle to receive the inputs in desired quantity and time despite they have to pay for the inputs of the service that public sector is promoting.

Lack of recognition of farmers' role in livestock improvement program

For the government, management of AI service is getting more expensive over years. In 2006/07 revenue generated from AI was 2.73 million against the expenditure of NRs 25.62 million to produce frozen semen and purchase and distribute liquid nitrogen. Even then, the demand for AI service was unmet due to short supply of semen and liquid nitrogen. The situation is not different even now. Many AI centers frequently run out of liquid nitrogen and temporarily suspend AI service. Such situation is very discouraging during the promotional phase of AI service. This is the single most reason that farmers solely do not rely on AI service. With assured delivery of inputs, AI service can be expanded tremendously.

Important to note is that AI service in Nepal is a private activity irrespective of who is the service provider. More than 90% of the inseminations are carried out at on-site where the services are paid and involve fee payment for the service. Farmers contribute through fee payment about NRs 19.7 million annually (As 90% of total inseminations involve fee payment with a fee of NRs 250 per AI the total amount equals 87,411 * 0.9 * 250 = NRS 19,677,475.0 for receiving the service. But, they have little role in management of the AI service in the country. Part of this fee is the revenue of NLBC from semen and liquid nitrogen that inseminators pay in advance. One school of thought is that selection of breeding stock of genetic merit and production and distribution of semen and liquid nitrogen could be improved tremendously if farmer is role is increased in the management of NLBC as an autonomous corporate organization.

4. FUTURE DIRECTIONS

For efficiency, effectiveness and sustainability of the AI service and improving its quality following strategies are recommended.

4.1 Involve private sector in organizational management of artificial insemination program

The situation discussed above demands for organizational revamping of the AI program at the national level to make it more scientific and farmer focused. Presently, farmers do not have any role in the management of AI program. The government investment and revenue generated from farmers are not converged for the scientific promotion of the AI program in totality. With initial promotional investment for about seven years along with convergence of resources collected from the beneficiaries, AI program can be operated as financially viable corporate business. The AI program possesses potential for complete privatization in the future. However, as it is at the promotional phase, it does not appear to be the priority area of the private sector. It is anticipated that due to current political instability, about seven years of interim arrangements are required to make policy change, strengthen private sector capacity, train and expand AI service, and transfer the public ownership to the corporate body. Genetic progression of the dairy animals will take place at quicker pace with farmers' participation in performance recording and in organizational management of NLBC. With this perspective, government should consider its role in emphasizing initial promotional investment and

then gradually shifting its role to ensuring quality and competitive services. To realize this, following interim arrangements are suggested:

- Prioritize AI service and declare, in breeding policy, that AI service will be promoted from the state for 7 years now on and as a corporate body thereafter.
- Develop 7 year plan for transforming NLBC into a corporate company under PPP principle
- During the interim period, strengthen private sector capacity and create an environment for level play among government and private inseminators.
- Form a National Livestock Breed Management Committee involving national experts, farmers, and stakeholders from private and public sectors. The Committee will manage semen and liquid nitrogen production/purchase and distribution during the interim period. The committee will also manage technical staff.
- Authorize the Committee for management of AI program including import and export of germplasms of genetic merit.
- Create a National Livestock Breed Improvement Fund (NLBIF). Revenue of semen and liquid nitrogen will revolve in the fund itself.
- Continue Government annual contribution not exceeding present annual budget of NLBC and RLBOs (the first year grant will be equal to present annual NLBC and RLBOS budget – revenue). Reduce gradually government's annual contribution based on annual review as the Fund capacity is strengthened. Phase-out government contribution after seven years.
- Expand AI program in all potential areas (service on demand basis). Aim and develop program for 500,000 inseminations annually within 6 - 8 years (this is a possible target).
- Phase out government's role in the above committee gradually ensuring a self-sustainable NLBI Fund as a financially viable corporate enterprise involving MPCs and private sectors.
- Declare NLBIF a corporate company and workout public and private share proportions based on valuation of the net- worth.

For example, if the current year inseminations of 160,000 (NLBC 2010, personal communications) is increased to 500,000 and NRs 50.0 per insemination is repaid as revenue in the NLBIF, the total annual revenue will be 25.0 million. This amount will form the major investment though not sufficient for operating a national livestock breeding program including artificial insemination. It is anticipated that farmer cooperative will fund partially for AI program if they have the stake in its management.

4.2 Supply the semen/bulls of proven genetic merit

The genetic merit of the frozen semen produced from NLBC is questionable though the quality based on seminal parameters is satisfactory. Commercial farmers are reluctant to use semen produced from NLBC. A stock of frozen semen of proven genetic merit must be available to farmers (including that of HF breed) at NLBC. To make sure that every farmer has access to broader selection of different sire semen from at least four bulls must be available to the inseminators with well documented pedigree record to avoid inbreeding. For the time-being part of the semen has to be imported. This should continue until NLBC will produce pedigreed frozen semen from the bulls selected from Dairy Cattle Improvement Project.

4.3 Expand private AI centers

Aim for 500AI centers from the existing 203 within three years and about 800 by the end of seventh year. (NB: some 100 additional AI centers are established from government/non-government sector during the CLDP period). **Supply semen and liquid nitrogen to official and private AI technicians and veterinarians with no preference and no price difference.**

4.4 Develop reliable source of liquid nitrogen

Production of liquid nitrogen from private sector has already begun. However, mechanism for distribution is lacking. Create liquid nitrogen storage depots at strategic locations involving MPCs and private sector. (CLDP has supported in creating one as a model at MBMAN, Urlabari, Morang that stores more than 200 liters of nitrogen at a time and sells to affiliated inseminators). NLBC should undertake feasibility study on selling nitrogen as a tradable commodity and establishing its distribution network at strategic locations from private sector.

4.5 Continue DCIP and execute a new one for buffalo

A project on buffalo breed improvement similar to Dairy Cattle Improvement Project should be developed and implemented by NLBC in collaboration with NARC.

4.6 Participate in sire evaluation scheme with NARC

The mandate for sire evaluation lies with NARC as it involves extensive data management and analysis. NARC has expertise on data management including genetic evaluation and ranking of bulls and cows based on performances. It should be an on-going collaborative activity between NLBC and NARC. NLBC should increase number of bulls required for semen collection (28 cattle and 12 buffalo). NLBC must purchase four times the number of bulls required for semen collection and select from among them after the evaluation of the sires. This necessitates the capacity to accommodate 40x 4 = 160 bulls. NARC also needs strengthening its capacity for research in new technologies for sire evaluation including gene marker assisted selection (MAS).

5. CHALLENGES

Enhancing milk productivity through genetic improvement is possible but following challenges must be perceived and addressed adequately along with genetic improvement programs.

- Breed improvement in smallholder farming system is tedious and needs commitment and dedication of experts until farmers fully understand the importance and benefits.
- Elite herds of cattle and buffaloes established from private sector might need additional technical support from the government.
- Sustaining the productivity of improved animals needs strong support for feeding, management and health care services. The supporting programs must go hand in hand.
- Government should continue its support for prevention, control and eradication of economically important diseases

ACKNOWLEDGEMENTS

The authors express their sincere thanks to all the farmers who interacted and contributed valuable information to prepare this paper. The authors express their sincere gratitude to Dr. Surya Bahadur Singh, DTL, FAO-TA/CLDP, for his critical comments and suggestions. Mr. S. N. Mahato and staff of NLBC deserve authors' special thanks for providing relevant information and sharing the ideas.

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THE EFFECT OF MEDICATED AND NON-MEDICATED UREA MOLASSES MULTI-NUTRIENT BLOCK (UMMB) SUPPLEMENT AGAINST NEMATODE INFECTION AND MILK PRODUCTION AND MILK COMPOSITION

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ABSTRACT

An experiment was conducted for 4 weeks on cross-breed lactating cattle at IAAS livestock farm to find out the effect of medicated and non-medicated UMMB supplementation on nematodes load, milk yield and milk composition. For this eight cross-breed cattle of 3rd to 4th parity and between 2nd to 3rd months of lactation were selected and were allocated into four equal groups. Group A was kept as control (on routine feeding), Group B was supplemented with UMMB 400gm/cow/day for 28 days, Group C with medicated pineapple UMMB on 1st day and same as group B, rest of the days and Group D with medicated albendazole UMMB on 1st day and same as group B, rest the of days. The blocks were prepared manually by hot method. The ingredients were 40% molasses, 10% urea, 4% mineral mixture, 6% mustard cake, 4% salt, 14% cement and 22% rice bran. For medicated UMMB pineapple leaves powder@200mg/kg body wt. and albendazole @7.5mg/kg body wt. was used. At the end of experiment, nematodes load decreased by 92.85%, 77.77% and 25% in albendazole, pineapple and plain UMMB group respectively. Milk analysis was done by Lacto scan and Milk yield was found to be increased by 12.19% in plain UMMB group, 13.81% in pineapple medicated group, 16.66% in albendazole medicated group and decreased by 6.25% in control group. Similarly milk fat, SNF, lactose and protein was increased by 24.32%, 2.93%, 2.57%, and 3.77% respectively as compared to control group. There was no significant change in milk composition between medicated and non-medicated groups.

INTRODUCTION

Agriculture plays a vital role in Nepalese economy. It contributes 39.4 percent of share to the Gross Domestic Product (GDP) and supports about 65.7 percent of the population (MOAC, 2009). Among the agricultural commodities, livestock plays significant role in the agricultural development and economic empowerment of the country. It contributes 31% to the National Agricultural Gross Domestic Product (CBS, 2004). Among various sub-sectors of the Nepalese economy, milk production is an important area. Dairying accounts for about two thirds of the livestock sub-sector. The average growth of milk production over the last decade was about 2.6% per year. The total animal population in Nepal is 46 million, among them population of dairy cattle and buffalo is estimated to be 7 million and 4.6 million which produces 14,45,000 metric ton of milk every year in which the dairy cattle contribute around 30% of milk and buffalo contribute around 70% of milk (MOAC, 2009).

In developing countries like Nepal, livestock are fed mainly on low quality roughages including natural grazing and agro-industrial by products such as cereal straws/stovers, sugarcane by products and other similar feeds, all of which contain large quantities of ligno-cellulose material. These feeds are deficient in protein, energy, minerals, and vitamins. In addition, at certain times in the year, the quantity of grazing and browse deteriorates substantially due to seasonal influences. Addition of foliage from tree leaves or supplementation with seed meals or urea-molasses multi-nutrient block, can improve the utilization of low quality roughages mainly through the supply of nitrogen to the rumen microbes. Attempts to increase the productivity of ruminants in developing countries encounter several constraints. Of the health constraints, bacterial and viral diseases can be successfully controlled through conventional vaccination and quarantine procedures. However, for parasitic diseases, these approaches are either not yet possible or impractical so chemotherapy along with grazing management is the only methods to control. But indiscriminate use of chemical anthelmintics develops resistance of helminthes to various anthelmintics compounds. For this reason screening of medicinal plants of anthelmintics property remains scientific interest. Herbs, shrubs, and trees are available in village areas of Nepal having anti-parasitic properties. Among them pineapple leaves is one of them

which is found abundant in Chitwan. So, determination of efficacy of potential herbs in controlling parasitic burdens of animals is very important for sustainable improvements of productivity of animals.

MATERIALS AND METHODS

The experiment was performed in IAAS livestock farm. The total of eight animals with same parity and of same lactation was chosen. The selected animals were ear tagged for identification.

The animals were divided into four equal groups as shown below:

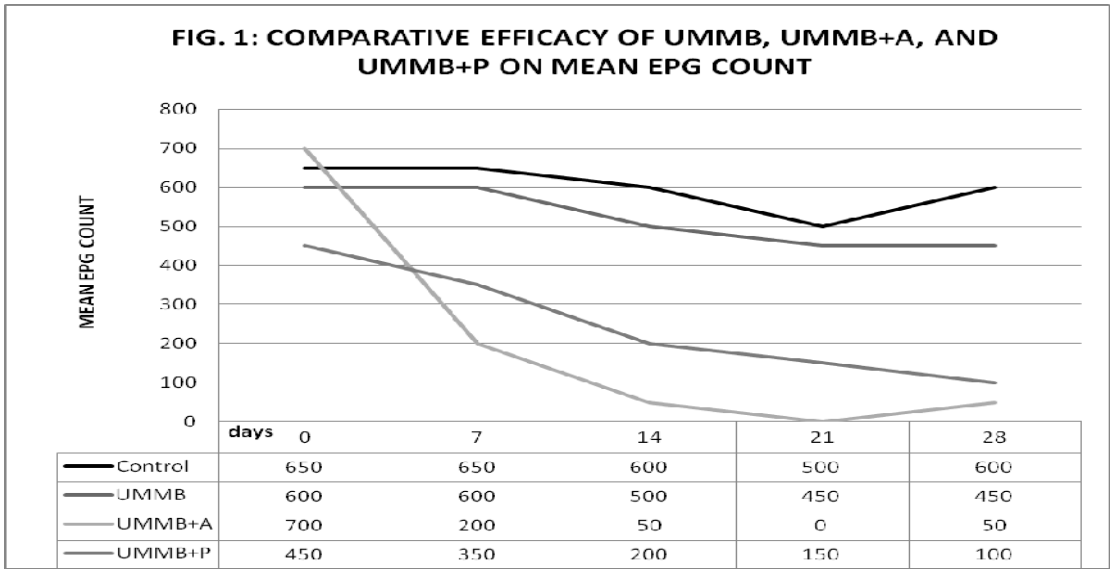
- GROUP A was kept as a control (on routine feeding)
- GROUP B was supplemented with 400 gm UMMB per cow per day for 28 days in addition to routine feeding.
- GROUP C was given medicated blocks with pineapple leaves in first day and then UMMB as group B
- GROUP D was given medicated blocks with Albendazole in first day and then UMMB as group B.

➢ UMMB was prepared by hot method and was fed to the cattle

➢ Milk was collected and measured at milk parlor with the help of measuring jar and milk composition (Fat, SNF, lactose, protein) were analyzed using ultrasonic milk analyzer at DLSO, Bharatpur. Milk was collected for analysis 7 days before feeding and at an interval of 7 days after feeding UMMB on 7,14,21&28 days.

RESULT AND DISSCUSION

Effect of Medicated and non-medicated UMMB on nematode load



Group of animals treated with albendazole medicated UMMB and pineapple medicated UMMB shows 92.85% and 77.77% decrease nematodes load respectively animals treated with plain UMMB and control shows 25% and 7.69% reduced load. In one study in Myanmar, Daing et al. (2006) reported efficacy of pineapple >79% and albendazole 94%.

On milk yield

Days	Control	UMMB	UMMB+A	UMMB+P
0	3.2	6.15	4.2	5.55
7	3.2	6.3	4.3	5.65
14	3.15	6.55	4.55	5.75
21	3	6.8	4.8	6.1
28	3	6.9	4.9	6.26

Similarly the group treated with albendazole medicated UMMB, pineapple medicated UMMB, plain UMMB, showed increase in Milk production by 16.66%, 13.81% and12.19% respectively and in control decrease by 6.25%. Sharma (2008) found that the milk yield was increased by 10.66% in UMMB treated group. Milk yield increased because the herbal antihelminthics have reduced the nematodes load of cow and consequently more nutrients were available to the animals for production purpose (Aktar, et al. 2003).

On Fat percentage

Animals treated with UMMB, increased fat by 24.32% as compared to animals treated with pineapple medicated UMMB (22.60%) and Albendazole medicated UMMB (20.59%). As milk yield increases Fat% and SNF decreases this may be the cause as milk yield was increased more in albendazole group and pineapple group than normal UMMB group. In control group slight fat% was increased this may be due to stage of lactation as stage of lactation advances fat% increases.

SNF

Similarly SNF increased by 2.93% in animals group treated with plain UMMB as compared to control that increased by 0.73%.and in UMMB, by 2.36% medicated albendazole group and by 2.57% in UMMB medicated pineapple group.This shows that there is no significant change in SNF among medicated and non-medicated group but change among treatment and control group.

Lactose

Lactose increased by 2.57% in animals group treated with plain UMMB as compared to control increased 0.92%.and in UMMB medicated albendazole group by 2.13% and in UMMB medicated pineapple group by 2.69%.This shows that there is no significant change in Lactose among medicated and non-medicated group but change among treatment and control group.

Protein

Protein increased by 3.77% in animals group treated with plain UMMB as compared to control that increased by 1.44%.and in UMMB medicated albendazole group by 3.01% and in UMMB medicated pineapple group by 3.06%.This shows that there is no significant change in protein among medicated and non-medicated group but change among treatment and control group.

CONCLUSION

Medicated UMMB was found effective against nematode loads, besides both medicated and non medicated UMMB was effective in increasing milk yield. There was drastic change in fat % in UMMB treated animal group as compared to control group but there was no significant changes in fat % among medicated and non -medicated groups. There was increased in SNF%, Lactose%, and Protein % among treatment and control groups with significant change between medicated and non-medicated block in other milk composition. Feeding medicated UMMB containing herbal antihelminthics to dairy cows under rural condition is more profitable than non-medicated UMMB and medicated UMMB containing albendazole.

In Nepal, low quality fibrous forage lack essential nutrient for optimal growth and production of livestock, so use of UMMB could be a better option. Nutrient deficiencies (particularly protein, energy and minerals) and parasites infestation are common in ruminant livestock raised by smallholders. Poor performance in terms of growth rate, milk yield, reproduction, and heavy mortality in young animals due to parasitic infection are factors that limit farmer's income. Limitations are generally attributed to poor nutrition of the animals as well as to poor understanding of the farmers of overall nutrition and feeding. The use of UMMB and medicated UMMB has been practical and effective in overcoming nutritional problem in dairy cattle.

RECOMMENDATION

- Further research work should be conducted at different dose rate of medicated block for longer duration of time.

- Dissemination of UMMB technology should be done at different levels by government, NGO's and INGO's to farmer's level.
- This technique is highly recommended in farms like IAAS, where livestock are supplied with straw based diet rather than concentrates.
- Further research on UMMB of different formulation should be made to know their effect on growth rate, feed intake, reproductive performance of cattle and buffalo.

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A CLINICAL STUDY ON ANESTRUS BUFFALOES IN SOUTHERN NEPAL

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ABSTRACT

Anestrus is one of the most important reproductive disorders in dairy buffaloes. The clinical feature of anestrus in buffaloes, however, has not been well described. The objectives of this study were to describe the causes of anestrus in buffaloes and their reproductive performance after treatment under the field condition in southern Nepal. Of the 135 anestrous buffalo cows, 61.4% had true anestrus with ovarian dysfunction and 33.3% had silent ovulation. In 111 buffaloe heifers, 76.6% were in true anestrus and 18.9% had silent ovulation. Duration of anestrus after calving was longer than 6 months in 83%. And 61.5% of the cows had durations longer than 10 months. The interval between the last breeding and diagnosis of anestrus was more than 5 months in 67.4% of cows and heifers. Treatment of anestrus with prostaglandin F $F_2\alpha$ in cows and heifers with the corpus luteum resulted in a higher pregnancy rate within one ($P<0.01$) and two months ($P<0.05$) after treatment as compared with treatment with vitamin/mineral mixture. Buffaloe cows and heifers with inactive ovaries bearing a dominant follicle were also successfully treated with gonadotropin releasing hormone, resulting in a pregnancy within one month after treatment ($P<0.05$). In conclusion, predominant causes of anestrus in dairy buffaloes in this region was true anestrus with inactive ovaries, and the duration of anestrus after calving as well as breeding was extremely long. Routine reproductive examination and adequate hormone treatment may improve the reproductive performance of these buffaloes.

INTRODUCTION

It has been commonly recognized that buffaloes have severe reproductive problems causing infertility. Anestrus and repeat breeding are two major reproductive disorders (Goley et al, 995; Pandya et al, 1989). This study aimed to show some clinical features of anoestrous buffaloes in Chitwan District, Nepal.

MATERIALS AND METHODS:

This study was conducted on Murrah graded buffaloes which were referred to infertility camps organized by the Veterinary Teaching Hospital, Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan District, southern region of Nepal, in collaboration with some of the dairy cooperatives and village development committee offices in the region during the breeding season in three consecutive years from 2001 to 2003. Three hundred and six buffaloes which had not been observed in estrus for more than two months after calving or after breeding were brought to the camps for diagnosis and treatment. Breeding history, milk yield, feed and fodders fed, were obtained by interviewing the owners before the gynecological examination. Body condition was checked and recorded as good, fair or poor. Among 306 buffaloes not observed in estrus after calving or after breeding 60 (19.6%) were diagnosed pregnant by palpation per rectum and were, therefore, excluded from the group of animals for further investigation. The 246 anestrous animals were subjected to different treatment based on clinical findings and the interval from calving to diagnosis and their age.

Parity of anoestrus buffaloes: Of 246 anestrous buffaloes 111 (45.1%) were heifers and 135 (54.9%) were cows. The parity of the buffalo cows ranged between one to nine, majority being one to four.

Breeding history: In buffaloes with postpartum anestrus the intervals in months between the last calving and diagnosis were longer than 6 months in 83% of buffaloes; 79 of 135 (61.5%) buffaloe cows were 10 months after calving or later (Table 1). Of 246 cows and heifers, 135 animals had post-breeding anestrus, not returning to estrus after breeding although they were not pregnant. Interval from last breeding to diagnosis in 66.0% of the heifers and 63.0% of the cows were longer than 5 months, and approximately 10.0% of the heifers and 12.0% of the cows were already 11 months after breeding or later (Table 2).

Milk yield and feeding: Buffalo cows were milked twice a day in the morning and evening. The lactation period ranges between 240 and 270 days. Buffaloes generally suckled their calves only when they were milked. Farmers allow the cows to suckle only for a few minutes to give the udder the stimulation before starting milking and again for another few minutes after the completion of milking. Then, the calves are separated. The duration of suckling is four to six months. Daily milk yield at the day of diagnosis was 1 to 2 kg in 19 buffaloes (28.4%), 3 to 6 kg in 31 buffaloes (46.3%) and 7 to 12 kg (25.4%) in 17 buffaloes. The buffaloes were fed with 5 to 10 kg of rice straw, 5 to 20 kg of green fodders, and 0.5 to 2 kg of wheat bran depending on their milk yield. Concentrate feed was not consistently fed to low producers (less than 2 kg per day), but for high producers (5 kg or above) 2 to 5 kg of concentrate feed was given.

Body condition: The body condition was poor in 42% of the heifers and 35% of the cows. All the other cows and heifers had moderate body condition.

Reproductive examination: All the buffalo with anestrus which were brought to the infertility camps during a period from 2001 to 2003 were palpated per rectum for the morphological examination of the reproductive tract by the authors themselves. In buffalo cows vaginoscopic examination was also conducted.

Diagnosis and treatment:
Buffaloes with corpus luteum (CL): anestrus buffaloe cows with a normal CL about 10 mm in diameter or larger were considered to be in luteal phase after silent ovulation. Among the anestrous animals, those which had been already 12 months after calving or more were injected intramuscularly with 25 mg prostaglandin $F_2\alpha$ ($PGF_2\alpha$; Pronargon F, tromethamin dinoprost, Pfizer, Tokyo). All the other animals were prescribed to administer per os vitamin/mineral mixture for three weeks [12]. Heifers with CL at the age of 2.5 years old or more were treated with $PGF_2\alpha$ and the others were treated with vitamin/mineral mixture.

Buffaloes with dominant follicle (DF): 10 to 12 mm in diameter or larger and without CL: Probably not cycling but having DF which may respond to GnRH. Anestrus buffaloe cows 12 months after calving with DFs greater than 12 mm and without CL were injected intramuscularly with 50 μg fertirelin acetate (Shering Plough Animal Health Co. Ltd., Tokyo). All the other cows were treated with vitamin/mineral mixture. Two and a half years old or older heifers with DF were injected with GnRH and the others were given vitamin/mineral mixture.

Buffaloes without CL and DF: buffalo cows and heifers with inactive ovaries were treated with oral administration of vitamin/mineral mixture. In the anestrus buffaloes with indications of cervicitis, one liter of 1% Lugol's solution was infused into the vagina. The anestrus buffaloes in which infection with internal parasites were suspected because of extremely poor nutrition were administered orally with 3 g oxyclozanide and 3 g tetramisole hydrochloride for deworming**.** Buffaloes with large follicles without CL: The buffaloes were considered to have had anestrus type of follicular cysts, when they had follicular structure 2.0 cm in diameter or larger without co-existence of CL. These cows and heifers were treated intramuscularly with 50 μg GnRH analog.

Mating and pregnancy diagnosis after treatment: all the anestrus buffaloes treated were observed for estrus behavior and signs for two months or longer. Breeding was conducted basically by natural mating after detection of estrus. The animals were checked for pregnancy 2 to 3 months after the last breeding. Statistical analysis: Difference in percentages between two groups was analyzed by Fisher's exact test.

RESULTS

Clinical findings
Ovaries: On palpation pre rectum, CL 10 mm in diameter or larger was found in 33.3% of anestrus buffaloe cows, DFs in 33.3%, and ovarian cysts in 5.2%. Twenty-eight percent of cows had neither CL nor DF. In heifers 18.9% had CL, 42.3% had DF, and 4.5% had ovarian cysts, while 34.2% had neither CL nor DF. Uterine horns: The diameter of the uterine horns estimated by palpation per rectum ranged between 1.0 and 2.0 cm in heifers. Of 135 cows 116 cows (85.9%) had uterine horns 1 to 2 cm in diameter and 19 cows (14.1%) had uterine horns 2.5 to 3.5 cm in diameter.Uterine contraction was

not detected in 66.7% of the heifers and 74.8% of the cows. Vaginal mucus discharge: Purulent discharge was detected on vaginoscopy in 2.7% of the heifers and 2.9% of the cows. No mucus was seen in 91.2% of the heifers and 73.3% of the cows. Effects of Treatment: since the effects of treatment in terms of reproductive performance did not differ between heifers and cows, data in heifers and cows were combined to compare the pregnancy rate after different methods of treatment (Table 3). Treatment of anestrous cows and buffaloes with CL using PGF2α resulted in a higher pregnancy rate within one month (P=0.0064) and two months (P=0.0239) after treatment than treatment with vitamin/mineral mixture. Buffaloes with inactive ovaries treated with GnRH showed a higher pregnancy rate within one month after treatment (P=0.0201) than those treated with vitamin/mineral mixture (Table 3).

CONCLUSION

In conclusion predominant causes of anestrus in dairy buffaloes in this region were true anestrus and silent ovulation, and the duration of anestrus after calving and breeding was extremely long. Routine reproductive examination and adequate hormone treatment may improve the reproductive performance of these buffaloes.

ACKNOWLEDGEMENTS

This study was partly supported by Heiwa Nakajima Foundation, Tokyo, Japan, and Grant- in- Aid for Scientific Research (B) (2), Ministry of Education, Culture, Sports, Science and Technology, Japan. Donation of $PGF_2\alpha$ by Pfizer, Tokyo as well as fertirelin by Shering Plough Animal Health, Tokyo, are acknowledged with great thanks.

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Table 1. Interval in months from last calving to diagnosis of anestrus in buffaloes

Months after calving	Numbers of animals	Percentage
2-3	7	5.2
4-6	16	11.8
7-9	29	21.5
10-12	27	20.0
13-15	13	9.6
16-	43	31.9
Total	135	100.0

Table 2. Interval in months from last breeding to diagnosis of anestrus in buffalo cows and heifers

Months after breeding	Numbers of animals	Percentage
3-4	44	32.6
5-6	38	28.1
7-8	20	14.8
9-10	6	4.4
11-12	14	10.4
13-	13	9.6
Total	135	100.0

Table 3. Reproductive performance in anestrous buffalo heifers and buffalo cows after treatment

Clinical findings	Number of buffalo heifers and cows treated						
	CL N=66		DF / No CL N=92		No CL / No DF N=76	F Cyst N=12	Total N=246
Treatment	$PGF_{2\alpha}$	Vit/M	GnRh	Vit/M	Vit/M	GnRH	
No. of heifers and cows treated	22	44	11	81	76	12	246
Cows in estrus / mated within one M	22	28	11	52	49	12	174
Estrus detection rate (%)	100.0	63.6	100.0	64.2	64.5	100.0	70.7
Cows conceived within one M	19	22	10	41	39	10	141
Pregnancy rate within one M (%)	86.4a	50.0b	90.9x	50.6y	51.3	83.3	57.3
Cows conceived within two M	3	13	1	25	24	2	68
Pregnancy rate within 2 M (%)	100.0x	79.5y	100.0	81.5	82.9	100.0	85.0

a,b Values in rows with different superscripts differ ($P < 0.01$)
x,y Values in rows with different superscripts differ ($P < 0.05$)

COMPARATIVE STUDY ON POVIDONE IODINE CREAM AND POVIDONE IODINE SOLUTION AGAINST SUB-CLINICAL MASTITIS OF DAIRY CATTLE UNDER FARMER'S MANAGEMENT IN NEPAL

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ABSTRACT

A field study conducted on lactating dairy cattle in Piple and Thakre VDC of Dhading district showed that both cream based and solution based preparations of Povidone Iodine were equally effective in reducing the level of sub-clinical mastitis in dairy cows. This was clearly evident by significant reduction in somatic cell counts in both groups as compared to the control animals. Furthermore, the somatic cell count decline was not different between cream based application and solution based application (teat dipping) of Povidone Iodine, which suggests that ointment based application was also equally effective as the Povidone Iodine teat dips against sub clinical mastitis of dairy cattle. The cream based application is easier for use in the field conditions minimizing the problem of spilling over, regular topping up and insufficient application of the chemical for teat dipping. As compared to dipping, ointment based preparation is cheaper, easier to handle and transport and compatible to the traditional practices of small holder dairy farmers, thus helping in reducing the prevalence of clinical and sub-clinical mastitis of dairy animals under Nepalese farming system.

INTRODUCTION

Nepalese dairy sector is characterized by predominance of small-holder production system with traditional and little or no preventive health care system adopted. Thus, high yielding animals have suffered from the high incidence of production diseases like mastitis causing serious losses in animal productivity and minimizing farmers' resources.

Subclinical mastitis is more common in animals but without any clinical symptoms except causing reduction in milk production. Thus, early detection of mastitis is important to reduce production losses and to enhance the prospects of recovery. Among the total loss due to mastitis, 70-80% is associated with sub clinical mastitis, while 20-30% is due to clinical mastitis (Philpot & Nickerson 1991). The economic losses sustained by dairy farmers due to mastitis are because of milk discarded due to its abnormal characters and poor quality, reduced milk production, decreased market value of cow due to damage of quarters and treatment cost (Chakrabarti, 2003) while subclinical mastitis has been reported to reduce the milk yield by about 25%.

Various studies in Nepal have recorded high incidence of mastitis in dairy animals. Joshi *et al.*, (1998) recorded 19% clinical mastitis in Murrah cross buffaloes and 9% in local cattle, while the prevalence of sub-clinical mastitis (SCM) has also been reported to be about 20% in cow and buffaloes in the eastern hills (Jha *et al*. 1993) and about 30% in western hills (Joshi & Joshi, 1997) and in Chitwan valley (Dhakal & Tiwari 1992). Isolation and identification of micro-organisms involved in mastitis revealed that more than 50% of the cases are caused by environmental bacteria (Joshi & Joshi 1997). Furthermore, about 78% of the clinical cases occur during the first week of calving, thus major risk period of clinical mastitis is of short duration (Joshi *et al.,* 1997). The prevalence of sub-clinical mastitis was recorded to increase with the advancement of lactation and about 15% of the sub clinical cases develop into clinical cases during the same or subsequent lactation (Joshi & Joshi 1997).

Somatic cell count in milk has been used as a useful indicator test to monitor the quality of milk. The cells consist of leukocytes, lymphocytes and epithelial cells. The normal number is 100, 000-300,000 cells/ml (Nielsen & Ullum 1989). Counts of less than 250, 000/ml are considered to be below the limit indicative of inflammation although most normal quarters show less than 100, 000/ml. the total count reflects the amount of gland involved in the inflammatory process whereas the neutrophil count

reflects the stage of the inflammation. A high total count (e.g. 1000,000/ml) and a high proportion of neutrophils (e.g., 90%) indicate acute inflammation affecting much of the quarter. A low total count (e.g., 500,000/ml) and a low proportion of neutrophils (e.g., less than 40%) indicate a small chronic lesion (Radostits *et al.* 2000). The normal cell count is between 100,000-250,000 cells/ml, excess of which is regarded as sub-clinical or clinical mastitis.

It is also reported that *in-vitro* sensitive antibiotics are not always successful during *in-vivo* application, which necessitate the introduction of preventive approaches to minimize the incidence of mastitis in high yielding dairy animals (Radostits *et al.*, 2000).

Post milking teat dipping in povidone iodine solution has been evaluated in Nepal in various milk production pockets of the country (Joshi & Joshi 2001; Joshi 2002; Joshi 2003). The responses to post milking teat dipping in povidone iodine has been found promising to reduce the incidence of sub clinical mastitis, reduction in somatic cell count and consequent improvement in milk yield of the animals. However, farmers complained of some practical difficulties in its regular application in the small holder production system. The fluid nature of the solution poses risk of spilling over and problem of regular topping up, which leads to irregular and insufficient application of the chemical for teat dipping reducing its effectiveness. Moreover, some farmers also complained of teat rash and dermatitis if emollient was not added in povidone iodine solution.

This study was thus undertaken to address these problems and to ease the application of povidone iodine under small holder production system. The approach was to introduce cream based preparation packaged in the collapsible tubes, which would be more appropriate and easy for the farmers for regular application. Hence, the study was carried out to evaluate the efficacy of cream based application of povidone iodine in comparison to povidone iodine teat dipping under the farmer's management.

MATERIALS AND METHODS

Description of the study area

The study was carried out in the dairy pocket areas of Piple, Thakre VDC of Dhading District. The dairy animals in the study area chiefly consists of cross bred cattle and buffaloes supplying milk to dairy development corporation (DDC), Kathmandu via local milk cooperatives.

Collection of milk samples

The animals were selected at random from a large population irrespective of age, parity and management system. Animals within first month of lactation and late lactation were excluded to avoid false positive results. All relevant information like farmer's name and address; age of animal, parity, date of calving and date of collection of samples were duly recorded. For California mastitis test (CMT) and Somatic cell count (SCC), four sterilized Mac Cartney bottles were used for each animal. The farmers were asked to carry out the hygienic measures during the collection of milk sample. First few streams of milk were discarded. Nearly 20 ml of milk sample from each quarter was collected in a separate bottle. The bottles were labeled as right fore (RF), right hind (RH) left fore (LF) and left hind (LH) as per the quarter from which the milk was collected. Altogether 364 milk samples (four samples from each animal) were collected from 91 cattle from the study area.

California Mastitis Test (CMT)

California mastitis test (CMT) is based on increased leukocyte count and increased alkalinity of the milk sample. These alterations are due to inflammatory exudates (leukocytes) and increased content of basic salt i.e. alkalinity (Chakrabarti 2003). CMT was performed on the freshly collected milk samples as per the procedure of Schalm & Noorlander (1957) employing the modified reagent of Pandit & Mehta (1969).

Examination of milk samples by CMT reagent

California Mastitis Test (CMT) was performed on the freshly collected milk samples as per the procedure of Schalm & Noorlander (1957) employing the modified reagent of Pandit & Mehta (1969) in the farmers' shed as a cowside test. Three millilitres of milk sample from each quarter and equal

amount of the CMT reagent was placed into each cup of CMT paddle and the content was mixed by gentle circular motion of the paddle in a horizontal plane. Sample with a slight slime with no tendency of gel formation as trace, and a distinct slime with no tendency to gel formation was regarded as weak positive (+) reactors. The mixture that thickened immediately with gel formation and on continued swirling, mass moving around the periphery leaving the bottom of the cup exposed was confirmed as distinct positive (++) reactors. Similarly a distinct gel formed, which tends to adhere to the bottom of the paddle and during the swirling a distinct central peak formed was regarded as strong positive (+++) reactors.

Somatic cell count (SCC)

Somatic Cell Count (SCC) is the total number of leukocytes or white blood cells per milliliter of milk. The alkaline pH is very favorable for multiplication of bacteria (Cruickshank *et al.,* 1975) and it is the basic principle of indirect test of SCC. The SCC of the milk sample was performed as described by Schalm *et al.* (1971) and Newman-Lampert stain was used for staining of the milk smears.

Preparation of Newman-Lampert stain

520 ml of Ethanol 95% and carbon tetra chloride (440 ml) were mixed and heated in water bath at 60^{o}C for 15 minutes. Methylene blue was added and the solution was refrigerated at 4˚C for 30 min, then concentrated glacial acetic acid (40 ml) was added. The solution was filtered using a filter paper and kept in dark bottle.

Preparation of milk films

Milk sample was taken in the test tube and kept between palms. It was thoroughly mixed so as to obtain a uniform distribution of the cells. Sample was allowed to stand for two to five minutes to permit air bubbles to raise and foam to disappear. A grease-free slide was placed on a level over a template to outline four 1 cm^2 area. Ten micro liter of mixed milk sample was drawn by micropipette and spread evenly over the template. This procedure was repeated with samples from each quarter. The film was air dried, duly protected from dust and kept in room temperature for 24 hours.

Staining of milk films

Slides were placed for 30 seconds to one minute in Newman's Lampert stain. Excess stain was drained off and slides were air-dried. The slides were then rinsed in distilled water until the stained slide were cleared leaving only the stained milk smear. The slides were air dried and kept in slide box for safety.

Calculation of the working factor for the microscope

a. A binocular microscope was used with 10× oculars and 1.8 mm oil immersion objective. The diameter of the field was measured with the help of stage micrometer.

b. Diameter of the microscope field (d)= 0.018 cm

   Area of the field= $\pi d^2/4$

   = [22/7× (0. 018) ^{2}]/4 = 0.00025 cm^2

c. Since 0.01 ml of milk is spread in 1 cm^2 area, the possible number of such fields which can be counted in 1 cm^2 = 1/0.00025= 4000

d. Milk volume represented by each field = 1/4000×1/100 ml

e. Hence, the microscopic factors (MF) = 400,000

f. Working factor (WF) = MF/ Number of fields counted

   = 400,000 / 10 = 40,000

g. The total number of cells per ml of milk = total number of cells counted × working factor i.e. 40,000

Counting of the cells

The stained slides were examined under the oil immersion objectives and the somatic cells were counted horizontally and vertically. Ten vision fields were counted for each milk sample. Total number of cells counted was multiplied by WF of the microscope to obtain the number of cells per ml of milk.

Group formation for treatment response trial

Out of 91 cattle examined, 39 cattle were found positive for subclinical mastitis on CMT and SCC. The positive animals were allocated to three groups viz. cream application (CA), teat dipping (TD) and control (C) groups. Each group consisted of 13 cattle. The three groups were made similar regarding average somatic cell count (SCC) before treatment, average daily milk yield, date of calving and parity.

Teat dipping and ointment application

Povicare Ointment was used for application on the teats and udder particularly at the opening of teats after each milking (morning and evening) with the help of tip of the fingers. Similarly, the animals within teat dipping (TD) group were subjected to teat dips in the wide-mouthed vessels containing a mixture of povidone iodine and glycerine [9 parts 5% povidone iodine solution (Betadine®) and 1 part glycerine] after each milking. Both treatments were carried out regularly for four months after the initiation of treatment.

Re-examination of milk:

The milk samples of all the animals from the three groups (two treatment groups and one control group) were examined via CMT reagent and SCC at one month, two months, three months, and four months post treatment to evaluate the treatment response. The number of somatic cells per ml of milk pre- and post treatment were analyzed statistically.

RESULTS AND DISCUSSION

Qualitative assessment of treatment response

In both the ointment based and dipping based application of povidone iodine, the number of samples that were positive on CMT reduced on subsequent months of treatment while it increased in the control group. In both the ointment group and dipping group, no animal was affected by clinical mastitis, while two cattle suffered from clinical mastitis in the control group during the fourth month of the study. In both the treatment groups, the farmers noticed softening of teats and easier hand milking of their animals possibly due to emmolient effect of glycerine.

Quantitative assessment of treatment response by Somatic cell count

The cell count data of all teats of the animal were combined together and were transformed to square root before analysis to normalize left skewed data and the count before treatment was used as covariate. The data were analyzed using general linear model with Minitab statistical package. The treatment responses are presented in Table 1 and Figure 1.

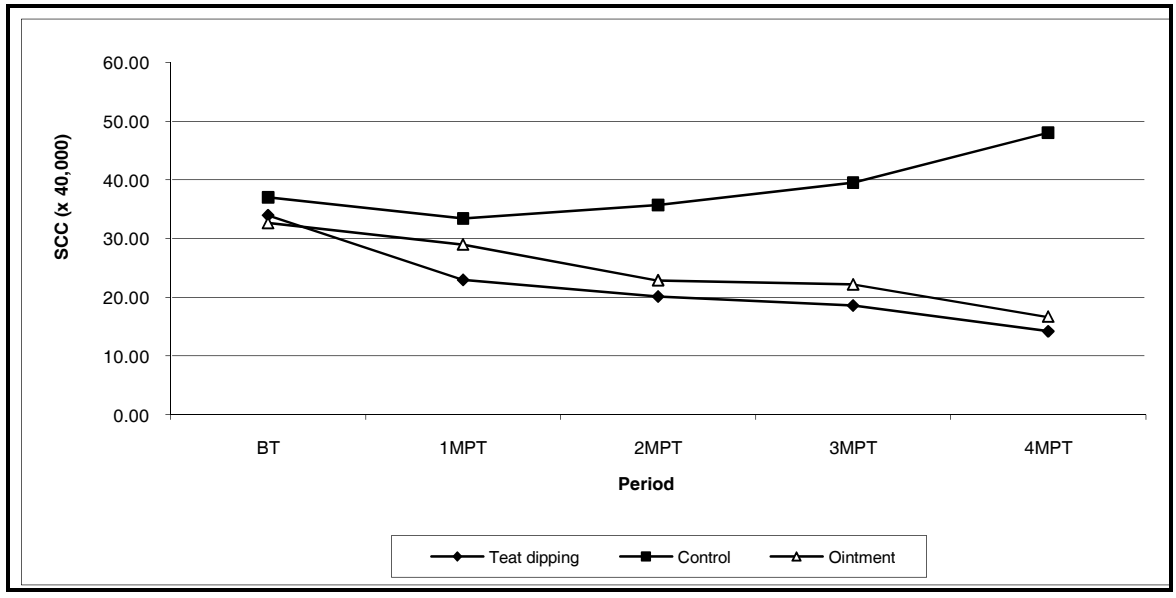
Table 1: Changes in somatic cell counts in ointment based and solution based povidone iodine application.

	Somatic Cell Count (mean ±se) × 40,000				
Group	Before treatment	One month post treatment	Two months post treatment	Three months post treatment	Four months post treatment
Teat dipped in povidone iodine solution	33.98±4.80	23.00±3.82[a]	20.17±2.63[a]	18.63±2.38[a]	14.25±0.99[a]
Ointment based povidone iodine	32.67±3.95	29.01±5.75[a]	22.89±3.23[a]	22.18±2.20[a]	16.66±2.41[a]
Control	37.00±5.92	33.43±3.77[a]	35.70±2.79[b]	39.57±4.59[b]	48.08±4.81[b]
Significance		NS	0.05	0.01	0.001

Note: Different superscript on the same column indicates significant difference. And 40,000 is a working factor. *The total number of cells per ml of milk = total number of cells counted × working factor i.e. 40,000.*

The data shows that before the treatment, there was no significant difference between all three groups and though there was considerable reduction in the number of cells in both teat-dipped and ointment applied animals than that of the control animals one month post treatment, this reduction was not significant. However, following the second month, this decline became significantly lower than that of the control animals, but there was no difference between both treatments suggesting that both teat dipping in povidone iodine and application of povidone iodine in cream based preparation were equally effective in reducing the somatic cell counts of the milk.

Figure 1: Changes in somatic cell counts in teat dipped and Povidone ointment applied animals



(Note: SCC= Somatic cell count, BT= Before treatment, MPT= Months post treatment**)**

The above graph shows that the level of somatic cell count decreased significantly in both the treatment groups while it increased in the control group. SCC continued to decrease towards the normal level in both treatment groups and it is expected that it would have reached to the normal value if the dipping and cream application would have continued for few more months. Similar responses were found in teat dipping in povidone iodine solution in earlier studies also (Joshi, 2002) and equitable responses of the cream application suggests that cream has been equally effective in reducing the severity of sub-clinical mastitis as was recorded with teat dipping on povidone iodine solution.

Limitations of the study

Despite the promising responses of the ointment in reducing the somatic cell count, the study has the limitation of its short term nature. It would have been more appropriate if the study period could have been extended for the lactation length of the studied animals. The other limitation of the study has been the smaller sample size available for the study and more number of animals in each group would have been better to increase the level of confidence. The whole study was carried out under farmers' management, so yield responses were difficult to measure, so a study under more controlled condition would have provided more data on responses of treatment especially the milk yield responses to both treatments.

ACKNOWLEDGEMENTS

The authors are very grateful to all participating farmers of the study area for their full cooperation during the entire study period. The support Dr. Poornima Manandhar, Mr. Tek Bahadur Air and Mr. Bal Bahadur Kunwar, Central Veterinary Laboratory, Tripureshwor, Kathmandu for somatic cell count and of Field recorders Mr. R.C. Bidari and Mr. D.R. Poudel in the field is highly appreciated. We are

also grateful to Medivet Pharmaceuticals Lab Pvt. Ltd., Kathmandu for providing the chemicals, ointments and supporting with field expenses.

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SUB-CLINICAL BACTERIAL MASTITIS (SCM) IN CATTLE OF EASTERN TERAI OF NEPAL

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ABSTRACT

This study was conducted from October 2006 to March 2007 in Regional Veterinary Laboratory, Biratnagar, Nepal to investigate the incidences of sub clinical mastitis (SCM) in Biratnagar sub-metropolitan city and nearby villages of Morang and Sunsari district. Altogether 190 lactating crossed bred cattle were selected on random sapling basis. After testing the milk sample of 190 cattle (760 quarters), by California Mastitis Test (CMT) and Modified White side Test (MWT) 13.6% animals and 5.9 % quarters were found to be affected sub clinically. Staphylococci (37.7%) were the most prevalent bacterial isolates found during the culture of positive samples. Enrofloxacin (88%) was found to be the most potent antibiotic in antibiotic sensitivity test.

INTRODUCTION

The economy of Nepal largely depends on the Agriculture sector. Livestock is integral part of agriculture in Nepal. It contributes 31% in agricultural gross domestic production (AGDP) and 15% in gross domestic production (GDP) of national economy. The population of lactating cattle and buffalo is 902206 and 1050977 respectively.

Sub-clinical mastitis is a herd problem, where as clinical mastitis is an individual problem. Sub-clinical mastitis (SCM) may cause milk loss up to 80% in a herd causing enormous economic loss. This is more than the clinical mastitis.

Mastitis is one of the most problematic disease and continues to have major economic impact on dairy industry throughout the world. Mastitis continues to be the most costly disease facing dairy producers today. Intramammary infection with bacterial organisms leads to substantial losses from both clinical and sub clinical mastitis. In addition, excessive negative energy balance in early lactation has become a major issue for dairy herds. Even though great technological advances have been made in dairy production systems, huge economic losses result from these problems

Dhakal and Thapa (2003) estimated the loss of Rs. 4287 or $ 63 per buffaloes per lactation due to mastitis in the Nepalese context. It was estimated that the total annual loss due to mastitis in the United States was nearly $2 billion, i.e. $181/cow/year (NMC, 1987).

The magnitude of the losses due to the disease warrants the need for continued work on different aspect of mastitis, especially its control. Apart from the cost benefit figure to dairy industry, mastitis is also of great public health significance. Diseases like staphylococcal food poisoning, Streptococcal infection, tuberculosis, brucellosis and Q-fever may be transmitted to human being through infected milk.
Clinical mastitis is easy to diagnose while SCM is difficult to diagnose as it does not exhibit any clinical symptoms. Not much attention has been paid on SCM, which causes loss in productivity and only limited study has been carried out in our country. Bovine mastitis is universally recognized as one of the most economic diseases confronting the dairy industry.
Sub-clinical mastitis, in lactating animal, is a condition characterized not only by the presence of pathogens in the udder but also changes in the biochemical profile in the milk. Mastitis has been recognized as one of the most important economic diseases of the buffaloes. In a study carried out by Dhakal (1995), showed that on an individual basis 27% of buffaloes in Nepal had sub clinical mastitis at the time of drying off and 16% post parturition (Dhakal, 1995). Buffalo having long teat and teat sphincter is also comparatively different to that of cows and thus predisposes for injuries and mastitis.

Study revealed that significant percentage of buffaloes, cattle are infected from mastitis in Asia. Bansal et al (1995) reported that 23% of buffaloes in India are having sub-clinical mastitis. Similarly,

Khalof (1983) also reported that 31% of buffaloes in Iraq are affected with sub- clinical mastitis. In Pakistan, 20% of Nili-Ravi buffaloes are reported to have sub-clinical mastitis (Rasool et al, 1983).

OBJECTIVE

- To find the incidence of sub clinical mastitis (SCM) in eastern Nepal
- To acquaint with the bacterial spectrum responsible for SCM
- To identify the most potent antibiotic to treat SCM.

MATERIAL AND METHODS

A total of 760 apparently healthy lactating quarters of 190 crossbred cows were selected for sub clinical mastitis test from different Villages of Morang, Sunsari, Saptari and Jhapa district of eastern Nepal.

Animals, for study, were selected on the basis of random sampling. History and relevant information's such as age of animal, lactation number, date of calving, previous mastitis history and date of collection of samples were duly recorded on a questionnaire sheet.

The experimental animals were allowed to wash and the shed was cleaned before taking the sample. The udders of the animals were thoroughly cleaned with water and allowed to dry. The apices of the teats were mopped with sterile gauge several times and finally with gauge soaked in 70 per cent alcohol.

After discarding first few streams of milk, nearly 30 ml of milk sample from each quarter was collected in two separate sterilized screw-capped test tubes. The collection tubes were marked as right front (RF), right hind (RH), left front (LF) and left hind (LH). These samples were kept in icebox at 4^0C. Milk samples from quarters exhibiting positive reaction in California Mastitis Test (CMT) and Modified White-side Test (MWT) were subjected for bacteriological studies.

Isolation of bacteria was done by streaking the sample on Mc Conkey's media, Nutrient agar, and 5% blood agar , which were incubated aerobically at 37^0 c for 24 hours. The isolates were identified on the basis of cultural, morphological and biochemical characteristics.
The antibiotic sensitivity test of the isolates was conducted against 8 drugs using antibiotic discs.These drugs include gentamycin, ciprofloxacin, cefotaxime, tetracycline, chloramphenicol, penicillin,

RESULT AND DISCUSSION

Screening of milk samples by CMT revealed that udder of 45 (13.6 %) of 190 animals were affected sub-clinically. This is in close agreement with the result drawn by Dhakal, et.al, who reported 15% of animals of IAAS livestock farm (Chitwan, Nepal) affected sub clinically.

However, in a study by Ghose and Sharma (2003) reported that 47.79% animals of Malwa Region of Madhya Pradesh, India was affected sub clinically.

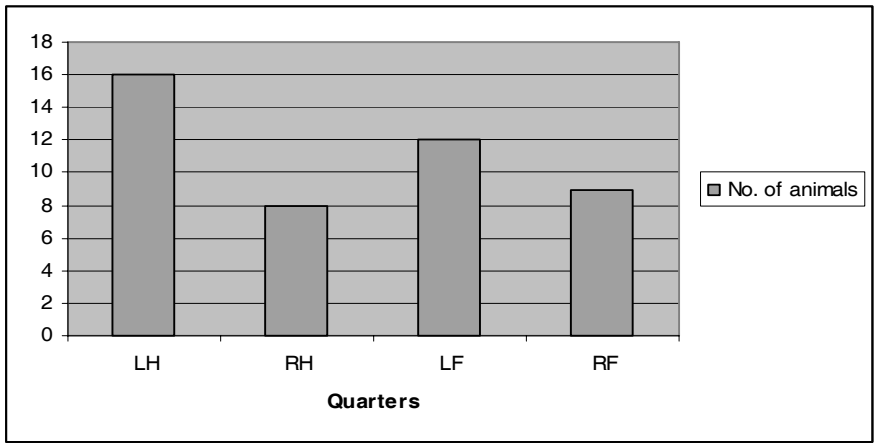
The quarter wise prevalence of SCM was found to be 26 (5.9%), which is in corroboration with findings of Chandan et. al (1989). The present investigation revealed the incidence rate of SCM in left fore, right fore, left hind, right hind quarters as 20 %, 17.7 %, 35.5 %, 26.6 % respectively .

Although the infection rate of fore and rear quarters did not differ significantly, the later were observed to be moderately more susceptible than the former. Quarter wise prevalence of SCM has been shown in figure 1. The study shows that 62.1% sample had hind quarter infection.

According to Joshi et al (1976), the incidence is more in hind quarters in both cows and buffaloes. The reason for this is due to fact that they are more exposed to dung and urine.

This result is in accordance with the findings of Gosh et al (2003), who reported that incidence rate of infection in LF, RF, LH, and RH as 22.67%, 19.24%, 23.4% and 23.46% respectively.

Figure 1: Quarter wise prevalence of SCM



The data of present study was analyzed in terms of number of quarters affected at a time. CMT and MWT of milk sample showed that only 7% of animal had the involvement of all i.e. four quarters at a time, whereas 3.8% animal had infection in three quarters only. Maximum percentage (46.15%) of animal had infection in single quarter followed by infection in two quarters (42.5%).

Tijarre and Singh reported single quarter involvement in 56.25% animals followed by two quarters involvement in 31.25% animals. Sharda et al. reported that 51.53 % cases had single quarter involvement.

Age and number of lactations

The selected animals were of age group between 3 years to 7 years. This investigation shows that older the animal, higher was the percentage of infection. Age wise prevalence rate of mastitis has been shown in table 1.

Cattle of 7 years of age had maximum percentage (18%) of incidence of infection. Out of 44 cows having 3 years of age, had minimum infection rate. There were 115 animals of more than 5 years of age. Out of them, 16.5% animals had SCM positive result.

Table1: Age-wise prevalence of SCM:

Age (Years)	No. of animals tested	No. of positive animals
3	**44**	**4 (9.0%)**
4	**31**	**3 (9.6%)**
5	**40**	**6 (15%)**
6	**25**	**4 (16%)**
7	**50**	**9 (18%)**
Total	**190**	**26 (13.6%)**

Incidence rate of sub clinical mastitis was higher in the animals having larger number of lactations. Out of 51, in cattle having number of lactations 4 or more, 21.5 % of animals showed SCM positive result.

Animals of two lactations showed the minimum percentage (10.34%) of positive result. Table 2 shows that with increased number of lactations, there is increased percentage of incidence of sub clinical mastitis. This result to a greater extent agrees with the fact drawn by Kapur and Singh (1978). They reported higher incidence of sub clinical mastitis with increased age and number of lactations. This has been due to the reason that resistance of animals might have lowered with advancement of lactation number (age) and insufficient sphincters.

Table2: Incidence of SCM based on numbers of lactations

No of lactations	No of animals tested	No. of positive animals
1	34	4 (11.7%)
2	58	6 (10.34%)
3	47	5 (10.6%)
4 or More	51	11 (21.5 %)
Total	190	26 (13.6%)

Bacteriological examination

On bacteriological examination of positive samples, 91.2% revealed bacterial growth, where as 8.8% were sterile. Ghosh and Sharda et al (2003) had reported 95.68% milk sample as culturally positive and 4.32 % sample as sterile in sub clinically affected cattle.

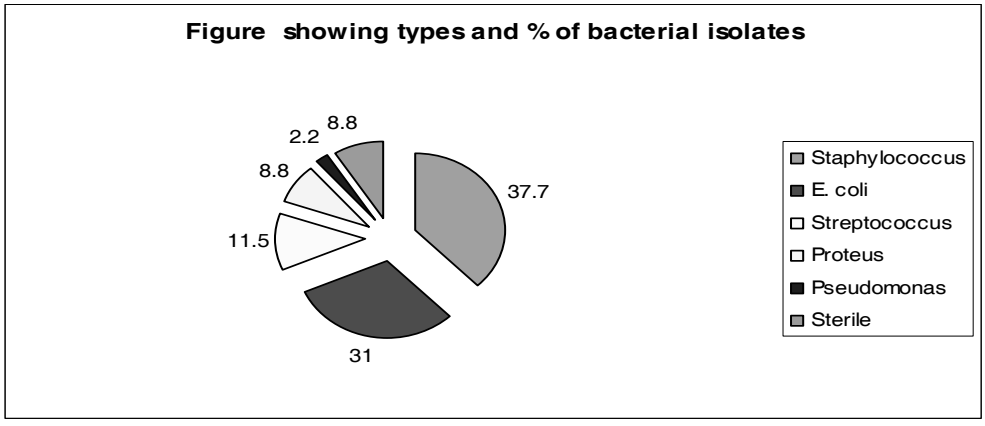
Amongst 41 culturally positive samples, 16 quarters (35.5%) were found to be infected with more than one type of bacteria. These results are in close agreement with the observation made by Singh et al., (1999), who recorded 28.6% and 36.1% quarters exhibiting mixed infections.

Bhattacharya (2002) reported that among the 72 culturally positive milk samples, 64 (88.8%) culture had single isolates where as 8 (11.1%) milk samples culture revealed mixed infection. In this study higher incidence of staphylococcal mastitis (44.4%) was observed

According to Radostits et al, (1994), the presence of more than one type of bacterium in udders usually is due to secondary invasion by opportunistic pathogens following a lowered resistance of the mammary tissue caused by the primary invaders.

The spectrum of isolates comprised of *Staphylococci* (37.7%), *Streptococci* (11.1%), *E. coli* (31.1%), *Proteus* (8.8%), and *Pseudomonas* (2.2%). This is in corroboration with the report of Chandra et.al (1997). *Staphylococci* were found to be the major cause of occurrence of SCM. *E.coli* was next to *staphylococci* followed by *streptococci* in the frequency of isolation from milk samples.

Figure: Spectrum of bacterial isolates:



Shukla et al., (1998) observed that the predominant infectious agent of mastitis, including in India, is now *Staphylococcus aureus* followed by streptococci.

Dhakal and Tiwari (1992) found that 30 per cent cows and 35.71 per cent buffaloes had sub clinical mastitis in western Chitwan. Dhakal (2000) reported that *Staphylococci* were the most prevalent organism accounting for 37.07 %, 28.26 % followed by *Streptococci* with 33.33 % and 25 % in IAAS and village buffaloes respectively.

On the other hand, coli form was the most frequently isolated organism from the clinical mastitis cases followed by *Staphylococcus,* non-coli form gram negative bacilli and *Streptococcus sps* (Dhakal, 1996).

In another study conducted by Jha et al., (1994), bacterial species isolated from the clinical cases of buffaline mastitis, the predominant species of organism was S*aphylococcus* spp (40%) with majority of *Staphylococcus* aureus followed by *E. coli* (17%), *Streptococcus* (12%), *Corynebacterium* (6%), *Proteus* (5%), *Micrococcus* (3%) and *Klebsiella* (2%)

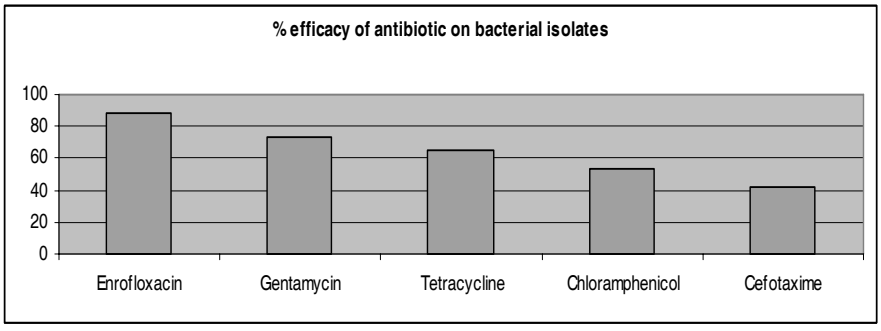
Singh and Bansal (2004) reported that *Staphylococcus aureus* was 58.60%, *Streptococci* 17.24% and *E coli* 3.45% among the 228 quarters from 57 lactating buffaloes.

749-quarter milk samples from 190 buffaloes with sub clinical mastitis in different herd size and milking management systems were studied. Among the bacterial positive samples, 32% were diagnosed as udder specific bacteria i.e. staphylococcus aureus, 25% streptococcus agalactia, 5% *Streptoccus dysgalactiae* and *Streptococcus uberis* 10 %.
(Thomas et al 2004).

Bacteriological examination of aseptically collected milk samples from mammary glands with sub clinical mastitis revealed *Staphylococcus aureus* (45.6%), coagulase-negative *Staphylococci* (13.0%), *Streptococci* (11.7%) and *Escherichia coli* 5.9%. (Shitandi Kihimbu, 2004).

Antibiotic Sensitivity test
The in vitro antibiogram studies of the bacterial isolates from SCM milk revealed enrofloxacin (88%) to be the most effective drug, followed gentamycin (73%), tetracycline (65%) and chloramphenicol (53%).

Figure3: Antibiotic sensitivity test



Bacteria may develop resistance either following continuous use of drug for a long time or due to injudicious dosage for course of treatment (Shukla et. al).Penicillin have been extensively used for treating mastitis which may have led to development of high resistance in bacteria against this antibiotic. So, obstacle in effective control of bovine mastitis is due to emergence of bacterial strain to various antimicrobial agents due to their wide and indiscriminate usage.

CONCLUSION

By this study, it can be concluded that nearly 15% cattle, though looking healthy, are suffering from sub clinical mastitis. Due to SCM, farmers are under great economic loss. Alike other research, this study also showed that animals having larger numbers of lactations had higher incidence of SCM. Streptococci are seemed to be the most prevalent bacteria affecting large number of animals. Enrofloxacin is the most potent antibiotic to treat the SCM affected animals.

RECOMMENDATIONS

- As in SCM, animals look to be healthy, it is necessary to have periodic milk test to find out affected animals in time.
- Dung and urine being major sources of contamination of udder, the floor of the shed must be regularly cleaned and disinfected.
- As milk is the medium for multiplication of bacteria, quarter of animals may not be an exception. So, complete milking of each and every animal is a must to avoid SCM.
- The udder of each lactating animal, prior to and after milking must be mopped with antiseptic solutions.
- Milkman may be the source of bacterial transmission. So, milkman must maintain greater level of personal hygiene.

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EARLY DETECTION OF SUBCLINICAL MASTITIS AMONG THE DAIRY BUFFALOES

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ABSTRACT

Mastitis is one of the most important economic diseases and continues to have major socioeconomic impact on dairy industry throughout the world. Subclinical mastitis (SCM), with no gross lesion is the most serious form of mastitis which is 15-40 times more common than the clinical mastitis. Buffaloes are the major source of milk production in Nepal; hence the study focusing on buffaline mastitis concerns a lot for the subsistence farming system like ours.

A total of 162 quarter of fore milk samples of buffaloes were collected to study the effectiveness of electrical conductivity in detecting the subclinical mastitis in dairy buffaloes. The mean electrical conductivity (EC) *of milk in different somatic cell count* (SCC) *threshold showed a continuous increase with increasing SCC threshold. Both parameters* SCC *and* EC *studied were increased for mastitic quarters with major and minor pathogen than in the quarter with no growth of organisms. Infected quarters had significantly higher mean values for the SCC ($P<0.001$) and EC ($P<0.001$) than non-infected quarters. Staphylococcal infection had significantly higher mean values for SCC ($P<0.01$) and EC ($P<0.01$) than for minor pathogen. The minor pathogen had also significantly ($P<0.01$) higher mean SCC, higher mean EC than normal quarters but the difference was non significant ($P>0.05$).*

INTRODUCTION

Mastitis is one of the most costly diseases faced by the dairy producers and continues to have major economic impact on the dairy industry throughout the world (Hwang *et al.,* 2000). A range of physical and chemical changes can be seen in the milk and pathological changes in the glandular tissues. It appears in both clinical and sub clinical forms and about 150 species of microorganisms were found as its etiological agent (Ebrahimi and Nikookhah, 2005). *Staphylococcus aureus, Streptococcus agalactiae and Mycoplasma bovis* are regarded as the major pathogens while *Streptococcus dysgalactiae, Streptococcus uberis, E.coli, Klebsiella spp, Enterobactor Serratia, Pseudomonas, Proteus* are known as major environmental pathogens. Similarly, cogulase-negative *Staphylococci* (CNS), *Corynebacterium bovis* and other uncommon mastitis pathogens are classified as minor pathogens.

The most serious form of mastitis is sub clinical mastitis (SCM). It harbors the inflammation but doesn't manifest any apparent clinical symptoms and gross lesions. It remains invisible for a considerable time and difficult to diagnose as mastitis. Subclinical mastitis is 15-40 times common than the clinical disease. It decreases the milk production and also degrades milk quality. Besides, farmers are less conscious about it. They ignore treatment of the disease as a result it causes colossal economic losses.

Mastitis causes an altered milk composition. There is an increased concentration of somatic cell count (mainly the leukocytes) and other inflammatory indicators. Its diagnosis is based on inflammatory indicators (Thomas *et al.,* 2004). Several mastitis indicators tests such California Mastitis Test, Modified Whiteside Test, Bromothymol blue, Somatic Cell Count etc are carried out for the screening of the dairy herds. Along with these inflammatory indicators, alteration can also be detected in the electrical conductivity, pH and other milk constituents.

MATERIALS AND METHODS

This study was carried out on 160 quarters of foremilk samples from the clinically healthy buffaloes of western Chitwan. Recently calved buffaloes (less than one month) and in late lactation (more than seven months) were not included in the study. They were randomly chosen from different location of Chitwan district with relevant information. Before taking the milk sample, the udder was thoroughly

cleaned with water and allowed to dry. The apices of the teats were sterilized with gauge soaked in 70 per cent alcohol. Nearly 30 ml of milk sample from each quarter were separately collected in each sterilized screw-capped test tubes after discarding first few streams of milk. These test tubes were numbered as right front (RF), right hind (RH), left front (LF) and left hind (LH). These samples were placed in icebox at 4 ^{0}C and then dispatched for culture within an hour. The *somatic cell count* (SCC) of the milk was estimated as described by Schlam *et al.,* (1971). From each quarter 0.01 ml (10µl) of warm (30-40^{0}C); thoroughly mixed milk sample was spread over one square cm area on greese free slide. After that, a thin film formed was dried, protected from dust and stained with Newman Lampert stain for one minute. During the preparation of the slide excess stain was drained off, rinsing thrice in tap water and air-dried. The SSC were counted by using the binocular microscope (Olympus).

Similarly, the (electrical conductivity) EC in milisiemens per cm (mS/cm) was estimated by using a hand-held device (Technipharm® "Milk Checker", Japan). Milk samples were put into the test wells of the device and then readings were recorded for each quarter with one decimal reading.

After that, these milk samples were cultured and isolated bacteria were identified using the routine method described by Claxton and Ryan (1993). At least one of these quarters infected with *Staphylococcus aureus, Streptococcus agalactiae or Mycoplasma bovis* is defined as being infected with a major pathogen. Likewise organisms like *Corynebacterium bovis, Staphylococcus epidermidis* and other than major pathogen were regarded as minor pathogen.

Regarding the analysis of the data Microsoft® Excel-2002 and SPSS-12 were used. A significant level (α, alpha) equal to 5 percent (*i.e.* P = 0.05) was used at which difference between values were considered statistically significant. Descriptive statistical information was calculated by using SPSS-12. Fisher's exact test was used for the estimation of level of significance (P). The mean difference of the factor within the quarter being infected or not infected was expressed as not significant, significant or highly significant on the basis of P values being more than 0.05, between 0.05 and 0.01, and less than 0.01 respectively.

RESULTS AND DISCUSSION

The distribution of mean EC at different SCC threshold has been presented in Table 1. Mean EC of milk having SCC up to 200, 200-500, 500-1000 and more than 1000 (x10^{3}cells /ml) were 3.70±0.67; 4.10± 0.03; 5.17 ± 0.24 and 7.06 ± 0.10 respectively.

Table: 1 Mean ± SE of EC at different SCC threshold.

SCC x10^{3}cells /ml Milk	N	EC (mS/cm) Mean ± SE
Up to 200	20	3.70 ± 0.67
200 - 500	81	4.10 ± 0.03
500 - 1000	46	5.17 ± 0.24
More than 1000	9	7.06 ± 0.10

Milk samples were cultured for detection of pathogenic organism-major pathogen and minor pathogen. The effect of different organisms on EC and SCC of milk has been presented in Table 2. The variation in the mean value, standard error and standard deviation of SCC and EC, in major pathogen detected, in minor pathogen detected and in non infected quarters quarter (Table 2).

Table: 2 Number of observation (n), mean and standard error (X±SE) of SCC and EC of milk from the quarters with no infection (NI), with major pathogen (MAP) and with minor pathogen (MIP) isolated.

Parameters	Non Infected (NI)		**Major Pathogen (MAP)**		**Minor Pathogen (MIP)**	
	n	**Somatic cell count (X±SE)**	n	**Somatic cell count (X±SE)**	n	**Somatic cell count (X±SE)**
SCC	51	326044.19 ± 23497.20	40	656774.65** ± 49378.63	32	433567.18* ± 46937.25
EC	51	3.85 ± 0.047	40	5.449 ± 0.422	32	3.99 ± 0.086

***Highly significant * Significant*

Table 2 gives the number of observation for each indicator and summarizes the corresponding values in quarters classified as NI, with minor pathogen and with major pathogen. Both variables showed lowest mean values in NI quarters. Highest mean values for SCC and EC was found in quarters with major pathogen (656774.65± 49378.62 and 5.44±0.42) and intermediate in minor pathogen (433567.18± 46937.25 and 3.99± 0.08). The difference between mean value of SCC is highly significant ($P<0.001$) for NI and MAP quarters significant ($P<0.01$) for MAP and MIP quarters and non-significant ($P>0.05$) for NI and MIP quarters.

Somatic Cell Count is the total number of leukocytes or white blood cells per milliliter of milk. The somatic cell count of the milk in this study ranged from 100147 to 1455077 with the mean of 473089.23 (Sd 28628.84). Blackburn (1965) has reported that the cell counts of 100,000 to 500,000 are considered to be doubtful, 500,000 to 5,000,000 as a sure case and above 5,000,000 as a severe case of a subclinical infection. Similarly the SCC from unaffected gland of buffaloes was found to be less than 200,000 and in case of subclinical mastitis, they were 200,000 per ml (Dhakal, 2004). However, SCC exceeding 300,000 and culturally positive samples from quarters are regarded as subclinical mastitis under Nepalese context (Dhakal, 1994). Likewise, the electrical conductivity (EC) of the milk ranged from 3.10 to 12.60 and mean 4.52 mS/cm with a standard deviation of 1.67. The EC of normal cow milk lies between 4.0 and 5.5 mS/cm (Wong, 1988) and that of buffalo milk is 3.76 mS/cm (Dhakal, 2005) at 25 °C. Many factors affect the milk EC; however, only intra-mammary infection is likely to influence EC of individual quarters (Dhakal, 2005). It may be changed during milking because of the changes in the electrolyte and fat concentration of the milk.

The relationship between mean EC of milk in different SCC threshold (Table 1) shows that there is continuous increase in the mean value of conductivity with increasing SCC threshold. Milk conductivity increases with increasing milk somatic cell count. Dhakal (2006) reported similar relationship with EC. The high SCC of mastitic quarters is attributed to the increased infiltration of leukocytes from the blood into the milk as a general response to the inflammation (Schalm *et al*., 1971). During inflammation, major increase in SCC is due to the neutrophils, that infiltrates into the milk to combat the infection (Sandholm, 1995) and the amount of neutrophils is the parameter considered to reflect the inflammation (Pillai *et al.,* 2001). Likewise the higher value of EC might be due the increased influx of Na+ and Cl- ions into milk through the damaged epithelium of mastitic quarters (Linzell and Peaker, 1971). The ion concentration in mastitic milk changes because of increased blood capillary permeability, the destruction of tight junction and the destruction of active pumping system (Kitchen *et al,* 1980).Increased EC is due to the damage of the mammary epithelium altering the balance of Na^+, K^+ and Cl^-. After the cell damage Na^+ and Cl^-, which have high concentration in extracellular fluid pour in to the lumen of the alveolus. In order to maintain the osmotic pressure K^+ and lactose concentration decrease in milk (Oshima, 1977).

The result of the study indicates that there is predomination of *Staphylococcus species* particularly *Staphylococcus aureus* (25.3%) followed by *Staphylococcus saprophyticus* and *Pseudomonas auruginosa* (5.7%) each. *Citrobactor*, *E coli*, CNS, and COPS other than *Staphylococcus aureus* are identified relatively lower frequency. In general these findings are similar with the observation made by

Bhattacharya (2002). Singh and Bansal (2004) reported that no such study was available for buffaloes where it had been tried to define the health status of quarter as per IDF criteria. Otherwise several workers have documented different rate of prevalence of mastitis with variable pattern of causative organism previously. There are difference in breed, management practices, milking procedure and adoption of various mastitis control measures at the farm which may influence the results.

Both SCC and EC were increased for mastitic quarters with major and minor pathogen than in the normal quarter. Table 2 shows that milk from infected quarter has significantly ($P<0.001$) higher mean values of SCC than in the non-infected quarters. The mean value of SCC is also significantly ($P<0.01$) higher in *S. aureus* infected quarters than in the quarters infected with minor pathogen. The difference in the mean value of SCC between non infected quarter and minor pathogen is non significant ($P>0.05$). The response of a quarter, i.e. increased SCC and EC may be due to the influx of bacteria. The actual response might be due to difference in virulence of the bacteria. Bacterial infection can mobilize neutrophils into mammary gland that attributes the increase in milk SCC. Hence the SCC in milk is an indicator of mammary infection. The EC of quarters infected with *S. aureus* was significantly ($P<0.01$) higher than that of infected quarters with minor pathogens and uninfected quarters ($P<0.001$). Woolford and Henderson (1988) reported similar finding for quarters infected with *S. aureus* where an increase in conductivity was observed ($P < 0.05$) from main flow to stripping fractions. This study found low EC (3.7± 0.000 mS/cm) in *E. coli* infected quarters.

CONCLUSIONS AND RECOMMENDATIONS

Though extensive researches have been done on bovine mastitis worldwide almost all of them are focused for dairy cattle only. Only a limited study on buffalo mastitis is conducted in Nepal although the buffaloes are the major source of milk production (MOAC, 2010). Since subclinical mastitis doesn't manifest clinical symptoms, it is even difficult to convince the farmer about the economic loss caused by this disease. When the disease appears in clinical form, there is likely to develop pathological changes in the glandular tissue that ultimately results in failure of medication. Hence the early detection of mastitis in subclinical form and initiating immediate treatment increases the prospect of recovery. The current study finding shows that S. aureus is the most frequently isolated organism and the recovery pattern of those organisms through antibiotic treatment is poor as suggested by the previous study reports. It might be the cause of poor prognosis of mastitis in several cases. Several approaches such as dry cow therapy; teat dipping and control of environmental pathogen are practiced for the prevention and control of mastitis. Although SCC has high sensitivity in detecting the disease, it is almost impractical technique to use as a screening field test. However, comparable sensitivity can be seen in EC which can be used as screening field test for the diagnosis of SCM. Commercial farmers who sell milk to dairy cooperatives or collection centers may get benefited if those collection centers use EC as screening test for receiving bulk milk as an alternative of bulk milk SCC. It may help them to detect the health and soundness of theirs animal's udder. Animals showing greater value of EC or increased reaction score are suggested to culture the milk samples and then to test for antibiotic sensitivity. It may not reduce incidence of developing clinical mastitis but also prevents the chance of contamination of bulk tank milk. This finding may be useful to prevent the clinical disease and ultimately to mitigate associated economic loss.

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EFFECT OF FORAGE PEANUT MEAL ON THE PRODUCTION PERFORMANCE OF LOHMANN LAYERS

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ABSTRACT

A study based on field situation analysis by employing System Learning Approach (SLA) was conducted during August 2008. Experiential Learning, System Analysis and Participatory Rural Appraisal (PRA) tools were used to explore and identify the key researchable issues related to poultry production focusing on feeding component and the nutrition of laying hens. Need of exploration of alternative protein feed source through possible legume forages was the key researchable issue identified through the SLA works. Incorporation of forage peanut (Arachis pintoi) meal at different proportions to study its effects on the performance and egg quality characteristics in the layer's diet for a period of two months (25 to 33 weeks age) were investigated. It was concluded that system approach was a valid tool to explore and analyze key researchable issues pertinent to poultry production. Incorporation of forage peanut meal at 2-5% level in the diet of layers resulted optimum production with no observable side effects. However, use of forage peanut meal in the layer's ration needs further research to cover the entire laying cycle before recommending its incorporation in poultry feed.

Key words: Experiential learning, system, protein, forage peanut, production, egg quality

INTRODUCTION

Feed cost is the major expenditure in poultry farming which accounts about 65-75% of the total cost (Parajuli, 1998). The cost of production can be reduced if alternative source of protein is supplemented in the diet of poultry. Moreover, the cost of fish meal is far higher than any of the vegetable protein sources available in Nepal (Singh, 1990). Protein is an essential nutrient for both meat and egg production. The use of meat and bone meal together with fish meal from the European Union has already been banned. This has opened a wider area to explore the use of alternative protein feed source, and preferably, forage legumes would be the better option.

There are evidences from the research that leaf meal prepared from some forage legumes such as Stylo, Berseem and Ipil Ipil have shown promising results in the performance of broiler and layers. It has been a routine practice to add upto 3% Alfalfa meal in the layer diet in many parts of the world. Maximum inclusion level of alfalfa meal in the Layers diet is reported to be 5 % (Beitler, 2000). Layers can even tolerate upto7.5% inclusion level (Bouwman, 1998). Feeding of poultry on pasture rich in legume forages has been popular in many parts of the world Forage legumes are rich in protein, vitamin A and B vitamins that are beneficial for layer chicken particularly for quality egg production (Robinson, 2007).

Forage peanut *(Arachis pintoi)* is a leguminous grass and is known by different names such as pinto peanut (Australia), mani forragero perene (Spanish), amendoim forrageiro (Portuguese), kacang pinto (Indonesia) and thua lisong tao(Thailand). It is native to South America (Brazil). It is a stoloniferous perennial herb developing a strong taproot on the older crowns and forming a dense mat of stolons. Stems are initially prostrate and become ascendant upto 50 cm height depending on the environment. Leaves are tetrafoliate with oval leaflets. Flowers develop on short axillary racemes and are yellow in colour. It grows well under the tree shades forming well developed swards (Cook et al., 2005).

Action Research is conducted through a series of “System Learning Approach (SLA)”. This is an approach rather than a methodology consisting of “Experiential Learning” and “Soft System methodology” for problem solving and situation improving (Kolb, 1984; Bawden and Packham, 1991). All farming systems are Agricultural Systems that can be considered as an ecosystem that has

specific boundary, components, functional relationships between the key components, input-output and human activity intervention.

Legume forages are good source of proteins and vitamins. Forage peanut in particular is rich in essential amino acids such as lysine, methionie, cystine, arginine, histidine, leucine etc. It can be used in the diets of chickens as an alternative source for protein feed ingredients.

Study on the problems and potentialities of poultry production system, focusing on feeding management in improving the nutrition of both rural and commercial chicken from systems perspective are still obscure. Therefore, exploring the potential use of forage peanut meal (FPM) in the layers diet was considered worthwhile, hence, this study was undertaken.

OBJECTIVE

To explore the effects of forage peanut meal on the production performance of Lohmann layers by employing System Learning Approach (SLA).

MATERIALS AND METHODS

The entire research was based on system learning approach (SLA) related issues and problems observed from the field situation. The research was completed through two stages namely, sociological study and biological experimentation.

Sociological study

Exploring the major issues, addressing the key issues for problem solving and situation improving through experiential learning and system analysis were employed in the initial phase of the research. Methods and approaches adopted for completing the research were as follows:

Household Survey

A survey was conducted among the poultry raising farmers of ward number 5, Madanpokhara VDC, Palpa. Thirty out of the 125 poultry farmers of the survey area were randomly selected for the survey. A set of twenty questions related with socioeconomic information and poultry production were included in the questionnaires. Findings from the survey were analyzed and the key issues addressed for scientific experimentation.

Group discussion

A group of thirty stakeholders representing from farmers group, District Fodder/Pasture Development Association, CBOs, NGOs and other line agency offices were involved in the group discussion. Poultry farmers, consumers, wholesalers, retailers, shopkeepers and local representatives were also present in the discussion. Issues and problems raised by the participants about poultry production were prioritized and discussed in detail.

Experiential Learning

The experiences were shared between the poultry entrepreneur and the researcher through a process of learning, an approach formulated by Kolb(1984). Issues and problems were explored, different views collected, conceptual idea generated and active experimentation made using the SLA tools. Four steps of Experiential learning namely Concrete Experience (CE), Reflective Observation (RO), Abstract Conceptualization (AC) and Active Experimentation (AE) were followed during the learning process (Figure 1).

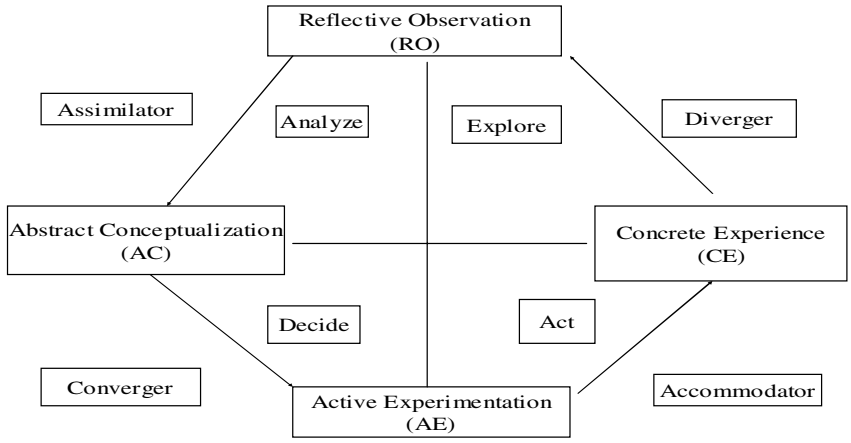

Figure 1: Experiential learning cycle derived from Kolb(1984)

System Analysis
Poultry Production was considered as a Farming System Model and hence its boundary, sub-systems, interrelationship between the key components, input, output and the context comprising the system properties were analyzed (Figure 2).

Production performance study

An experiment was conducted using 120 Lohmann layers employing completely randomized design with five treatments and three replications in a private layer farm at Mangalpur VDC Chitwan for a period of 8 weeks. Fresh forage peanut meal prepared after sun drying was incorporated in the diets at 2, 3.5, 5 and 6.5 % levels. Daily and weekly observations were made for feed consumption, egg production, egg mass output, body weight gain, feed conversion ratio and livability of the birds..

RESULTS AND DISCUSSION
System analysis

Household survey and group discussion revealed that raising of cattle, buffalo, goat, pigs and poultry were the key components of the livestock production in the survey area. Forage peanut was the newly introduced perennial legume grass intercropped within the coffee plants and fruit orchards. Farmers said that local hens fed with extracts from forage peanut produced more eggs than they had previously. Farmers had less technical know how about protein source feed ingredients. Commercial feeds were costlier.

Several issues and problems were explored and analyzed through **Experiential Learning** as one of the powerful tool of System Learning Approach (SLA). They are discussed under the following sub-headings as follows:

Concrete Experience (CE): Low productivity of animals, costlier feeds and feed ingredients(specially the protein source) and lack of technical know how about the feeding value of forage peanut were the issues identified from experiences the of farmers.

Reflective Observation (RO): Different views of farmers, CBOs, NGOS and government institutions were expressed for improving the current situation of animal production system. Government program for pasture development, group organization and mobilization were the strength while lack of knowledge about the proper utilization of the local resources was the weakness of the farming system. Costlier protein source feed ingredients were the threat for optimum cost of production where as availability of forage peanut in the farmer's field was the opportunity to incorporate as a protein source feed ingredient in the diet of poultry (SWOT analysis).

Abstract Conceptualization (AC): A consensus was made to explore the potential use of forage peanut meal in the diet of laying hens.

Active Experimentation (AE): A study was conducted using different proportions of forage peanut meal in the diet of commercial layers in Mangalpur, Chitwan. Details of the findings are discussed under the production performance.

Poultry production of Madanpokhara VDC was taken as a model of farming system for system analysis. Four system components identified for the discussion were animal subsystem, feeding subsystem, management subsystem and the marketing subsystem.

Animal subsystem
Cattle, buffaloes, sheep, goat and poultry were raised by majority of the farmers in the study VDC. A total population of 733 cattle, 1326 buffaloes, 66 sheep, 3857 goat, 1043 pigs and about 15000 poultry (layers 5000, broilers 10000) comprised the animal component.

Feeding subsystem
Commercial feeds are utilized by the farmers who raised layers and broilers. Local chickens were raised under the scavenging system. Farmers were unable to afford commercial feeds in

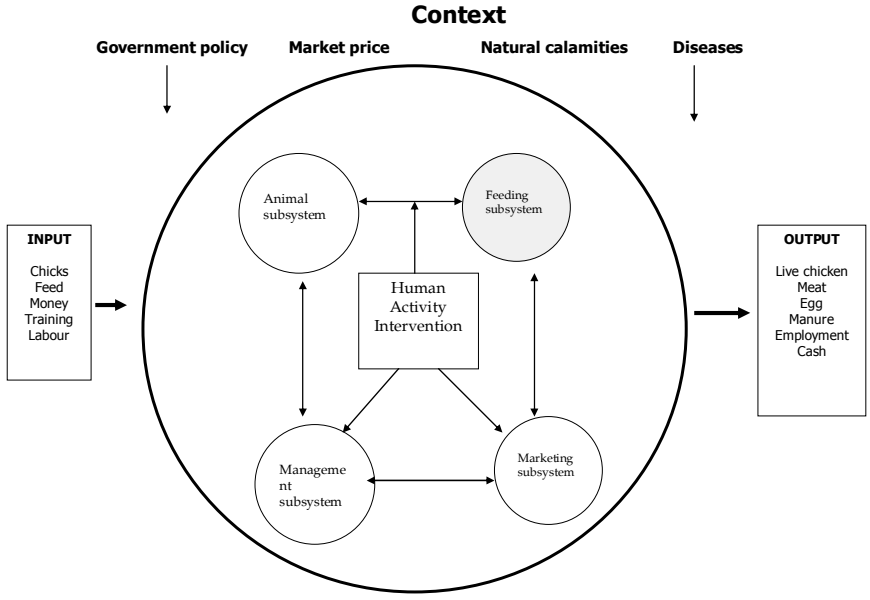

Figure 2: Poultry production in System's Perspective View

terms of price and availability of the feed at the door steps. Protein source feed ingredients were found costlier than any other feed ingredients. Therefore, farmers were in search of better alternatives that could be incorporated in the poultry diets.

Management subsystem
Commercial layers and broilers were raised under intensive system of management while local chicken are raised under free range system. Farmers lack adequate knowledge about the stocking density, space requirements for feeding and watering etc.

Marketing subsystem
Marketing of feeds and chicks takes place through the dealers and sub dealers of the feed industry and hatcheries. Eggs and meat were marketed through the wholesalers and retailers. Marketing system was well co-coordinated between the producers, middle man and the consumers.

Interrelationship between the system components and human activity

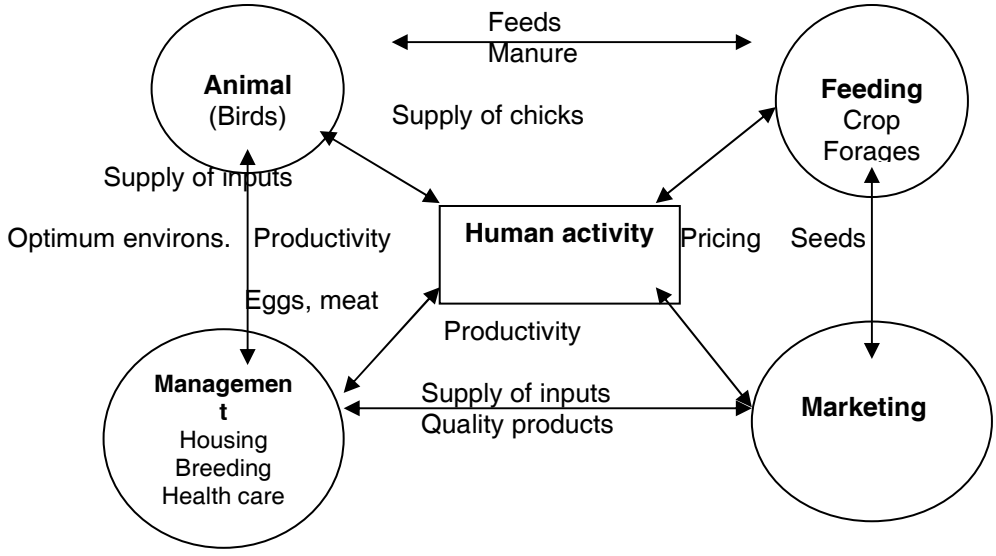

Figure 3: Interrelationships between the key system components of poultry production

Key system components, interrelationships between the key components, flow of output and input, the context and the human activity intervention are presented in Figure 3.

Technology generation, flow of inputs and out puts, settlement of market price and development approaches of various institutions were the major human activity interventions operating the systems dynamics. Though, there was a great potentiality to improve the production system in the study area, the role and responsibility of the institutions involved were still unclear in terms of system's properties such as stability, equity, sustainability and viability. Therefore, a broad vision of system's approach had facilitated the stakeholders for the betterment of poultry production and development in the coming days.

Production performance

Feed consumption, egg production (laying percentage), egg mass output, FCR and livability of the hens fed diets with different proportions of FPM are presented in the following table.

Table 1: Production performance of Lohmann layers fed diets supplemented with different proportions of forage peanut meal

S.No.	Parameters	Control	2%FPM	3.5%FPM	5%FPM	6.5%FPM
1	Feed consumption,g	127	128	128	129	129
2	Egg production, %	88.9^{a}	85.7^{a}	83.9^{ab}	84.5^{ab}	78.2^{b}
3	Egg mass output,g	48.9^{a}	46.5^{a}	46.7^{a}	47.3^{a}	42.4^{b}
4	FCR	2.63^{b}	2.80^{b}	2.73^{b}	2.71^{b}	3.07^{a}
5	Livability, %	100	100	100	100	100

Means within the rows having different superscripts differ significantly ($P<0.05$).

The results revealed that there was non-significant ($P>0.05$) difference in feed consumption among the control and treatment groups. It indicated that FPM will not negatively affect the feed consumption of the laying hens. Similar results were also reported by Berry and Mello(1981) with diets fed with Ipil ipil leaf meal. Laying percentage did not vary significantly ($P<0.05$) between the control and the diet supplemented with 2 % FPM when analyzed by ANOVA (M-STAT). Though the production was higher in the control group (88.9%), it did not vary significantly with the diet supplemented with 3% FPM (85.7%). Similarly, egg production of hens fed diets with 3.5 and 5 % did not vary but there was significant difference in the diet fed with 6.5 % FPM. Mean egg production of control and diets

supplemented with 2, 3.5 and 5% did not vary significantly. It indicated that inclusion of FPM upto 5 % level will have no adverse effect on egg production.

The findings were also similar with the results of previous researchers (Kingan and Sulivon, 1964; Berry and Mellow, 1981) who also reported non-significant effects with Ipil ipil and alfalfa leaf meal. It was observed that the mean FCR was non-significant between the control and the diets upto 5% inclusion level but varied significantly at 6.5% level with greater FCR. Livability percentage was similar in all the groups indicating that FPM did not produce any serious effects on the health of the laying hens.

CONCLUSIONS AND RECOMMENDATIONS

From the above study, following conclusions and recommendations were made:

1. Experiential learning and System analysis is one of the powerful tools of System Learning Approach (SLA). It is pertinent to exploring the key researchable issues from the field situation and useful in generating data and information

2. Incorporation of 2-5% forage peanut meal in the layer's ration would result effective egg production considering quality of the eggs (egg weight, shell thickness, albumen weight, yolk weight, albumen index, yolk index, yolk colour) where as more than 6 % inclusion revealed to produce adverse effect on egg production, egg mass output, albumen weight and feed conversion ratio.

Study of the incorporation of forge peanut meal in the diet of layers would require a longer period covering the entire laying cycle of the hens to make a complete final conclusion. Therefore, further tests and verifications regarding the proportions of forage peanut meal in the diet of layers are strongly recommended before delivering this technology to the poultry farmers.

ACKNOWLEDGEMENT

I offer my sincere thanks to Professor Dr. Maheswar Sapkota, Chairman of the Advisory Committee and Associate professors Dr. Naba Raj Devkota and Dr. Ishwari Prasad Dhakal for their valuable guidance to conduct this research. Co-operation and helps provided by Mr. Buddhi Poudel and his family, owner of the poultry farm at Mangalpur, Chitwan are highly acknowledged.

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ISOLATION AND IDENTIFICATION OF SALMONELLA SPECIES FROM THE POSTMORTEM SAMPLES OF POULTRY AT REGIONAL VETERINARY LABORATORY, POKHARA

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ABSTRACT

This study was conducted from March to July 2009 at Regional Veterinary Laboratory (RVL), Pokhara with attempts to find out the prevalence of salmonellosis in poultry. Isolate and identify Salmonella species in post mortem (PM) samples and perform the antimicrobial sensitivity tests of the Salmonella isolates. One hundred eighty one (181) live birds brought at Regional Veterinary Laboratory were tested by rapid plate agglutination test using S. pullorum colour antigen, out of which 61 (33.70%) showed positive reaction. A total of 64 samples (liver, Spleen, heart, lungs, gallbladder and intestine) were collected from the Postmortem cases of the positive reactors and from other cases with lesions suspected to be positive for Salmonella infection for the isolation of bacteria in different media, and identification was performed based on the staining, cultural and biochemical properties of Salmonella species. Out of 64 samples, 17 (26.56%) isolates of Salmonella species were isolated. The isolates were subjected to antimicrobial sensitivity test for different antimicrobials using disc diffusion method which revealed enrofloxacin (100%) to be most sensitive antimicrobials followed by gentamicin (94.11%), cotrimoxazole (94.11%), chloramphenicol (88.23%) and doxycycline (76.47%). Tetracycline (58.82%) was moderately sensitive, cefotaxime (29.41%) was least sensitive and ampicillin was found insensitive. The result showed that the poultry farms at Kaski were in threats of Salmonella infections and necessary measures needs to be taken for its control and prevention.

Key words: Isolation, Identification, *Salmonella,* Prevalence, Antimicrobial, Sensitivity

INTRODUCTION

In Nepal poultry farming has been commercialized within few decades. It is developing as one of the commercial enterprises in the private sectors since 1972. A dramatic growth rate in chicken population was observed from late 1988/89 to 1992/93, (Dhakal, 2002). Evolution of high yielding breeds, advancement in management, nutrition and disease control measures have contributed for the development of poultry industry to develop as a major sector of agribusiness. It shares 8% of AGDP and 4% of GDP (Karki, 2005). So it has significant contribution on national economy. The annual growth in commercial chicken eggs and meat were estimated 10.6% and 18.3% respectively up to year 2001 (Shrestha, 2005). Though poultry farming is quite promising and beneficial enterprises, outbreak of some of the diseases reduce production at different level thereby causing economic losses to small as well as large scale farmers (Calnek *et. al.,* 1999).

With the commercialization of the poultry industry, more and more diseases are becoming prevalent. Accordingly the disease pattern is also changing. Among diseases, *Salmonella* infection has been a concern not only because it causes loss in poultry but also it infect to human beings. *Salmonella* is more often isolated in poultry and its products. Host adopted *Salmonella* (*S. pullorum* and *S. gallinarum*) are responsible for severe systemic diseases in poultry whereas numerous serotypes of non-host-adopted paratyphoid *Salmonella* are often carried sub clinically by poultry and thereby may contaminate poultry products (Swayne *et al.,* 1998).

In recent years, an extensive use of antimicrobial drugs has been led to an increase in the occurrence of antimicrobial drug resistant *Salmonella* species in many countries. *S. typhimurium* has been found to be resistant to most antibiotics such as ampicilin, tetracycline, streptomycin chloramphenicol and sulphonamide. This phenomenon of multiple resistant represents a worldwide problem for veterinary and public health sectors.

MATERIALS AND METHODS

This study was conducted from March to July, 2009 in commercial broiler breeds at Regional Veterinary Laboratory, Pokhara. In this study, a total of 578 poultry brought for Post Mortem (PM) examination at RVL, of which 181 were brought alive. Blood samples from live poultry were collected and tested for *Salmonella pullorum* antibody by Rapid Plate Agglutination Test (RPAT) using *S. pullorum* color antigen. For culture, 64 samples were taken from the postmortem (PM) cases aseptically in a petridish. For culture, samples of liver, spleen, heart, lungs, intestine, and gall bladder were taken. Samples which were suspected for salmonella from the PM lesions and those yielded positive in RPAT were taken for culture. Before inoculating the samples in selective enrichment media, they were minced and directly inoculated in Buffered Peptone Water (BPW) and incubated for 24 hours at 37°C. 1.0 ml of the culture from the BPW was transferred to 10 ml of Tetrathionate (TT) enrichment broth and incubated for 24 to increase the recovery rates. From TT broth, a loopful of inoculation was streaked in Mac Conkey agar, Brilliant Green Agar (BGA) with sulfa supplement and Xylose lysine deoxycholate (XLD) Agar medium. The *Salmonella* suspected colonies from the culture plates were picked up with sterile loop and inoculated in nutrient agar to obtain pure culture. After plating, a colony was picked from the plating media and inoculated in Triple Suagr Iron (TSI) and Lysine Iron Agar (LIA) media by stabbing the butts and streaking the slants in one operation. All the culture plates were incubated for 24 hours at 37°C. The suspicious colonies were then subjected to various biochemical tests which narrowed down the suspects towards confirmation. Antibiotic susceptibility test of all the isolated *Salmonella* species was done using disc diffusion methods.

RESULTS

Table 1: Results of whole blood or Rapid plate agglutination tests

S.N	Place	Number of birds tested	No. of Positive	Percentage of positive
1	Birauta	10	2	20.00
2	Batulechaur	20	6	30.00
3	Lekhnath	17	7	41.17
4	Nayagaun	15	6	40.00
5	Sisuwa	9	1	11.11
6	Purandhara	13	6	46.15
7	Puranchaur	7	1	14.28
8	Simpani	9	2	22.22
9	Hemja	22	9	40.09
10	Chauthe	12	8	66.66
11	Lamachaur	13	5	38.46
12	Sarankot	8	1	12.50
13	Other	26	7	26.92
	Total	181	61	
	Prevalence			33.70

Out of 181 birds belonging from more than thirty poultry farms of different parts of Kaski district tested by RPAT, 61 (33.70%) birds showed positive reaction with *S. pullorum* antigen.

Table 2: Results of Isolation and Identification of *Salmonella* species

S.N	Sample Type	No. of samples	No. of isolates	Percentage of isolates
1	Lungs	10	1	10.00
2	Liver	13	6	46.15
3	Heart	14	4	28.57
4	Spleen	13	5	38.46
5	Intestine	12	1	08.33
6	Gallbladder	2	0	00.00
	Total	64	17	26.56

Different organs from the postmortem cases which were positive for *Salmonella* antigen and cases with lesions suspected for *Salmonella* infections were taken for isolation and identification of the organism. Out of 64 samples subjected for culture, 17 (26.56%) isolates were identified.

Table 3: Results of Antibiotic sensitivity tests of the isolates

S.N	Antibiotic disc tested	Disc potency	Sensitive	Intermediate	Resistant	Total
1	Enrofloxacin	10 mcg	17 (100.0%)	-	-	17
2	Gentamicin	10 mcg	16 (94.11%)	-	1(5.88%)	17
3	Chloramphenicol	30 mcg	15 (88.23%)	1 (5.88%)	1 (5.88%)	17
4	Tetracycline	30 mcg	10 (58.82%)	2 (11.76%)	5 (29.41%)	17
5	Doxycyline	30 mcg	13 (76.47%)	2 (11.76%)	2 (11.76%)	17
6	Cotrimoxazole	25 mcg	16 (94.11%)	1 (5.88%)	-	17
7	Ampicillin	10 mcg	-	8(47.05%)	9 (52.94%)	17
8	Cefotaxime	30 mcg	5 (29.41%)	8 (47.05%)	4 (23.52%)	17

Sensitivity of commonly available antibiotics was conducted to the isolated 17 *Salmonella* species. The result showed that all the isolates were found to be 100% sensitive to enrofloxacin, while gentamicin cotrimoxazole, chloramphenicol and doxycycline were other highly sensitive drugs which were 94.11%, 94.11%, 88.23% and 76.47% sensitive respectively. Tetracycline was moderately sensitive, cefotaxime was least sensitive and ampicillin was found resistant.

DISCUSSION

In this study the prevalence of *Salmonella* infection from the whole blood test was found to be 33.70% which is similar to the findings by Shrestha (2005) and similar to the serological data recorded by the RVL in annual serological test report (Annual Report, 065/66). This higher rate of prevalence may be due to that the cases brought to RVL are all clinically affected, birds from *Salmonella* infected hatchery. Infection by coliforms, *Micrococcus, Streptococcus* were found to be responsible for the cross reaction in chicken (Calnek *et al.,* 1999). The higher rate of prevalence may also be due to the fact that affected farms were tested while others were left. Here in this study, the prevalence rate would have been lowered to just 10.55% when the total cases (578) of poultry disease diagnosed at RVL were taken which is in accordance to cases recorded at RVL and other researchers of Nepal (Singh *et al.,* 2000; and Gupta, 2006).

The recovery rate of *Salmonella* species in liver, lungs, heart, spleen, intestine, and gallbladder of PM samples of birds was 26.56%. The findings revealed that the recovery rate of liver, spleen and heart sample were higher than the intestine, lungs, and gallbladder sample. Among the 17 isolates, 6 samples were isolated from liver (n=13), 4 from heart (n=14), 5 from spleen (n=13), 1 from lungs (n=10) and 1 from intestine (n=12). These findings revealed that liver and spleen is the main target for the isolation of *Salmonella,* which is in close agreement with Hossain et al. (2006).

In the present study, specific biochemical media were used for the detection of *Salmonella.* All of the isolates fermented dextrose, mannitol, and xylose but didn't ferment lactose and sucrose and all of the isolates were indole negative, Methyl Red (MR) positive, Voges Proskaer (VP) negative, catalase positive, oxidase negative, urease negative which are special biochemical characters for the *Salmonella* species that are previously suggested by a number of scientists and authors (Quinn *et al.,* 1998; Swayne *et al.,* 1998, and OIE manual, 2008).

In this study, the colony characters of *Salmonella,* colourless colonies in Mac Conkey agar, pinkish colony on BGA, and black colonies on XLD, production of H_2S with red alkaline slant and yellow acid butt in TSI agar and purple alkaline butt in LIA were corresponded with the characters of *Salmonella* as described by several authors. Similarly in Gram's staining, the morphology of the isolated bacteria were small rod, Gram negative, single or paired in arrangement which also corresponded with morphological characters as described by many authors (Quinn *et al.,* 1998; Swayne *et al.,* 1998, and OIE manual, 2008).

In the present study, 15 of the isolates were non motile and 2 were motile. Motility test was the fundamental basis for the identification of motile and non motile *Salmonella* organism. Non motile organisms were considered to be either *S. pullorum* or *S. gallinarum.* The motile organisms were considered as other species of *Salmonella* under paratyphoid group (OIE manual, 2008).

The ability or inability of *Salmonella* to ferment different carbohydrates was used as fundamental basis for their isolation but species identification was difficult (Freeman, 1995). In the present study dulcitol fermentation was not performed due to unavailabilty of the reagent as dulcitol fermentation test is performed to differentiate non motile *S. pullorum* and *S. gallinarum* worlwide (Quinn *et al.,* 1998). Ornithine decarboxylase test was performed, from which 8 sample isolates were found positive and considered as either S. pullorum or other and 9 samples were found negative and considered as *S. gallinarum* (Quinn et al., 1998). Based on the motility, reaction on ornithine decarboxylase and other biochemical tests, among 17 isolates, 9(52.94%) were considered as *S. gallinarum* (didn't decarboxylated ornithine and non motile), 6 isolates were *S. pullorum* (decarboxylated ornithine and non motile), and 2 isolates were other species of *Salmonella* under paratyphoid group (motile and decarboxylated ornithine).

The occurance of *Salmonella* species in the present study was 26.56% which were relative higher than the reports by other scientists in Nepal (Singh *et al.,* 2000; Dhakal, 2003, and Gupta, 2006). This is probably due to the fact that the samples used in the present study were either positive to *S. pullorum* colour antigen or suspected for salmonellosis by necropsy examination.

In this study, all 17 isolates were subjected to antibiotic sensitivity test by the disc diffusion method using 8 different antimicrobial agents. All isolates were found to be 100% sensitive to enrofloxacin, while gentamicin cotrimoxazole, chloramphenicol and doxycycline were other highly sensitive drugs which were 94.11%, 94.11%, 88.23% and 76.47% sensitive respectively. Tetracycline was moderately sensitive, cefotaxime was least sensitive and ampicillin was found resistant. The isolates were most sensitive to enrofloxacin which is in close agreement to the findings by many researchers (Lee, 2003, and Gupta, 2006). Gentamicin, Cotrimoxazole, chloramphenicol and doxycyline were other sensitive antimicrobials which is in close agreement with findings by Roy (2001) Oliveira (2005) and Shrestha (2008). Tetracyline was moderately sensitive which is in accordance to the findings by Oliveira (2005). Cefotaxime and ampicillin were found resistant which is in close agreement to the findings by Shrestha (2005).

In vitro study of antibiogram of the isolates revealed susceptible to most of the commonly used antimicrobials in poultry but their inefficacy in vivo may be due inappropriate and imprudent use of multiple drugs against certain diseases without the convincing etiological diagnosis.

CONCLUSION

The overall prevalence of *Salmonella* infection from the whole blood test was found to be 33.70% and isolation of the organisms could be made in 26.56% of the total sample of 64. Fowl typhoid was found more prevalent than that of pullorum and fowl paratyphoid. The liver and spleen could be the choice for the isolation of *Salmonella* infection in the clinical cases. Most of the commonly used antibiotics used in poultry were found sensitive in vitro study. Hence it can be concluded that the poultry farms in Kaski district are not free from *Salmonella* infection and are at greater risk.

RECOMMENDATION

- Buying of chicks *from Salmonella* fee certified hatcharies
- Use of biosecurity measures in hatcheries and farms
- Infected and carrier birds should be removed and disposed by regular screening test in the flock
- National *Salmonella* eradication plan should be implemented by the government to reduce and eventually eliminate *Salmonella* infection in poultry
- Serological test and serotyping of the isolated samples should be done
- Rational use of antibiotics to decrease selective pressure on bacterial strains by antimicrobial agents and thus decrease antimicrobial resistency

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ENTEROCOCCUS: THE MAJOR CAUSE OF WEAKNESS AND MORTALITY IN DAY OLD CHICK

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ABSTRACT

A total of 96 liver and 75 yolk sacs samples from 118 day old dead chicks, were examined for the presence of bacterial and mycotic infection. Enterococci were positive in 30.4 % liver samples and 27.1% of yolk sac samples. In the same way 145 un-piped embryos were tested and 20% of embryos were found to be infected with Enterococci. Mixed infections were not found in both dead day old chicks and dead embryos. Only 2 species of Enterococci were detected from liver and yolk sac samples representing 86.9% E. faecalis and only 13.1 % E. faecium. Prevalence of E. faecalis was found to be 89.6% in dead chicken embryos and remaining 10.4% were other species of Enterococci. During the pathogenicity test with E. faecalis, a maximum of 45% of embryos found to be un-hatched and un-piped and almost 32% of the hatched chicks were weak and 78.6% of weak chicks died within 48 hours of chicks' life and E. faecalis were re-isolated from 90.9% of dead chicks. This study showed Enterococci was one of the major causes of weak chicks, early chick mortality and E. faecalis was the major cause of embryonic death of chicken embryo.

Key words: Embryo, liver, mortality, pathogenicity, yolk sac.

INTRODUCTION

Enterococci infection is one of the important bacterial diseases of human infection. It has taken much importance due to multi drug resistance characteristics (Simjee *et al.,* 2002). Though some experience has been gained in the field of antibiotic susceptibility of Enterococci in veterinary field but part of pathogenicity has been neglected. So, a further study on epidemiology, pathogenicity, and virulence is still needed.

Poultry industry is one of the fast growing industries in all over the world. Much focus is being paid to produce quality chicks and to reduce embryomnic death as well as early chick mortality. Although hatching eggs are having enough physical barriers like cuticle, shell, albumen, yolk etc. and chemical like high pH, lysozyme and ovotransferin; against many of the invading microorganism but some of the organisms are able to cross these barriers and infect the embryo. It has been already reported and known that *E. coli* has been associated with hatchery losses and early chick (Venugopalan, *et al*., 1974; Falade, 1977; Orajaka and Mohan 1986; Kabilika and Sharma 1997). Reports regarding chick mortality caused by Salmonella (30-35%) next to Colibacillosis and less responsible are Staphylococcus (6-8%), Streptococcus (5-6%), Klebsiella (3-4%), Citrobactor (2-3%) and *Bacillus spp* (1-2%) (www.poultry.solution.com). Avian Mycoplasma serotype I is associated with embryos mortality (McClenaghan *et al*., 1981).

In this study we have focused on the role of Enterococci in mortality of young and weak chick production. Different etiological factor was suspected for this problem. For this reason, different batches of weak and dead day old chick samples as well as un-piped embryos were collected and tried to isolate suspected pathogens. The interest is focused on Enterococci in this study due to frequent isolation of pure Enterococci from the serum neutralization test contaminants which was run in the presence of antibiotics e.g. penicillin, gentamycin and streptomycin on cell culture.

MATERIAL AND METHODS

Population Study

1. *Day-old chicks*: A total of 118 commercial and breeder dead day old chicks from seven different batches of eight sources were examined for the presence of Enterococci in theirs liver and yolk sac.

Table: 1. Number of organs tested form different types and sources of bird samples

Batch No	Source	Breed	Type	Nos of sample	Organ tested	
					Liver	Yolk sac
1	A1	Ross	Broiler	40	40	24
2	A2	Hubbard	Broiler	26	26	17
3	B	Ross	Breeder	16	16	16
4	C	Hubbard	Breeder	8	8	8
5	D	Hubbard	Breeder	4		4
6	E	Hubbard	Breeder	6	6	6
7	F	Ross	Broiler	18	16	10
Total	7	2	2	118	96	75

2. *Un-piped embryos*: A total of 145 piped and un-piped chicken embryos were received from three different sources. Embryos were received from the same hatcheries from where dead chicks were received.

Table: 2. Examined nos of un-piped embryos from different breed and types of birds to isolate Enterococci.

Batch no	Sources	Breed	Type	Examined nos
1	A1	Ross	Broiler	71
2	A2	Hubbard	Broiler	29
3	A3	Hyline brown	Layer	13
4	B	Ross	Breeder	17
5	G	SPF	SPF	15
Total	3	4	3	145

Isolation and Identification

1. *Day old chicks*: All the dead day old chicks were dipped into 3% of Virkon-S (Bayer Korea Ltd.) solution for about three to five minutes. Antiseptic water was drained keeping chicks in slanting position in a box for three to five minutes. Birds were opened and samples were taken with sterilized cotton bud from liver and yolk sac separately after sterilization of surface of liver and yolk sac with red hot spatula. Samples were inoculated in Enterococcosel Agar (EA) (Difco) and Tryptic Soya Agar (TSA) (Difco) providing opportunity to grow Enterococci as well as other bacteria.

2. *Un-piped embryos*: Almost 90% un-piped embryos were from the same batches of examined dead day old chicks. All the tested un-piped embryos were cleaned with 70% ethanol externally twice in five minutes interval before taking sample. Embryos were opened with flamed sterilized forceps and sample were taken inserting sterilized cotton bud in-to the embryos. Each sample was inoculated in three different medium plates e.g. TSA, EA and Sabouraud Dextrose Agar (SDA) (Difco) to observe the different types of bacteria as well as fungi. Inoculated media plates were incubated at 37^0C for 24 hours for BHIA, up to 72 hours for EA and for 7 days at room temperature for SDA.

Black pigmented colonies on EA were compared with simultaneously inoculated TSA and SDA plates to differentiate pure and mix culture. Black pigmented colonies from EA were tested with conventional test e.g. Gram stain, catalase test and growth in brain heart infusion (BHI) broth (Difco) containing 6.5% sodium chloride to identify as genus Enterococcus. Enterococcus species were identified with the help of conventional sugar fermentation test (Facklam and Collins 1989) as well as Vitek (BioMerieux) GPI card. Motility of the Enterococci was tested by using MIO (Difco) medium.

Biochemical tests of several suspected different Enterococci colonies were tested separately from each positive sample plates to differentiate mixed Enterococcal infection.

Reproducing of disease and observation of day old chicks

Two sets of experiment were performed to produce disease artificially by injecting *E. faecalis* directly in to 1) Egg white, and 2) Air sac. Two sets of control were kept 1) diluted broth inoculated control in respective routes in 10 embryos each and 2) un-inoculated control 10 embryos. One milliliter syringe Greenject-1 (Green cross medical, Korea) was used to inject broth of *E. faecalis*. Phosphate buffer saline (PBS) was used to dilute overnight *E. faecalis* broth in BHI. Total volume injected was 10ul containing required cfu of *E. faecalis*.

Before inoculation of bacteria, shell of embryos was cleaned with 70% ethanol twice in five minutes interval to make sure that the shell was free from other organism. Disease was produced by inoculating *E. faecalis* directly in egg white or in air sac in zero day old embryos. Embryos after injecting via respective route were shield with melted wax and were incubated at 100^0F of temperature and 60% humidity (PN Incubator and Hatchers, Korea) till 18 days. Embryos were transferred in hatching chamber on 19th day. Embryos were checked on 21st day of incubation to detect hatchability, un-hatched and un-piped embryos. Weak chicks were separated and raised separately in a cartoon box providing water and starter feed adlibitum to provide safe environment from other active chicks. All the chicks were kept in well ventilation and supplied extra heat to maintain brooding temperature.

Re-isolation of *E. faecalis*

Un-piped, un-hatched embryos and dead chicks within 48 hours after hatching were tested for presence of causative agents causing abnormal situation during incubation as well in early age of chicks. Same protocol was used to re-isolate the *E. faecalis* which was used in the case study

RESULT AND DISCUSSION

Gross Lesions in the Chicks

Yolk sac infections, swollen gall bladder with thick bile, nephritis were the main gross lesions in the Enterococci positive samples. These were mild enlargement and few necrotic foci in the liver of day old chicks and dead matured embryos. Peculiar fowl smelling with rotten shell membrane was characteristic of Enterococci infected embryos.

On an average 30.5% of the livers and 27.1% of yolk sacs (Table no 3) were positive for Enterococci. No other organisms were isolated from the livers and yolk sacs of the dead day old chicks.

Table: 3. Isolation of Enterococci from the liver and yolk sac of dead day old chicks

Batch	Liver	+ve nos (%)	Yolk sac	+ve nos (%)	Remarks
1	40	7 (17.5)	24	7 (29.2)	
2	26	8 (30.8)	17	5 (29.4)	
3	16	6 (37.5)	-	-	
4	8	2 (25.0)	8	0 (0)	
5	-	-	4	4 (100)	Severe YS infection
6	6	3 (50.0)	6	3 (50.0)	
7	16	8 (50.0)	10	4 (40.0)	Severe YS infection
Total	112	25 (30.5)	85	23 (27.1)	

A total of 20% of un-piped embryos were positive for Enterococci. No other bacteria were isolated from those embryos.

Table: 4. Isolation percentage of Enterococci from un-piped matured embryos

Batches	Embryos nos	Enterococci +ve (%)	Remarks
1	71	14 (19.7)	
2	29	0 (0)	
3	13	5 (38.5)	
4	17	7 (41.2)	
5	15	3 (20.0)	
Total	145	29 (20.0)	

Prevalence of Enterococci in Day Old Chicks and Un-piped Embryos

1. *Day old chicks*: 0nly two species of Enterococci were isolated from liver and yolk sac representing 86.9% of *E. faecalis* and 13.1% of *E. faecium*. This finding indicated *E. faecalis wa*s more pathogenic in comparison with *E. faecium*. *E. faecium was* found to be very common isolates among the blood isolates followed by *E. faecalis* in our study (data not shown).

2. *Un-piped embryo*: A total of 89.6% of embryos were infected with *E. faecalis* and other 10.4% were other species of Enterococci. Prevalence frequency of *E. faecalis* also resembles with the frequency of *E. faecalis* in liver and yolk sac of day old chicks.

Reproducing of Disease in Hatching Embryos and Observation of Hatched Chicks

Table: 5 Detection of mortality of weak chicks within 48 hours after hatching and isolation of Enterococci form un-hatched embryos, and dead weak chicks inoculated with 400 cfu of *E. faecalis* in egg albumen and air sac at 0 day old embryos.

Exp groups	Smp size emb	Inoculum route	H nos	Pip UNH	UNP	Weak chicks	Dead chicks within		Enterococci +ve Nos (%)
							24 hrs	48 hrs	
1.1. *E. faecalis*	40	EW	22	3	15	7	4/22	2/18	DE: 12/18 (66.67%) and WDC: 5/6 (83.33%)
	50		28	3	19	8	5/28	2/23	15/22 (68.2 %) and WDC 6/7 (85.7 %)
1.2. *E. faecalis*	10	400/EW	6	0	4	1	1	0	DE: 3/4 (75%) and WDC: 1/1 (100%)
1.3. *E. faecalis*	10	40/EW	7	0	3	1	0	1	DE: 1/3 (33..33%) and WDC: 1/1 (100%)
1. Control	10	ODB/EW	9	1 PO	0	0	0	0	
1. Control NI	10	NI	9		1	0	0	0	DE: 1/1 (100%)
2. *E. faecalis*	50	210/AS	41	0	9	4	2	0	DE: 6/9 (66.67%), WDC: 2/2 (100%)
B. Control	25	DB/AS	23	1	1	1	0	1	WDC: 1/1 (100%)

Exp = experimental, Smp = sample, emb = embryo, cfu = colony formation unit, H = hatched, nos = numbers, UNH = un-hatched, UNP = un-piped, hrs = hours, +ve = positive, EW = egg white, DB = diluted broth, NI = no inoculation, AS = air sac, PO = pure other, DE = dead embryo, WDC = weak dead chick

Gross observation of embryos and chicks

Almost all the shell membranes of un-piped and un-hatched embryos were rotten and had yolk sac infection. Foul smells from those un-piped and un-hatched embryos were not strong as in naturally infected embryos. All the weak chicks did have yolk sac infection as well as slight swollen and mild necrotic foci on liver. Kidney of all dead weak chicks had nephrosis and hemorrhages

Bacteriology

From this study, it is proved that even 40cfu of *E. faecalis* via egg white is enough not to pip the embryo and ultimately killing of embryos and also cause the weakness of young chicks ultimately killing. As many as inoculums dose increased the number of un-piped, un-hatched embryos and weak chicks were increased irrespective of the routes indicating that penetration of quantity of *E. faecalis* is important to cause un-piped, un-hatched and weak chicks.

This study also proves that *E. faecalis* is one of the major cause of weak chicks during early age of chicks as well as mortality of young chicks which is justified by extremely very high frequency of re-isolation (83%-100%) of *E. faecalis* from 1-2 day old dead chicks as well as very high % (9.76-32.82) of weak chicks during the time of hatching. It is very interesting that *E. faecalis* was isolated from liver of weak chick of control group inoculated diluted broth via AS route and from the dead embryo of un-inoculated control group which indicates the possibility of cross contamination of organism during incubation. No other than *E. faecalis* was isolated from the dead chicks and experimental embryos except in one control group inoculated via egg white where pure other than Enterococci was isolated.

There may be the different reasons for the weak chicks and death of the chicks in early age e.g. incubation temperature and humidity, health status of parent stocks, too crowded, fasting for long time, chilling brooding temperature, suffocation but in this situation isolation of bacteria may not be possible from visceral organ of the chicks. Nevertheless these conditions will enhance the severity of the mortality during early age of chicks. With the result of pathogenicity test of *E. faecalis* in chicken embryo showed that even up to 30-45% loss of chicken embryos could happen with *E. faecalis* infection. As well as there could be up to 32% of weak chicks due to the cause of *E. faecalis* infection in embryos.

With the result of this study it is concluded that Enterococcus infection is the major cause of weakness of day old chicks and early chick mortality.

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FEASBILITY STUDY ON USING NON-SURGICAL STERILIZATION AS A MEANS OF STREET DOG POPULATION CONTROL IN KATHMANDU, NEPAL

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ABSTRACT

Rabies is a major public health concern in Nepal. An average of 25,000 people receives post-exposure treatment for rabies and more than 100 people die from rabies each year. As the guidelines of World Health Organization, control of the street dog population is one major component of rabies control. Both poisoning and surgical sterilization have been used in Kathmandu, Nepal in attempts to reduce the street dog population. The aim of this study was to analyze the feasibility of using an alternative population control method, non-surgical sterilization, in Kathmandu, and, in doing so, explore potential societal challenges regarding the introduction of this novel control method. A total of 60 community and 20 veterinary questionnaires were conducted to collect data on the social attitude towards the Kathmandu street dog population and its control methods. Although respondents expressed general acceptance and support for non-surgical sterilization, the study identified multiple concerns that must be addressed if a non-surgical sterilant is to be introduced. Lack of community awareness regarding dog overpopulation and lack of government involvement in dog population management indicate the need for a government-led policy on rabies and dog population control that includes public education. The data collected in this study will facilitate future implementation of non-surgical sterilization in Kathmandu, and it may also serve as a model for potential implementation of non-surgical methods in other developing countries.

INTRODUCTION

Rabies has been successfully eliminated in the domestic dog reservoir in certain parts of the world through mass canine vaccination and canine population control. However, it is a growing problem in the developing world, where economic instability, inadequate biomedical infrastructure, and political turmoil limit the implementation of rabies control techniques (Rupprecht *et al*., 2006). Rapidly growing dog populations and increasing urbanization and density of human populations also contribute to the rising incidence of rabies in these areas (Anon., 2004). Accordingly, the World Health Organization (WHO) reports that dog bites account for more than 99% of human rabies cases worldwide and more than 99% of all human deaths from rabies occur in Africa and Asia (Anon., 2004).

In Nepal, rabies is a zoonotic disease of major public health concern. However, the lack of a national policy for rabies vaccination, inadequate funding, political instability, and the absence of easily accessible human and veterinary medical care all constitute major barriers to rabies control and prevention (Joshi *et al.,* 2007). Annually, nearly 25,000 people receive post-exposure treatment and an estimated 100 people or more die from rabies in Nepal (Gongal and Rai, 2001). According to one study, more than 96% of rabies patients between 1991 and 2000 in Nepal were exposed to a rabid dog (Gongal and Rai, 2001). It is currently estimated that there are 25,000-35,000 dogs in the Kathmandu Valley (Anon., 2004b). Although this estimate includes the entire Kathmandu Valley, the street dogs of Kathmandu proper constitute the majority of this count.

Non-surgical sterilization is a potential alternative to these methods. Examples of non-surgical sterilants include intratesticular injection (Neutersol®, Addison Biological Laboratory, Inc.), immunocontraception (SpayVac™, SpayVac™-for-wildlife, Inc.), and hormonal down-regulation using exogenous hormone (Delvosteron, Intervet) or gonadotropin releasing hormone (GnRH) analogs (Suprelorin®, Peptech Animal Health) (Kutzler and Wood, 2006).As non-surgical sterilization is an emerging field in the small animal sciences, little literature is available on its applicability and its effects

on controlling street dog populations. At present, an ideal non-surgical sterilant does not exist for use in small animals. According to the Alliance for Contraception in Cats and Dogs, a nonprofit animal welfare organization working to advance non-surgical methods of sterilization for cats and dogs, the ideal non-surgical sterilant product would be 100% safe and effective in dog and cats of both sexes; it would provide permanent sterilization with a single dose; and it would provide the same health benefits as spay/neuter (Anon., 2006). Research is being done to develop such a product.

The potential benefit of non-surgical sterilization is that it may be economical safe and time saving. The purpose of this study was to investigate the social and veterinary attitudes toward Kathmandu's street dog population and its control methods with the goal of analyzing the feasibility of potential implementation of an alternative population control method, non-surgical sterilization. It is intended that this study will facilitate future implementation of non-surgical sterilization in Kathmandu, and also serve as a model for potential implementation of non-surgical methods in other developing countries. The hypothesis of the study was that non-surgical sterilization is more socially acceptable method of population control compared to other control methods.

MATERIALS AND METHODS

Study sites

The study was conducted between June 2009 and August 2009 throughout Kathmandu Metropolitan City, Nepal. The city is divided into 35 wards based on political divisions. For community surveys, 3 wards were chosen as study sites based on areas in which KAT works and 3 more wards were chosen based on areas in which KAT does not work. The purpose of doing so was to later statistically compare whether any differences in community attitude regarding dog population and dog population control existed between wards in which KAT serves the community and wards in which it does not serve the community. Veterinary surveys were conducted throughout Kathmandu, as well as in the neighboring cities of Lalitpur and Bhaktapur.

Study design

The study was based on standardized questionnaires. Four socio-demographic questions were asked of every respondent, including gender, age, occupation, and education level. One standardized survey was administered to community members, while another, different standardized survey was administered to veterinarians. Both questionnaires included open and close-ended questions.

Study respondents

A total of 60 community surveys were conducted throughout the 6 chosen wards in Kathmandu. Using maps of the wards, 10 major neighborhoods were chosen as study sites within each ward (Anon., 2009). Upon arriving at a study site, a major intersection was chosen at which to interview the first visible person. By selecting 10 major neighborhoods within each ward, a broad cross section of the Kathmandu population was sampled.

A total of 20 veterinary surveys were conducted throughout Kathmandu and the neighboring cities of Lalitpur and Bhaktapur. Veterinarians of diverse backgrounds were sought so as to obtain a broad range of veterinary perspective regarding dog population issues in Kathmandu. Respondents were asked to state what the majority of their practice consisted of: eight respondents were primarily small animal clinicians, 7 were public health-related, 2 were large animal related, 1 was involved in research, 1 was a police dog related, and 1 was involved in poultry medicine.

RESULTS

Community attitude regarding dog population, its control method, and non-surgical sterilization

The sixty community respondents were first asked basic questions regarding the Kathmandu street dog population, its control, and public health issues. These questions served to garner public perspective and knowledge concerning these issues. The respondents were first asked what they believe to be the different populations of dogs in Kathmandu. When asked if they knew of any diseases that humans can get from dogs, 95% of the respondents knew that a person can get rabies from a dog. A majority of the respondents (86.7%) believed there to be too many dogs in Kathmandu,

and a nearly unanimous proportion of the respondents (98.3%) believed that it is necessary to control the dog population

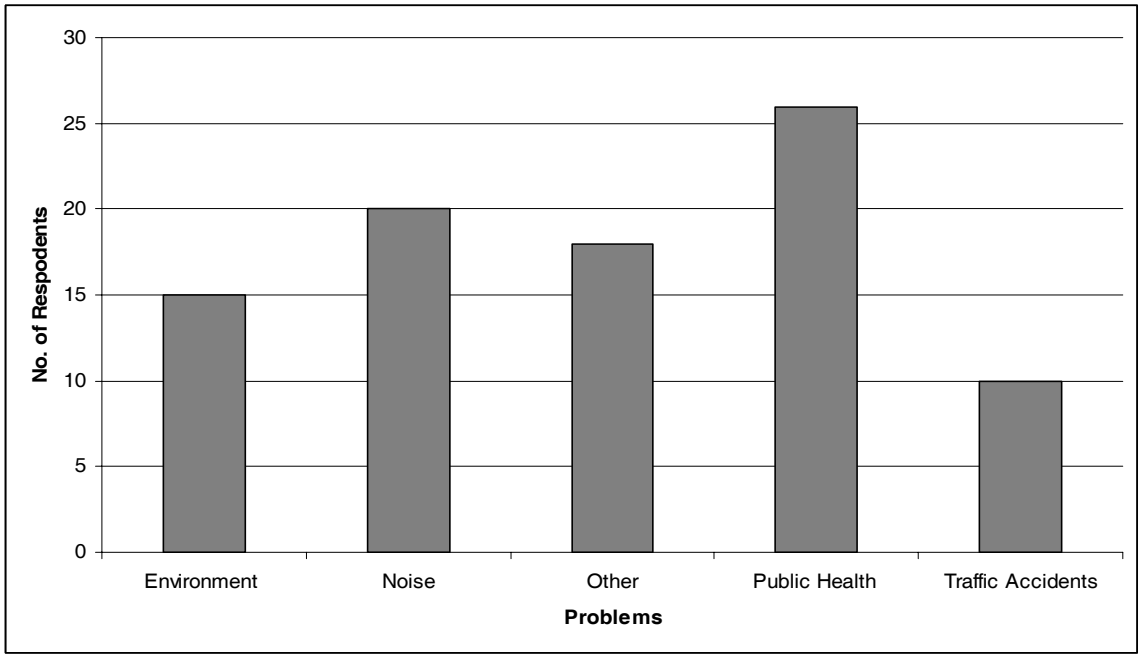

Figure1. Classification of community responses when asked of the problems associated with street dogs.

Table1. Community responses to questions regarding the use of poisoning as a dog population control method.

	Yes # (%)	No # (%)	Uncertain # (%)
Familiar with poisoning?	49 (81.7)	11 (18.3)	-
Is poisoning an effective population control method?	14 (23.7)	38 (64.4)	7 (11.9)
Should poisoning be continued?	6 (10.2)	50 (84.7)	3 (5.1)

Table 2. Community responses to questions regarding the use of surgical sterilization as a dog population control method.

	Yes # (%)	No # (%)	Uncertain # (%)
Familiar with surgical sterilization?	47 (78.3)	13 (21.7)	-
Is surgical sterilization an effective population control method?	56 (93.3)	1 (1.7)	3 (5.0)
Should surgical sterilization be continued?	58 (98.3)	1 (1.7)	-

Table 3. Veterinary responses to questions regarding the use of poisoning as a dog population control method.

	Yes # (%)	No # (%)
Familiar with poisoning?	18 (90.0)	2 (10.0)
Is poisoning an effective population control method?	6 (31.6)	13 (68.4)
Should poisoning be continued?	1 (5.0)	19 (95.0)

Table 4. Veterinary responses to questions regarding the use of surgical sterilization as a dog population control method. One respondent's answer is lacking for the last question.

	Yes # (%)	No # (%)
Familiar with surgical sterilization?	20 (100.0)	-
Is surgical sterilization an effective population control method?	20 (100.0)	-
Should surgical sterilization be continued?	19 (100.0)	-

DISCUSSION AND CONCLUSION

This is the first study carried out in Kathmandu, Nepal concerning the potential use of non-surgical sterilization to control the city's street dog population. Through analyzing the perceptions of both Kathmandu community members and veterinarians towards the street dog population and its control, this article offers insights into possible implementation issues for non-surgical sterilization. The majority of both community and veterinary respondents believe there to be a true dog overpopulation problem in Kathmandu. They both also recognize the connection between dog overpopulation and the public health threat of rabies. With the help and approval of community members and veterinarians, the introduction of non-surgical sterilization could provide a solution to both of the issues of dog overpopulation and rabies.

Both a majority of community and veterinary respondents believed that the community or a combined effort consisting of the community along with the government or an NGO should be responsible for the control of community dogs. When asked about who should be responsible for stray dog control, the majority of both community and veterinary respondents stated that the government or a combined effort led by the government should be held accountable. These responses indicate that if non-surgical sterilization is introduced in Kathmandu, the government would have to take initiative and lead the effort in street dog population management.

Although a majority of community members were aware of some efforts to control the dog population, no significant difference in awareness arose between the responses of community members within KAT working wards compared to non-KAT working wards. The reason for this is because the community respondents are only vaguely aware of these population control efforts. They were unaware of the details concerning these efforts or who is responsible for them. This indicates that there is a lack of public education and involvement with regard to the specifics of dog population control. WHO states that a key to a successful and sustainable population control effort is to involve

the local community in these efforts (Anon., 1992). Therefore, this lack of public awareness must be addressed by both the government and any NGOs if a non-surgical sterilization program is to be introduced in Kathmandu. With regard to surgical sterilization both community and veterinary respondents stated that a major negative aspect of this control method is that it is costly. Veterinary respondents also noted that surgical sterilization is time-consuming and requires technical expertise. Non-surgical sterilization could mitigate these public and veterinary concerns.

There is a general support for non-surgical sterilization in both the community and veterinary populations. Community members are interested in a non-surgical sterilant that is less costly and not as risky as surgery and does not require post-operative care as surgery does. A majority of community members stated that they would be willing to contribute money or time to a non- surgical sterilization campaign for stray dogs, if a product becomes available. This willingness to help indicates that the community views dog population control, perhaps particularly in relation to rabies, as an effort worthy of support.

Although the veterinary community supports the introduction of safe and effective non-surgical sterilization, there are some socio-economic issues that must be addressed. For example, one veterinary respondent stated that surgery is the financial mainstay of his clinical operation and he was therefore concerned that a low-cost non-surgical sterilant might devastate the economic dynamics of his clinic. This is a significant concern, because, for instance, a low-cost non-surgical sterilant might be cost-effective for an NGO to utilize for street dog population control, but it may not be cost-effective for a private practitioner as it may hinder his/her financial gain. This economic issue must be addressed to gain full support of the veterinary community for introduction of a non-surgical sterilant.

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E. COLI 0157: H7 INFECTION AN EMERGING THREAT TO PUBLIC HEALTH: ITS EPIDEMIOLOGY, PREVENTION AND CONTROL; A REVIEW

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ABSTRACT

Escherichia coli are normal and usually harmless inhabitants of the intestinal tract of man and animals. However, a few strains are pathogenic and cause distinct diarrheal syndromes. The four main categories of pathogenic E. coli include enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), enterotoxigenic E. coli (ETEC) and enterohemorrhagic E. coli (EHEC). E. coli 0157:H7 has been recognized as a widely emerging food borne pathogen since it was first implicated in food borne disease in 1982. E. coli 0157:H7 produce Shigha Like Toxin (SLT) or Verotoxin (VT) which are potent cytotoxins responsible for diarrheal syndrome. Illness caused by this organism can range from self-limiting, watery and bloody diarrhea to life-threatening manifestations. Mode of transmission is primarily through food, however, person to person transmission have also been implicated. Cattle are the major reservoir of the E. coli 0157:H7; however, it has been also isolated from other animals. Proper cooking can kill the E. coli 0157:H7. Therefore, the infection is greatly influenced by food habits of the people.

INTRODUCTION

E. coli are normal and usually harmless inhabitants of the intestinal tract of man and animals. However, a few strains are pathogenic and cause distinct diarrheal syndromes. The four main categories of pathogenic E. coli include enteropathogenic E. coli (EPEC), enteroinvasive E. coli (EIEC), enterotoxigenic E. coli (ETEC) enterohemorrhagic E. coli (EHEC) (Levine, 1987). E. coli 0157:H7 has been recognized as a widely emerging food borne pathogen, since it was first implicated in food borne disease in 1982, which produces enterohemorrhagic type of diarrheal syndrome with wide range of virulence mechanism. The organism produce potent cytotoxins referred to as Shigha like Toxin (SLT) or Verotoxin (VT) which causes diarrheal syndrome. Infection due to 0157:H7 serotype ranges from asymptomatic infection, diarrheal illness to fatal complicated hemmorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Karmali et al., 1989), thrombotic thrombocytopenic purpura (Griffin and Taxue, 1991) with high morbidity and mortality in man (Spika *et al.,* 1986). Hemolytic uremic syndrome occurs most frequently in children of less than 4 years (Pavia *et al*., 1990; Spika *et al*., 1986) and in very old persons (Ryan *et al*., 1986; Carter *et al*., 1987).

The purpose of this paper is to review the epidemiology, pathogenesis, clinical features, detection, and prevention and control of the E. coli 0157:H7 infection.

CHARACTERISTICS OF THE ORGANISM

Physiological and biochemical characteristics

Most biochemical reactions of *E. coli* 0157:H7 are typical of *E. coli*: such as the H_2S negative, indole positive, MR positive, VP negative, acid and gas formation from glucose and lactose. However, it is atypical of the *E. coli* group of organisms such as the inability to ferment sorbitol (Ryan, 1986), and the absence of β-D glucuronidase activity (Krishnan *et al*., 1987). *E. coli* 0157:H7 is MUG (4-methylumbelliferyl β-D- glucuronide) assay negative where as 93% of *E. coli* strains are MUG positive which is the basis for development of a rapid fluorogenic essay for *E. coli* (Feng *et al*., 1982). This assay uses MUG as an indicator that is hydrolyzed to a fluorogenic product by the enzyme β-glucuronidase (Rippey *et al*., 1987 as cited by Padhey and Doyle, 1992).

Growth requirements

The organism grows rapidly between 30-42^{0}C (Doyle and Schoeni, 1984). Since the *E. coli* 0157: H7 grows poorly at 44-45^{0}C the standard procedures with incubation at 44.5^{0}C cannot be used to detect *E. coli* 0157: H7 (Raghubeer and Matches, 1990). Thus it is possible that *E. coli* 0157: H7 is omitted in normal screening for fecal coliforms by standard procedures with incubation at 44.5^{0}C. Therefore, the classical definition of fecal coliforms with growth at 44.5^{0}C does not fit *E. coli* serotype 0157: H7 (Raghubeer and Matches, 1990). Raouf *et al*. (1993) found significant increase in population at 21-30^{0}C with the pH 5.55-5.94.

Survival/resistance of the organism

E. coli 0157: H7 can be killed at 57.2^{0}C (Doyle and Schoeni, 1984). It can survive well in ground beef during frozen storage at –20^{0}C for 9 months without any apparent drop in numbers after nine months (Padhye and Doyle, 1992). Raouf *et al*. (1993) reported rapid death of the organism at 50^{0}C and at pH 4.7. It can survive at a pH below 4.0 for 20 days at 8^{0}C (Besser *et al*., 1993). Doyle and Schoeni (1984) found that the organism did not grow within 48 hours at 4, 10 or 45.5^{0}C. Pasteurization easily destroys the organism (D' Aoust *et al*., 1988). Irradiation by doses of 0.27 kGy at 5^{0}C and 0 .42 kGy, at –5^{0}C destroys the organism (Thayer and Boyd, 1993).

Antimicrobial resistance

Krishnan *et al*. (1987) found *E. coli* 0157: H7 strains susceptible to the ampicillin, ticarcillin, tetracycline, gentamycin, polymyxin, sulfamethoxazole, trimethoprim-sulfametoxazole, chloramphenicol and nirofuranton. In another study, (Wells *et al.,* 1991) found *E. coli* 0157: H7 susceptible to all antibacterial tested such as chloramphenicol, trimethophrim-sulfamethoxazole, cephalothin, tetracycline, sulfasoxazole, nalidixic acid, ampicillin, kanamycin, streptomycin, getnamycin and trimethoprim. There is no evidence that antimicrobial agents are of benefit in *E. coli* 0157:H7 infections, in spite of the fact that virtually all strains tested have been highly susceptible (Pavia *et al*., 1990).

The use of antibiotics prior to onset of symptoms, during a nursing home outbreak, was considered to be a risk factor for acquiring the infection (Carter *et al*., 1987). However, Remis *et al*. (1984) as cited by Giffin *et al*. (1988) found the mean duration of 7.5 days illness for patients who received antimicrobial to which the organism was sensitive and 8.5 days for those who did not receive antimicrobials. The mechanism by which antibiotics increased the risk of infection and complications may involve enhancement of toxin production or an alteration in the normal bowel flora, leading to an overgrowth of *E.coli* 0157:H7 (Dorn, 1993). In one outbreak five of eight treated with trimethoprim-sulfamethoxazole developed HUS, compared with none of seven who had no subsequent complications (Pavia *et al*., 1990). It may be due to the endotoxin, which has major role in the pathogenesis of HUS that is liberated by antibiotic induced bacterial cell lysis (Pavia *et al*., 1990).

EPIDEMIOLOGY

Incidence

Since 1982, cases of hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura with death in children and elderly due to *E. coli* 0157:H7 infection have been recorded in different countries. Outbreaks have occurred in nursing homes, daycare centers and schools. The foodborne outbreak of 47 cases of hemorrhagic colitis (HC) due to verocytotoxin producing *E. coli* 0157:H7 was first documented in Michigan and Oregon, USA in 1982 (Riley *et al*., 1983). In Ontario, Canada, in September 1985, a severe outbreak of hemorrhagic colitis due to contaminated sandwich affected 55 residents and 18 staff members at a nursing home. Among the 55 residents affected 19 died due to severe diarrhea (HC) and 11 developed HUS. In 1986 in Walla Walla of Washington State, 2 died among 37 cases; in Dayton 3 cases with HC and in other areas 10 cases with hemolytic uremic syndrome or thrombocytopenic purpura were recorded (Ostroff, 1990).

In 1990, from the village of Tarves in Grampian, UK, 4 patients were infected with *E. coli* 0157 (Dev *et al*., 1991). In 1987, 93 cases were reported on statewide disease surveillance in the United States. Among them 95% cases developed bloody diarrhea, 12% of the patients developed either hemolytic uremic syndrome or thrombotic thrombocytopenic purpura and one died (Ostroff, 1989). A large waterborne outbreak of *E. coli* 0157:H7 in between 15 Dec. 1989 and Jan.1990 in Missouri, USA

associated with bloody diarrhea and death has been reported. Among 243 cases 86 had bloody diarrhea, 4 died and 2 had the HUS (Swerdlow *et al*., 1992).

There are evidences that incidence of hemolytic uremic syndrome is increasing, especially in children. In USA, HUS increased from 1.6 cases in 1979 to 5.8 cases in 1988 per 100000 in children of less than 5 yrs (Griffin and Taxue, 1991). In United Kingdom it increased from 54 in 1983 to 188 in 1989 (Griffin and Taxue, 1991).

The most common food vehicle is ground beef; however, other food vehicle and person-to-person transmission are also common.

Reservoir/Principal Animals Involved

The principal reservoir of enterohemorrhagic *E. coli* 0157:H7 is the intestinal tract of cattle (Waites and Arbuthnott, 1990). *E. coli* serotype 0157:H7 has been isolated from cattle in the United States (Wells *et al*., 1991; Ostroff *et al*., 1990), United Kingdom (Chapman *et al*., 1989), Germany (Montenegro, 1990), and Egypt (Dorn, 1993). The rate of isolation has been 1% or less in most surveys with generally higher rates up to 5.3% in heifer and calves (Griffin and Taxue, 1991). Most of the 0157:H7 serotypes were isolated from apparently healthy animals (Dorn, 1993; Wells, 1991). The isolation of *E. coli* 0157:H7 from the feces of healthy dairy cattle demonstrates that cattle are potential reservoirs for this organism.

In Canada*, E. coli* 0157:H7 was isolated from the calves on a farm where raw milk of cows implicated in an outbreak among kindergarten children (Borczyk *et al*., 1987 as cited by Griffin and Tauxe, 1991). Results of numerous studies suggest that claves may harbor VTEC capable of causing enteric infections in people. *E. coli* 0157:H7 and other serotypes have been isolated from retail pork, lamb and poultry products (Doyle and Schoeni, 1987) however, little are known about the prevalence in those animal species of strains potentially pathogenic to human beings. *E. coli* 0157:H7 has been isolated from chickens, swine and sheep (Doyle and Schoeni, 1987). Beery *et al.* (1985) as cited by Doyle and Schoeni (1987) revealed that *E. coli* 0157:H7 can readily colonize the ceca of chickens and excreted in the feces several months, which suggests that chickens may also be a reservoir of the organism.

The organisms are isolated more commonly from dairy calves than from beef calves which may be due to immune deficiency caused by inadequate colostral intake, which is more likely to occur in dairy operations. The data supports that the *E. coli* 0157:H7 colonize in cattle and it also shows that *E. coli* 0157:H7 is more commonly colonize in healthy than in cattle with disease (diarrhea).

Geographic distribution

E. coli 0157:H7 infection is more common in United States, Canada and United Kingdom, however, it has been isolated from other developed countries such as Ireland, Belgium, Germany, Italy and Czechoslovakia, Australia, Argentina, Japan, China, South Africa (Griffin and Taxue, 1991). The reason behind it is not clear that whether it is due to better developed epidemiological investigation infrastructures in these countries necessary to identify outbreaks and sporadic cases and isolation of organism and lacking of the same in other countries to identify it and might go unnoticed.

Temporal relationship

The infection is seasonal and most prevalent in summer (Karmali *et al.,* 1989; Pai *et al*., 1988). In Canada and USA sporadic cases occurs highest in the summer (Todd, 1990). Pai *et al*. (1988) found striking seasonal variation with *E. coli* 0157:H7 infections. He found 106 (77.4%) of 137 cases occurred in the month of June to September, with a peak in June and August.

Food vehicle

E. coli 0157:H7 has been recognized in sporadic as well as in outbreaks, as an important cause of diarrhea and hemorrhagic colitis (Riley *et al.,* 1983). Ground beef has been implicated as the major food vehicle in most of hemorrhagic colitis outbreaks. The major source of the organism is undercooked hamburger meat (Todd, 1990) and outbreaks of hemorrhagic colitis have occurred due to the consumption of undercooked hamburger patties (Carter, 1987; Ryan, 1986; Belongia *et al*, 1993). Water (Swerdlow *et al*, 1992), unpasteurized milk (Martin *et al*., 1991) and unpasteurized apple

cider (Besser *et al*., 1993) has been implicated as a vehicle of organism. The other food vehicles such as ham, turkey, cheese sandwiches, turkey roll sandwiches were also implicated (Carter *et al.,* 1987). Doyle and Schoeni (1987) isolated the *E. coli* 0157:H7 in 2% beef, poultry, and pork and lamb samples.

MODE OF TRANSMISSION

Ingestion

The major source of E. coli 0157:H7 infections are undercooked food of animal origin (Karmali et al., 1989). Most E. coli 0157:H7 transmission is by ingestion of contaminated ground beef and raw milk (Padhye and Doyle, 1992) although outbreaks also have been associated with fresh pressed apple cider (Besser et al., 1993), unchlorinated drinking water (Swerdlow et al., 1992).

Person to person transmission

There are increasing evidences that the infection may also be acquired through person-to-person transmission (Carter *et al*., 1987; Spika *et al*., 1986; Giffin and Tauxe, 1991). Person-to-person spread of *E. coli* 0157:H7 infection has been observed between children in a day-care center (Spika *et al*., 1986), from residents to staff in a nursing home (Carter *et al*., 1987) and from a child to a nurse in Toronto (Karmali *et al*., 1989).

Direct transmission

Renwick *et al.* (1993) has mentioned the direct transmission of *E. coli* 0157:H7 from animal to human. This was happened in a 13-month old from a calf in Southwestern Ontario in USA.

PATHOGENESIS

The mechanisms of pathogenesis of *E. coli* 0157:H7 illness has not fully defined, however, several factors have been associated with the virulence of the organism. *E. coli* 0157:H7 produces large quantities of verotoxin also known as Shiga-like toxin I (immunologically similar to Shiga toxin) and SLT-II (immunologically distinct from Shiga toxin) (Whipp *et al*., 1994) which plays a major role in the pathogenesis of infection (O'Brien and Holmes, 1987 as cited by Barrett *et al*., 1992). The bacteria adhere and colonize the cecum and colon and elaborate toxins which cause degeneration, necrosis and erosion of epithelial cells (Padhye and Doyle, 1992). Tzipori *et al.* (1987) found distortion of the epithelium and destruction of the microvilli which leads to diarrhea. Virulence factor other than SLT such as adhesions, and enterohemolysins were implicated, however, roles for those factors in the pathogenecity of these organisms have not been defined (Whipp, 1994). Another virulelnce factor a 60-MD a plasmid that confers the attachment of the organism in the intestine (Karch *et al*., 1989 as cited by Padhye and Doyle, 1992) was also implicated.

The SLT produce the endothelial injury, which may be due to release of cytokines (Pavia, 1990). Berry *et al*. (1985) as cited by Padhye and Doyle (1992) found colonic vascular disturbances, extreme thinning of colonic wall and luminal accumulation of blood. The verotoxins produced by the *E.coli* 0157:H7 damages vascular endothelial cells, thus triggering the clotting mechanism. The resulting microthrombi completely or partially clot the capillaries of the kidneys and other organs, which results in accumulation of waste products in blood (Cleary, 1988 as cited by Padhye and Doyle, 1992). Wadolowski *et al.* (1990) found the death of the mice, by feeding *E. coli* 0157:H7, solely due to SLT-II which caused the acute renal cortical tubular necrosis. This indicates a critical role of SLT-II in renal damage associated with *E. coli* 0157:H7 infection.

CLINICAL FEATURES

E.coli 0157:H7 has been shown to produce a spectrum of illness ranging from asymptomatic infection and uncomplicated diarrhea to hemorrhagic colitis, HUS, thrombotic thrombocytopenic purpura and death (Karmali *et al*., 1989; Pai *et al*., 1988; Griffin and Tauxe, 1991). Some of the patients also develop vomiting and fever (Briffin and Taxue, 1991). Bloody diarrhea (hemorrhagic colitis) is a distinct clinical syndrome characterized by sudden onset of crampy abdominal pain followed within 24 hours by watery diarrhea. It is then followed by grossly bloody diarrhea described as "all blood and no stool"

(Padhye and Doyle, 1991) due to lower gastrointestinal bleeding (Griffin and Taxue, 1992). The abdominal pain has been described as being equal in intensity to labor pains of pregnant women and worse than appendicitis (Padhye and Doyle, 1991). In different outbreaks the proportions of patients with bloody diarrhea to total cases ranged from 31-65%. Hemolytic uremic syndrome (a triad of acute renal failure, hemolytic anemia and thrombocytopenia) is a leading cause of acute renal failure in children. Clinical manifestation in HUS appears jaundice and hypertension. Spika (1986) observed 7% complication that developed HUS in children. In a nursing home outbreak complication with HUS developed in 12 (22%) of 55 elderly residents (Carter, 1987). The incubation period has been recorded differently in different outbreaks. It ranged from 3-9 days with a mean of 4 days (Riley *et al.* 1987), 4-8 days with a mean of 6 days (O'Brien, 1987), 1-14 days with a mean of 8 days (Dungan *et al*. 1987 as cited by Karmali *et al*., 1989).
Pai *et al*.(1988) found the duration of excretion (defined as the period from the onset of symptoms to the first negative stool culture) of *E.coli* 0157:H7 ranged from 4- 43 days, with about 50% of the patients becoming culture negative at 10 days from the onset (Pai *et al*., 1988). However, in other studies he found the duration of excretion more than 3 weeks.

Persons at risk
Very young children (Spika, 1986) and elderly persons (Pai, 1988; Ostroff et al., 1989) has the higher rate of illness. Pai (1988), Ostroff (1989) found more infections in females than males over the age of 15 years. However, the reason has not been defined. Persons with occupational exposure to cattle, ground beef, and clinical stool specimens may also be at increased risk of illness (Ostroff et al., 1989).

Risk factors for HUS and thrombotic thrombocytopenic purpura
The reported risk factors for illness caused by *E.coli* 0157:H7, other than ingesting contaminated food or water and person to person spread are very young age (Griffin and Taxue, 1988; Pavia *et al*., 1990), very old age (Griffin and Taxue, 1988), female sex (Rowe *et al*., 1991 as cited by Griffin and Taxue, 1991), mental retardation (Pavia *et al*., 1990), bloody diarrhea (Carter *et.al*., 1987) fever (Pavia *et al*., 1990), toxin type of *E.coli* 0157:H7 (Ostrioff *et al.,* 1990), antimicrobial therapy (Ostrioff *et al.,* 1990).

DETECTION AND ISOLATION

Different methods have been developed for the detection and isolation of *E. coli* 0157:H7 from food and clinical samples since its recognition as a cause of hemorrhagic colitis in 1982 and hemolytic uremic syndrome in 1983. Most standard methods used traditionally to detect fecal coliforms and *E.coli* from food use incubation temperatures in the range of 44-45^{o}C as a selective factors (Padhye and Doyle, 1992). However, *E.coli* 0157:H7 grows poorly in this particular temperature range (Doyle and Schoeni, 1984).

The three cultural characteristics distinguish E. coli 0157:H7 from other E. coli are the inability to ferment sorbitol in 24 hours the inability to produce p- glucoronidase and poor growth at temperature above 42^{o}C (Doyle and Schoeni, 1984). E.coli 0157:H7 does not hydrolyze MUG (4-methylumbelliferyl P-D glucoronide) where as other 93% of E.coli strains posses the enzyme P- glucoronidase such that a rapid flurogenic assay for the detection of E.coli 0157:H7 can not be used (Padhye and Doyle, 1992). Shiga like toxin producing strains of E.coli other than E.coli 0157: H7 may be important cause of HUS in different countries which are, however, in contrast to E. coli 0157:H7 are sorbitol positive and β-glucoronidase positive (Gunger, 1992 as cited by Johnson et al., 1995).

Culture on Sorbitol MacConkey
Lack of sorbitol fermentation in 24 hours has been considered a stable phenotypic characteristic of E. coli 0157:H7, and media which contain sorbitol such as MacConkey- sorbitol agar are used as differential media for selection of E. coli 0157:H7 from other enteric. Negative sorbitol, positive raffinose and negative β-glucoronidase tests appears to be constant markers for the detection of E. coli 0157:H7 (Krishnan et al., 1987).

Tissue culture test for verotoxin
Fecal filtrate can be tested on vero cells monolayers (Krishnan et al., 1987). Examine daily for cytopathic effects, ie, rounding, shriveling and detachment of cells.

US Food Safety and Inspection Service (FSIS) method
US Food Safety and Inspection Service (FSIS) have developed an official method for the detection, isolation and identification of E. coli 0157:H7 (Johnson et al., 1995). According to this method sample is enriched in modified EC medium with novobiocin as enrichment medium for 6 hrs and 10 fold dilutions is prepared and inoculated into petrifilm plates and incubated for 18 hrs at 42^{o}C. Then the test is performed for the presence of 0157 antigen using HEC direct blot ELISA. If the test is negative the test is stopped and the positive sample is further analyzed by reacting colonies with direct blot ELISA suspended in 0.85% of saline solution. It is then plated on to MSA-BCIG (5-brom-4-chlro-3 indoxyl–β–D glucuronide) and incubated at 42^{o}C for 24 hrs which is negative to sorbitol MacConkey agar and β-D glucuronide.

PREVENTION AND CONTROL

Successful prevention and control of E. coli 0157:H7 infection needs a multifaceted approach. Since cattle have been implicated as the principal source of E. coli 0157:H7 infection (Wells et al., 1991) more information about ecology and epidemiology of the organism in cattle and cattle farms is needed to know whether colonization of cattle corresponds to any specific farming and husbandry practices (Chapman, 1995) and to determine whether intervention strategies can be used to prevent transmission through the food chain to man.
Since cattle are the important reservoir of organisms, reducing the number of animals carrying E. coli 0157:H7 entering the food chain is very important. For that the presence of E. coli 0157:H7 should be routinely monitored in animal production and meat processing. Likewise it is also important to prevent fecal contamination of meat during slaughter. Thus, control measures should be intended to:

a. Preventing/avoiding contamination of food during production
Contamination of meats may arise during slaughter from the gut contents of animals (Chapman, 1995) such that proper inspection at slaughter for proper evisceration and to avoid fecal contamination of carcasses should be performed and equipment and tools should be clean. The hazard analysis and critical control point (HACCP) programs should be practiced as a preventive measure.

b. Destruction of microorganisms in foods
Proper cooking of food
Improper cooking of food is a major factor leading to transmission of VTEC 0157 to man in the United States (Bean, et al., 1990 as cited by Chapman, 1995). Thus ground beef products should be cooked properly such that all parts of the food should be heated at least 160^{o}F. The partially cooked patties should be labeled stating partially cooked: for safety, cook until the internal meat temperature is 160^{o}F (Dorn, 1993).

c. Inhibiting the growth of organism

VTEC E. coli 0157:H7 does not grow at 4^{o}C (Raouf et al., 1993). Therefore strict temperature control throughout production would be beneficial. The organism is sensitive to organic acids such as acetic acid and lactic acid which are effective in reducing contamination (Raouf et al., 1993).

d. Pasteurization of milk
Milk-borne infection with E. coli 0157:H7 can be reduced by pasteurization of milk as pasteurization easily destroys the organism (D' Aoust et al., 1988).

e. Irradiation of meat
Irradiation is an effective method to control this food born pathogen. Irradiation of beef is capable of killing E. coli 0157:H7 (Thayer and Boyd, 1993). However, public acceptance and adoption of this

technology may be low. Thayer and Boyd (1993) found that 90% of the viable E. coli 0157:H7 was eliminated by dose of 0.27 kGy at 5^{o}C and 0.42 kGy at – 5^{o}C.

f. Washing apples and vegetables

Since apples and vegetables transmit E. coli 0157:H7, consumers can reduce their risk by consuming properly washed apple cider and vegetables (Besser et al., 1993).

g. Use of preservative

Besser et al. (1993) found that addition of sodium benzoate 0.1% prevented growth at 80C and reduced the counts to undetectable levels within 7 days.

h. Education

Education to the consumers and food industry personnel about bacterial resistance, sources of infection, contamination, refrigeration and general sanitation should be given.

CONCLUSION

E. coli 0157:H7 is now recognized as newly emerging important bacterial cause of gastroenteritis and bloody diarrhea which can range from self-limited, watery and bloody diarrhea to life-threatening manifestations. Several studies suggest that infections with E. coli 0157:H7 are increasing. Even though infection is mainly described from developed countries it also occurs in other countries of the world. However, the status of E. coli 0157:H7 infection in Nepal is not known. Cases of diarrheal diseases are increasing in Nepal; therefore there is a need to investigate the possibility of the presence of infection of E. coli 0157:H7. Mode of transmission is primarily through food, however, person to person transmission have also been implicated. Many features of E. coli 0157:H7 infections remains poorly understood. It has been isolated from other animals; however, cattle are the major reservoir of the E. coli 0157:H7. Therefore more information about ecology and epidemiology of the organism in cattle is needed. Further research is needed to determine the pathogenesis of diarrhea and hemolytic uremic syndrome and the treatment models. There is a need for a cheaper rapid diagnostic test for the detection of E. coli in food and clinical samples, and research reagents must become commercially available. Proper cooking can kill the E. coli 0157:H7 such that infection is greatly influenced by food habits of the people. Therefore, it is necessary to educate the food service personnel and consumers.

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PREVALENCE OF TAENIA SOLIUM CYSTICERCOSIS IN SWINE IN KATHMANDU VALLEY AND ITS IMPACT ON PUBLIC HEALTH

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ABSTRACT

Pig production and pork consumption has increased in Nepal during the last decade. With an increase in pigs and consumption by small holder, there have been problems with zoonotic parasitic diseases especially porcine cysticercosis. It has remained a major health and socioeconomic problem in Nepal and many low-income countries and is the one of the main causes of epileptic seizures in many less developed countries and is also increasingly seen in more developed countries because of immigration from endemic areas. A study was conducted among 200 pigs from nine different slaughter slabs from Kathmandu valley during June to August 2009. The objectives of this study were to determine prevalence of Taenia solium cysticercosis in swine by carcass, lingual and ELISA examination, to collect data retrospectively on episode of Taeniosis and epilepsy/ Neurocysticercosis (NCC) in humans in major hospitals of Kathmandu valley, and to analyze the questionnaires for the possible risk factors and public health impact. The prevalence rate of cysticercosis by lingual examination, carcass examination and ELISA was found out to be 0.0%, 0.005% and 35.5%, respectively. The collected cysts were confirmed as Taenia solium cyst by the histopathology and microscopic examination. Neurocysticercosis (NCC) patients were found at an overall rate of 9.8% (179) out of 1839 epilepsy patients from the survey of five hospitals of Kathmandu valley viz. TUTH, Bir, Patan, Norvic and NMC. The age wise distributions of NCC patients were 55.63%, 24.02% and 22.35% for 15-35yrs, 0-14 yrs and above 35 yrs respectively. The distribution of NCC in male and female were 66.5% and 33.5% respectively. This study has indicated for strong enforcement of the Meat inspection Act for the prevention of meat borne zoonoses and lessen the economic burden in the people of Nepal and other developing countries.

Keywords*: Taenia solium, Cysticercosis, Pigs, ELISA, NCC/ Epilepsy*

INTRODUCTION

Infectious diseases remain a major health and socioeconomic problem in many low-income countries (Boraschi *et. al.,* 2008). Taenia solium cysticercosis is a parasitic disease endemic in developing countries where pigs are in close contact with human feces. Humans are the only definitives host and harbor the adult tapeworm. Taeniasis occurs after ingestion of improperly cooked pork and tapeworm carriers disseminate eggs in their feces. Cysticercosis occurs with the ingestion of larvae or cyst, and both people and pigs can become infected by fecol-oral contamination (Lescano *et. al*., 2009). The infective stage of *T.solium* is *Cysticercus cellulosae* that develops in pig and adult form is an obligatory intestinal parasite for man as reported by Joshi *et. al*. (2007). In humans, cysts often locate in the central nervous system (CNS) causing neurocysticercosis (NCC). Seizures are NCC's main clinical feature, although manifestations can range from asymptomatic, mild headaches and seizures to death. Neurocysticercosis imposes a heavy financial burden to cases and their families, and treatment costs and productivity losses account on average for 53% of an annual minimum wage salary in the first year of treatment (Lescano *et. al.* 2009). Trade and industry losses due to condemnation or cheap value of infected pork are another economic burden of cysticercosis (Acevedo, 1982; Flisser, 1988; Carabin *et al*., 2006 and Ngowi *et. al*., 2007). While it is well known that harboring a tapeworm or living with a carrier are factors associated with increased cysticercosis risk and disease burden (Lescano *et. al.* 2009).

It is reported that human and porcine taeniasis/cysticercosis is one of the major zoonotic diseases in Nepal. Some of the ethnic groups, up to 25% of the total population of Nepal, are pig farmers and pork consumers having very low sanitation and hygienic practices, and have no power over pig husbandry

and slaughtering (Joshi *et al*., 2007). It is now emerging as a major public health problem of worldwide dimensions (Sciutto *et al,* 2000).

Agrawal (2006) reported 66 cases of Neurocysticercosis (NCC) at Neurology service T.U. Teaching Hospital. Individuals of 16-25 years of age were mostly affected. 77.2% presented with seizures of one or other type, 40.9% had weakness of the limbs and 18% presented with headache alone and 9% had signs of increased intracranial pressure. Out of 66 cases 42 cases (63.6%) showed single ring enhanced lesion and 36.3% (24) showed multiple ring enhanced lesions. And most of the lesions were seen in parietal region (63.6%) followed by frontal (13%), temporal and occipital (9% each). Dorny *et al* (2004) in a study of 868 slaughtered pigs at Lusaka, Zambia found the sensitivity and specificity of lingual examination, meat inspection, Ab-ELISA and Ag-ELISA as 0.210, 0.221, 0.358 and 0.867, and 1.000, 1.000, 0.917 and 0.947, respectively. Souza and Hafez, (1999) reported serodiagnosis as the major reliable technique than meat inspection. ELISA detected the highest percentage of porcine cysticercosis.

Joshi *et al* (2006) conducted a survey of porcine cysticercosis where 200 pigs slaughererd were subjected to lingual examination, antibody detection by ELISA and arcass examination with 10.5, 22.5 and 20.5% found positive, suggesting lingual examination method for detecting porcine cysticercosis is easy, inexpensive and could be utilized as a surveillance tool in developing countries like Nepal where technical resources and technological capacity are very limited.

MATERIALS AND METHODOLOGY

Study Area

Kathmandu valley was elected as the study area which shares its boundaries with Kavrepalanchok district on east, Dhading on west, Nuwakot and Sindhupalchok districts on the north and Makwanpur on the south. The valley is located at an altitude of 1300 m from the sea level and extends about 25 km east to west and 20 km from north to south. The temperature in summer (May, June, July) ranges from 19.5ºC to 28.1ºC and in winter (October, Jan., February) ranges from 3ºC to 19.3ºC (NTB, 2009).

Lingual examination

The tongues of pigs were palpated, were sliced by a knife and the cut surfaces were examined thoroughly for the detection of any cyst if present after slaughter.

Carcass examination

Total 200 pigs were examined for cysticercosis from all nine slaughter slabs. The head, carcass and viscera were thoroughly examined visually as per the OIE guideline. Meat inspection was done by visual inspection of the carcass and its cut surfaces for the detection of cyst. The muscles of diaphragm, heart, shoulder, thigh and abdomen were thoroughly examined visually; similarly the masseters and the pterygoid muscles were examined on incisions.

Sampling schedule

Sample was collected during the period of June to July 2009.

Collection of Blood samples for serology

About 10 ml blood sample was collected in a sterile blood-collecting vial without EDTA directly from the Jugular vein after the slaughtering of pigs and from the heart at the places where stabbing or hammering was done. The vial was numbered appropriately and the detail record was maintained. Then the blood was centrifuged at 5000 rpm for 10 minutes and the serum was separated in a serum-collecting vial (eppendorf tube) for serology with the help of a micropipette.

Serological study (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of circulating antigen (Ag-ELISA) was conducted at National Zoonoses and Food Hygiene Research Centre (NZFHRC), Kathmandu. For this study, the sandwich antigen-ELISA as described by Dorny *et al* (2000) and adapted by Dorny *et. al.* (2004) was used. This test makes use of the IgG type monoclonal antibodies developed for the diagnosis of *Taenia saginata* cysticercosis (Van Kerckhoven et *al.* 1998), but cross-reaction makes it

possible to use these antibodies for the diagnosis of *T. solium* cysticercosis as well (Brandt *et al.* 1992).

Microscopic and Histopathological examination of cyst

The microscopic examination of the cyst was done at NZFHRC. Thawing of frozen cyst was done. The cyst was kept in 10% HCl for 2 minutes to dissolve the outer layer. The cyst was kept in between the two slides and pressed so that the scolex was separated. Then the slide was examined under the microscope at 100x magnification (Eye piece 10X, Objectives 10X). The Scolex was searched and the hooks were counted. The Size of hooks was measured with the help of an ocular micrometer, which was fitted in the eye piece. For the histopathological studies, infected meat samples were kept in the 10% buffered formalin and transported to National Avian Lab, Bharatpur. The tissues was dehydrated by passing on ascending series of graded ethanol, cleared by Xylene followed by infiltration and impregnation and blocking with the help of Leuckhart's L pieces. The section was cut at 4-6 µm thickness on rotary microtome and stained with Hematoxylin and Eosin stain. Finally, the slide was examined under the microscope.

Questionnaire survey to pig farmers, abattoir workers and meat sellers

A suitable questionnaire was developed after pre-testing in 10% (20) of the respondent. The level of education, types of pig raised and slaughtered, information regarding housing of pigs, deworming, preparation of pork meat, pork consumption pattern, toilet use pattern, history of seizures or epileptic fits were obtained.

Hospital records survey

Hospital records from five major hospitals of Kathmandu valley (Norvic, TUTH, Patan, Bir, NMC) were used together the information of patients admitted or examined with complaints with seizures/ epilepsy.

RESULTS

Results of Laboratory analysis (ELISA)

Out of 200 serum samples of pigs, 72 were found positive (35.5%) by ELISA.The OD (Optical Density) value for five set of ELISA was 0.061-9.885 (Set I), 0.046-9.911 (Set II), 0.052-9.929 (Set III), 0.525-9.796 (Set IV) and 3.015-83.33 (Set V).

Table 1: Age wise distribution of cysticercosis among pigs

Age (months)	Positive	Negative	Total
0-3	0	0	0
3-5	1	0	1
5-7	33	51	84
7-9	29	58	87
9-11	9	16	25
>12	0	3	3
Total	72	128	200

Table 2: Sex-wise distribution of cysticercosis among pigs

Sex of the pigs	Positive	Negative
Male	66	118
Female	06	10
Total	72	128

Results of Lingual and Carcass examination

Out of 200 lingual examinations, none of the tongues of pig was positive. The prevalence of Cysticercosis by lingual examination was found to be 0.0%. Similarly, out of 200 carcass examination, only 1 (0.005%) was found to be positive to cysticercosis (Cysts of *Taenia solium* in muscle).

Histopathological Result

The microscopic examination of the cyst showed a prominent scolex with four large suckers and a rounded rostellum armed with two different sized sickle-shaped hooks, 12 small and 12 large hooks. The morphological characteristics i.e. size and types of the rostellar hooks were comparable to those of *Cysticercus cellulosae* (Soulsby, 1982).

Table 3: Results of Hospital Survey From 2002-2008 in Kathmandu valley.

Name of Hospitals	Total Epileptic patients	Total NCC patients
TUTH	890	36
Bir	68	35
Patan	380	31
Norvic	321	55
NMC	180	22
Total	1839	179

Age and sex wise distribution of NCC patients

The age wise distribution of NCC cases shows the highest prevalence in the age of 15-35 years having 96 out of 179 (53.63%) followed by 43 out of 179 (24.02%) in 0-14years and 40 out of 179 (22.35%) in above 35 years age group. Out of total 179 NCC cases, the proportion among male is 119 (66.5%) and among female is 60 (33.5%).

DISCUSSION

The study shows that most of the farmers and abattoir workers are literate up to primary school but they lack adequate knowledge about the sanitation and hygiene. Low level of sanitation and hygiene are considered risk factors for the transmission of zoonotic diseases (Ratala, 2006). Since 98% of the farmers, abattoir workers/ meat sellers use the toilet, the risk of pigs getting access to faeces is low though the risk factors for the spread of disease as Ngowi *et al.* (2004) in Tanzania and Diaz. *et al.* (1992) in Peru reported a statistically significant association between latrines and incidence of porcine cysticercosis. In free range husbandry system, pigs have a much higher access to human faeces in communities with few or no latrines were the main risk factors associated with the porcine cysticercosis (Sikasunge *et al*., 2007, Sarti *et al*., 1997 and Pouedet *et al*., 2002). Most of the pigs (53%) are rearing without deworming. These entire factors are responsible for the incidence of cysticercosis.

The prevalence rate of porcine cysticercosis was found as 0.0, 0.005 and 35.5% based on lingual, carcass and serum examination (ELISA) of 200 pigs, respectively. Joshi *et al.* (2006) reported 10.5% and 20.5% of porcine cysticercosis on lingual and carcass examination of 200 pigs in Kathmandu valley in 2005, respectively. Pandey (2007) reported 0.63%, 0.94% and 19% prevalence rate of porcine cysticercosis by lingual, carcass and serum examination, respectively. Sapkota (2005) reported 0.99% of porcine cysticercosis on carcass examination in Kathmandu valley. The increase in prevalence rate obtained in this study may be due to different sources of slaughtered pigs, poor management of pig farming and adoption of poor sanitation and hygienic practices.

The local pigs are exposed to the contaminated forage and water. Moreover, local pigs have scavenging habit on the human faeces. Sometime in certain community people don't construct latrines. Child defecates on the premises, which are consumed or swept away by dogs, pigs and hens. It helps in the maintenance of the life cycle of organism in the environment. If the local environment harbors the tapeworms the persistence of cyclicity is maintained.

During 2002-2008, cases of NCC and epileptic patients were found to be 179 and 1839, respectively, in 5 hospitals (Norvic, TUTH, Patan, Bir, NMC) of Kathmandu valley. In the same hospitals, the occurrence of NCC was 9.7 % of total epileptic admission episodes. This finding is lesser than the findings of Sapkota (2005) where 18.7% cases of NCC were reported out of epileptic admission episodes from a survey of 6 hospitals in Kathmandu and that of Pandey (2007) with 13.34% of total epileptic admission episodes. This decrease in NCC in human may be due to the increase level of sanitation and hygiene, deworming practice and eating of well cooked pork.

This study as well as the review clearly shows that NCC was the major contributory factor in the occurrence of epilepsy in Nepal. The high percentage of NCC in males may be due to the fact that males are more mobile than females in Nepal and the more consumption of raw pork and green salad along with drink by male tends to contribute for taeniasis and autoinfection leading to NCC.

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EPIDEMIOLOGICAL SITUATION OF ANIMAL RABIES AND ITS CONTROL STRATEGY IN NEPAL

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ABSTRACT

Rabies is an acute, fatal, preventable viral disease of mammals most often transmitted through the bite of a rabid animal and impacts public health, livestock, and wildlife. Rabies is endemic in Nepal and is maintained in two interrelated cycles namely urban and sylvatic. With more than 200 people dying in Nepal annually, rabies is a serious public health concern. The animal rabies situation from 2000 to 2009 was analyzed and control strategy has been proposed on the basis of the epidemiological information.

Large ruminants were found to be the highest number dying among the animals clearly indicating the economic importance of rabies in Nepal. Hills were found the most affected among the eco-zones while month-wise, February showed the highest number of cases with Jhapa district being most affected.

Mass vaccination of the dog with effective management of dog population in community participation, public awareness and effective epidemiological surveillance backed by legislation shall have positive impact in reducing the cases of rabies both in livestock and human.

Key Words: Rabies, Public Health, Mass Vaccination, Public Awareness, Community Participation.

INTRODUCTION

Rabies is an acute, fatal viral disease of mammals most often transmitted through the bite of a rabid animal and impacts public health, livestock, and wildlife. Rabies costs governments and the people millions of rupees for diagnosis, investigation of animal bites, treatment of humans who have come into contact with rabid animals, livestock losses, vaccination, running of rabies laboratories, and animal birth control programs. In addition, each year thousands of people are suffered by anxiety, fear, and trauma associated with potential or actual rabies exposure to themselves and their domestic animals (Rupprecht *et al*. 1995). Despite implementation of aggressive rabies management strategies in many countries, rabies still results in 30,000 to 55,000 human deaths mostly in developing countries around the world.

Rabies in Nepal is maintained by two interrelated cycles viz. sylvatic and urban. Sylvatic cycle is mainly maintained by foxes and jackals. However, the major cause of rabies in both human and livestock is due to urban cycle i.e. dog bite.

The earliest records suggest rabies was present in dogs about 2300 B.C., but the disease probably evolved before recorded history (Blancou, 2003). Despite its long coexistence with humans, rabies is a public and animal health problem that annually results in 50,000 to 70,000 deaths a year worldwide (WHO, 1992).

Rabies is not, in the natural sense, a disease of humans. Human infection is incidental to the reservoir of disease in wild and domestic animals; therefore, a more accurate projection of the impact of rabies on public health should include an estimate of the extent to which the animal population is affected and the expense involved in preventing transmission of rabies from animals to humans (CDC, 2006).

Annually, 3.5 million post exposure treatment (PET) cases are estimated in the world and in Nepal More than 30,000 populations receive PET in human and 200 hydrophobia cases are estimated. More

than 94% of the reported human rabies cases are due to dog bites, 4% due to jackal and the rest due to mongoose, cat and other domestic animals' bites. The risk of human rabies is more on densely populated in terai, inner terai and mid hills. (Shrestha, 2009).

Limitations of the Study

Due to the lack of laboratory confirmation for all cases dying from dog/fox bite, animal dying after dog/fox bite showing clinical signs of rabies is also considered as rabies case.Many cases occurring at the rural areas of the country might have been left out due to the problem of under-reporting.

MATERIAL AND METHODS

The monthly epidemiological reports received at Veterinary Epidemiology Center from all the District Livestock Services Offices from 2000 to 2009 were analyzed to have an overview of animal rabies trend in Nepal from the following perspective.

4. Year-wise trend of animal rabies cases.
5. Month wise distribution of animal rabies cases.
6. Eco-zone wise distribution of animal rabies cases.
7. Region-wise distribution of animal rabies cases.
8. District-wise distribution of animal rabies cases.
9. Species-wise distribution of animal rabies cases

Control methods have been proposed based on epidemiological findings and literature review.

RESULTS AND DISCUSSION

The epidemiological analysis of animal rabies data shows that rabies is highly endemic in Nepal and has been persistent in all the eco-zones and regions, throughout the year and almost in all the districts.

The distribution of animal death due to dog/fox bite during 2000 to 2009 has been presented in Fig.1.

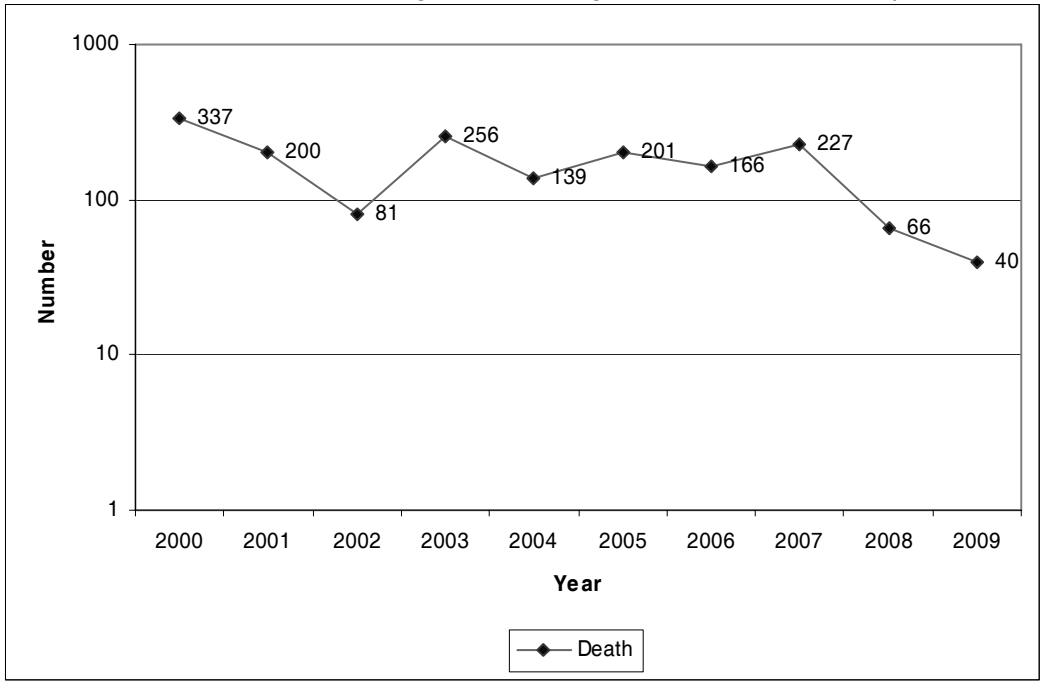

Fig. 1: Cases of Death of Animals due to Dog / Fox Bite Cases, Nepal, 2000-2009

Fig. 1 shows that the number of animals dying from dog / fox bite is in erratic trend year-wise. The highest death was observed in 2000 numbering 337 with lowest cases in 2009 that is 40. However, it

can be inferred that on an average more than 200 animals die annually due to dog / fox bite. But the actual number might be much higher in the light of poor coverage of reporting.
The distribution of animal death cases month-wise due to bite of dog / fox is presented in the Fig.2. Month-wise distribution pattern shows the highest cases in February followed by June and August. Cases ranged from 89 to 251. More cases in February may have been observed after an incubation period of animal bites during the activities related to breeding seasons of dogs.

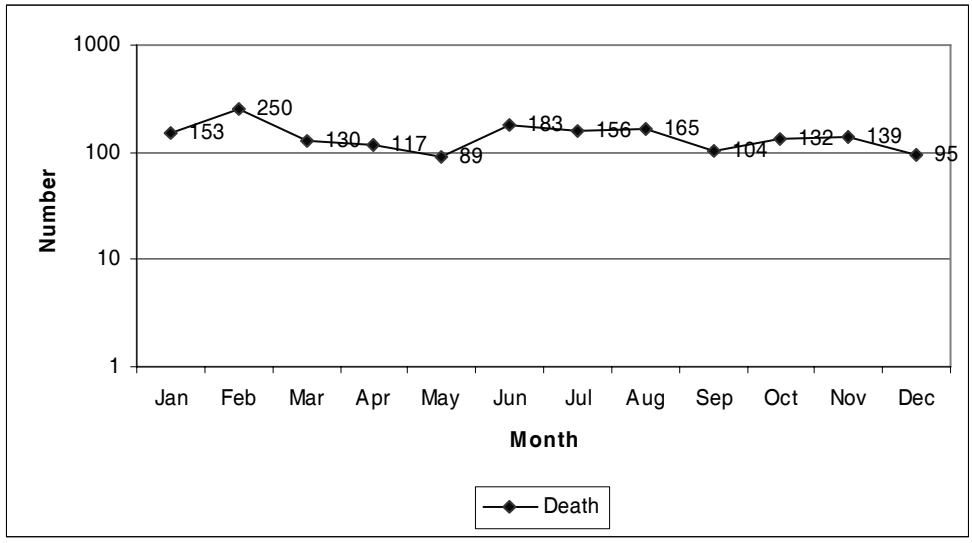

Fig. 2: Month-wise Distribution of Rabies Cases, Nepal, 2000-2009

The distribution of animal death cases district-wise due to bite of dog / fox is presented in the Fig.3.

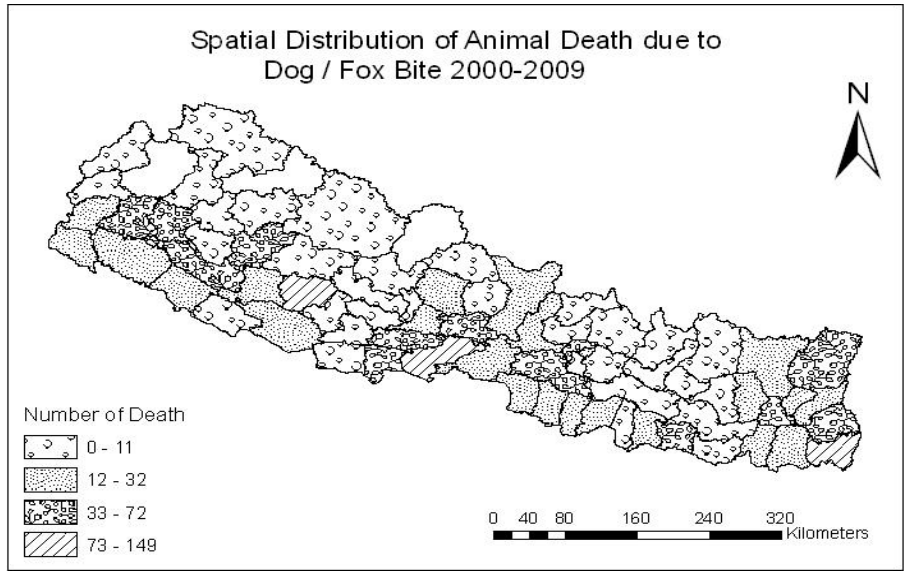

Fig. 3: District-wise Distribution of Rabies Cases, Nepal, 2000-2009

District wise distribution of animal death due to dog / fox bite clearly shows that almost all the districts are affected by rabies. Animal death due to dog / fox bite has been reported from 72 out of 75 districts. Animal death due to dog / fox bite has not been reported from Bajhang, Mugu and Mustang districts. These districts being the higher mountainous regions, low dog population might be the reason for absence of cases. The other reason might be no reporting. Jhapa, Rolpa and Nawalparasi showed the highest number of cases with substantial number of cases in Kathmandu itself.

The distribution of animal death cases eco-zone wise due to bite of dog / fox is presented in the Fig.4.

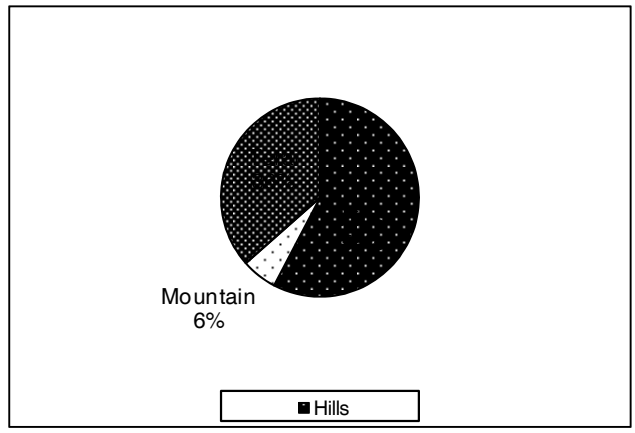

Fig.4. Eco-zone wise distribution of Animal Deaths due to Dog / Fox Bite from 2000 to 2009

Eco-zone wise distribution of animal death due to dog / fox bite clearly shows that highest cases were observed in hills accounting for 58% of the total cases followed by terai (36%) and mountain (6%). The higher cases in hills and terai might be attributed to the higher density of livestock and dog population. The distribution of animal death cases region wise due to bite of dog / fox is presented in the Fig.5.

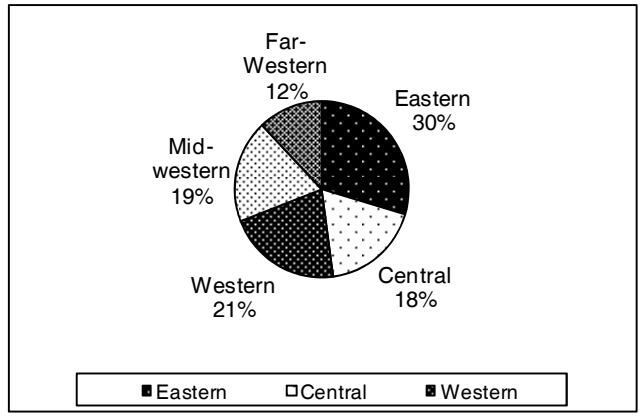

Fig. 5: Distribution of Animal death region wise due to dog / fox bite from 2000 to 2009

The highest number of cases was observed in eastern region accounting for 30% of the total cases followed by far western region (21%), mid western region (19%), central region (18%) and western region experiencing the lowest number of cases that is 12%. The distribution of animal death cases species wise due to bite of dog / fox is presented in the Fig.6.

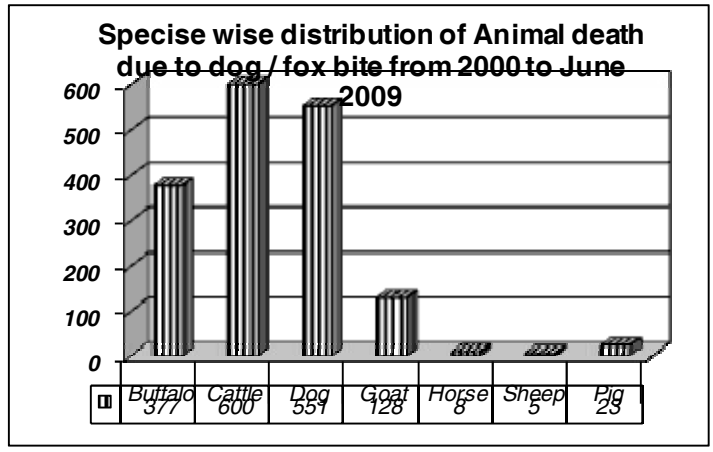

Fig. 6: Species wise distribution of Animal death due to dog / fox bite from 2000 to 2009

It is obvious from Fig.6 shows that the highest number of animal dying from dog / fox bite was cattle followed by dog themselves, buffalo, and goat with small number of cases among pigs, horses and sheep. The significant number of cattle and buffalo dying shows the economic importance of rabies.

CONTROL MEASURES

There are no as such universal methods for the control of rabies. It might differ according to the country, region, socio-cultural settings, financial availability etc. Rabies control is a complex phenomenon. Varying ecological, behavioral and biological attributes of diverse meso-carnivore and bat reservoir species introduce new challenges to contemporary rabies control programs that underscore the need for interdisciplinary collaboration among wildlife professionals, veterinarians, physicians, public affairs specialists, economists, and others. The abundant and widely distributed rabies reservoirs among wild mammals complicate rabies control measures, including effective prevention of the virus in humans and many domestic animal species. The diversity of rabies reservoirs in wildlife makes prevention and control quite complex (Velasco-Villa et al. 2002).

In addition to the expense of rabies biologicals are expenditures for the physician, hospital, the loss of income as a result of the need to physically visit a clinic, and the emotional and psychological trauma of PEP (WHO, 2005).

Promotion of responsible dog ownership and animal birth control (ABC) program in urban areas will have positive impact in reducing human rabies incidence. Involvement of NGOs, CBOs and veterinary educational institutions in risk communication and execution of mass vaccination and ABC program will be vital to improve vaccination coverage and encourage community participation. The introduction of oral rabies vaccination will be essential to control sylvatic rabies in the future (Gongal, 2005).

Legislation to control rabies is the need of the country. National Rabies Control Strategy should be prepared for the systematic control of rabies. Integrated approach by livestock, public health and wildlife authorities is necessary for the implementation of effective rabies control program. Massive inoculations of the canine population with community participation, public awareness, and effective surveillance are necessary for the rabies control. Though birth control is against the law of nature, animal birth control program for stray dogs can also be one of the measures for the control of rabies. Oral vaccination as practiced in other countries can be applied for the rabies control in wildlife even though this is expensive for countries like Nepal.

The capacity of the rabies vaccine production laboratory should be increased to produce more doses of anti-rabies vaccine to cater the national demand.

CONCLUSION

Rabies is an endemic zoonotic disease in Nepal. Regardless of the eco-zone, region, district, season or month, rabies has been seen in Nepal throughout the year and everywhere. Rabies is one of the most important diseases as there is no cure once the sign of rabies develops. However, the disease is preventable. Rabies is also economically important pursuing to the loss due to death of livestock, post exposure treatment cost in animals and humans. More than this, the psychological trauma associated with it in humans is immeasurable.

Hence, Rabies control program should be in priority. For this strong collaboration among the Veterinary, Public Health and Wildlife authorities is necessary. Formulation of National Rabies Control Strategy backed by strong legislation and mobilization of NGOs, communities, dog owners and private sector can have some positive impact in reducing the cases of rabies. It has been said that to reduce the incidence of rabies in human, the most cost effective way is to reduce the rabies cases in dogs as most of the rabies in human is attributed to dog bite. For this, mass inoculation of the dogs with animal birth control program seems vital.

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IDENTIFICATION OF TWO RABIES VIRUS LINEAGES OF NEPAL

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ABSTRACT

A study was performed to identify the Rabies Virus genomes circulating in Nepal in 2009. Three brain samples, collected from carcass of buffalo, human and dog infected with Rabies were sent to Pasteur Institute, in France for molecular characterization and identification. The entire nucleoprotein genes (NG) of these samples were sequenced and analyzed phylogenetically. Sample from buffalo was identified as Indian subcontinent lineage where as samples from human and dog were identified as Arctic lineage of Rabies virus.

INTRODUCTION

Rabies is a fatal viral zoonotic disease which affects all worm blooded living beings. It is recorded as an endemic disease in Nepal. It is spread all over country as well as occurs through out the year. The incidence of Rabies has been reported in bovine, caprine, ovine, canine and equine species of animals. Over the past five years (January 2005 to December, 2009) 411 outbreaks and 700 death of animal has been recorded in 45 districts of Nepal (Veterinary Epidemiology Centre, 2010). Laboratory diagnosis of Rabies is only limited to Central Veterinary Laboratory (CVL), Kathmandu and disease been confirmed in human, dog, buffalo, cattle, sheep, goat and mice. A total number of 149 samples were submitted to CVL for laboratory confirmation from July 16, 2004 to July 15, 2009 and 66% were positive (Annual Technical Report 2004-2009). Both Sylvatic and urban epidemiological cycles of Rabies virus exist in this country giving potential risk from jackal, fox and dog respectively. Dog is considered as a principal reservoir or vector and also 99% responsible to transfer disease in human. Rabies is the cause of death for 100 people and 200 animals every year in this country. Thirty five thousand people get post exposure treatment against rabies. Rabies Vaccine production Laboratory, Kathmandu has been producing Tissue Culture Anti Rabies Vaccine (TCARV) for animal use in the country since 2006 however it produces only 20,000 doses of vaccine which is not enough to meet the national demand. This disease is panic and spread all over the country therefore molecular epidemiological study is essential to identify the genome of Rabies virus existing in the country. This article is preliminary study of molecular characterization of rabies virus in Nepal.

MATERIAL AND METHODS

Sample collection and dispatch

Three brain samples infected with rabies virus were submitted at CVL for confirmation from different institutions in different time. Human brain was collected from Lalitpur by Patan Hospital on 30/07/2003 from 12 years school girl after death. Buffalo brain sample was collected from Kaski by Regional Veterinary Laboratory, Pokhara on 11/07/2003. Dog brain sample was collected from Kathmandu by CVL on 08/09/2008. All of those samples were tested at CVL by performing Negri body test, FAT and, mice inoculation and also Rapid test as soon as possible and found positive for Rabies (Annual Technical Report, 2003). The portion of positive samples were provided to Rabies Vaccine Production Laboratory on request and, devided into several containers and stored at -80° C until use. A part of those samples were dispatched to Pasteur Institute, France according IATA instruction 602 on July 21, 2009.

Molecular characterization

Total RNA was extracted from clinical samples using TRIzol or by using commercial RNA extraction kits. Standard reverse transcription polymerase chain reaction (RT-PCR) was used to amplify the complete N gene. PCR product were purified and sequenced with automated sequencing system and finally phylogenetic analysis was performed by following the method described by Bourhy *et al.,* 2008.

RESULT

All three samples were positive Rabies viral RNA in RT-PCR. Dog and human brain sample were detected as Arctic lineages of Rabies where as buffalo brain was identified as Indian subcontinent lineage on phylogenic analysis. The sequences of RABV collected from dog, human and buffalo are given below.

Dog_Nepal_Nucleoprotein_Complete

ATGGATGCCGACAAGATTGTATTCAAAGTCAATAATCAGGTGGTCTCTTT
GAAGCCTGAGATTATCGTGGATCAATATGAGTACAAGTACCCTGCTATCA
AGGACTTGAAGAAGCCCAGTATCACCCTAGGGAAAGCCCCCGATTTGAAC
AAGGCATACAAGTCAGTCTTATCAGGTTTGAATGCTGCCAAGCTTGATCC
TGATGATGTATGTTCCTACTTGGCAGCTGCGATGCAGTTCTTCGAGGGGA
CATGTCCTGAAGACTGGACCAGCTATGGGATCTTGATTGCACGAAAAGGA
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TTCCTGAGCATGCGTCTTTAGTCGGTCTTCTCTTGAGCCTGTATAGGCTG
AGCAAGATATCTGGGCAAAACACCGGTAACTATAAAACAAATATTGCAGA
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ACCATACTCTAATGACAACTCACAAGATGTGTGCCAATTGGAGTACGATA
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CTCCTCATGAGATGTCCGTCTTAGGGGGCTATTTGGGGGAGGAGTTTTTT
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AGCCCCGAAGCTGTTTATGCCCGAATCATGATGAACGGAGGCCGACTAAA
GAGATCGCACATACGGAGATATGTTTCTGTCAGCTCCAATCACCAGGCTC
GTCCGAACTCATTTGCCGAGTTTCTAAACAAGACGTATTCCAGTGATTCA
TGA

Human_Nepal_Nucleoprotein_Complete

ATGTAACACCTCTACAATGGATGCCGACAAGATTGTATTCAAAGTCAATA
ATCAGGTGGTCTCTTTGAAGCCTGAGATTATCGTGGATCAATATGAGTAC
AAGTACCCTGCTATCAAGGACTTGAAGAAGCCCAGTATCACCCTAGGGAA
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GTATTCCATTGATTAATGAAAGGC

Bovine_Nepal_Nucleoprotein_Complete
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TAA

DISCUSSION

Dog associated RABV forms distinct phylogenetic group that has six major cluster identified as the Africa 2, Africa 3, Arctic-related, Asian, Cosmopolitan and Indian subcontinent clades. Among these 6 clades of RABV Artic and Indian subcontinent lineage were identified in Nepal in this study. Although Arctic clade of RABV was also identified in Nepal in 1998 in 6 brain samples collected from one female goat, four dogs and one mongoose in Kathmandu (Personal communication with Dr. J.N. Rai, Consultant, Rabies Vaccine Production Laboratory, Tripureshwor, Kathmandu). The Arctic- related clade has also been isolated from dog, raccoon dog, arctic fox, red fox, striped skunk and wolf. It is circulating in Russia, Nepal, North India, Korea, Greenland and North America (Hyun *et al*, 2005). However Indian subcontinents clade was first identified in Nepal in 2009. Therefore this is the first report that provides scientific evidence for the presence of Indian Subcontinent clade circulating in buffaloes at Kaski district of Nepal. Earlier study revealed that Indian Subcontinent clade of RABV was considered to be distributed only within Southern India and Sri Lanka (Nanayakkara *et al*, 2003). This is very important finding because it is notable because it is one of the first to diverge. The status of other lineages of RABV is known as limited number of samples was tested therefore there is enough room to continue this study to identify different lineages of RABV circulating in different part of country in different species of animal.

CONCLUSION

Arctic and subcontinent lineages have been found circulating in Nepal to date.

ACKNOWLEDGEMENT

The author would like to thank Central Veterinary Laboratory, Veterinary Epidemiology Centre for providing positive samples and epidemiological information for this study. The technical help and support provided by Institute of Pasture, Paris for molecular characterization is highly appreciated.

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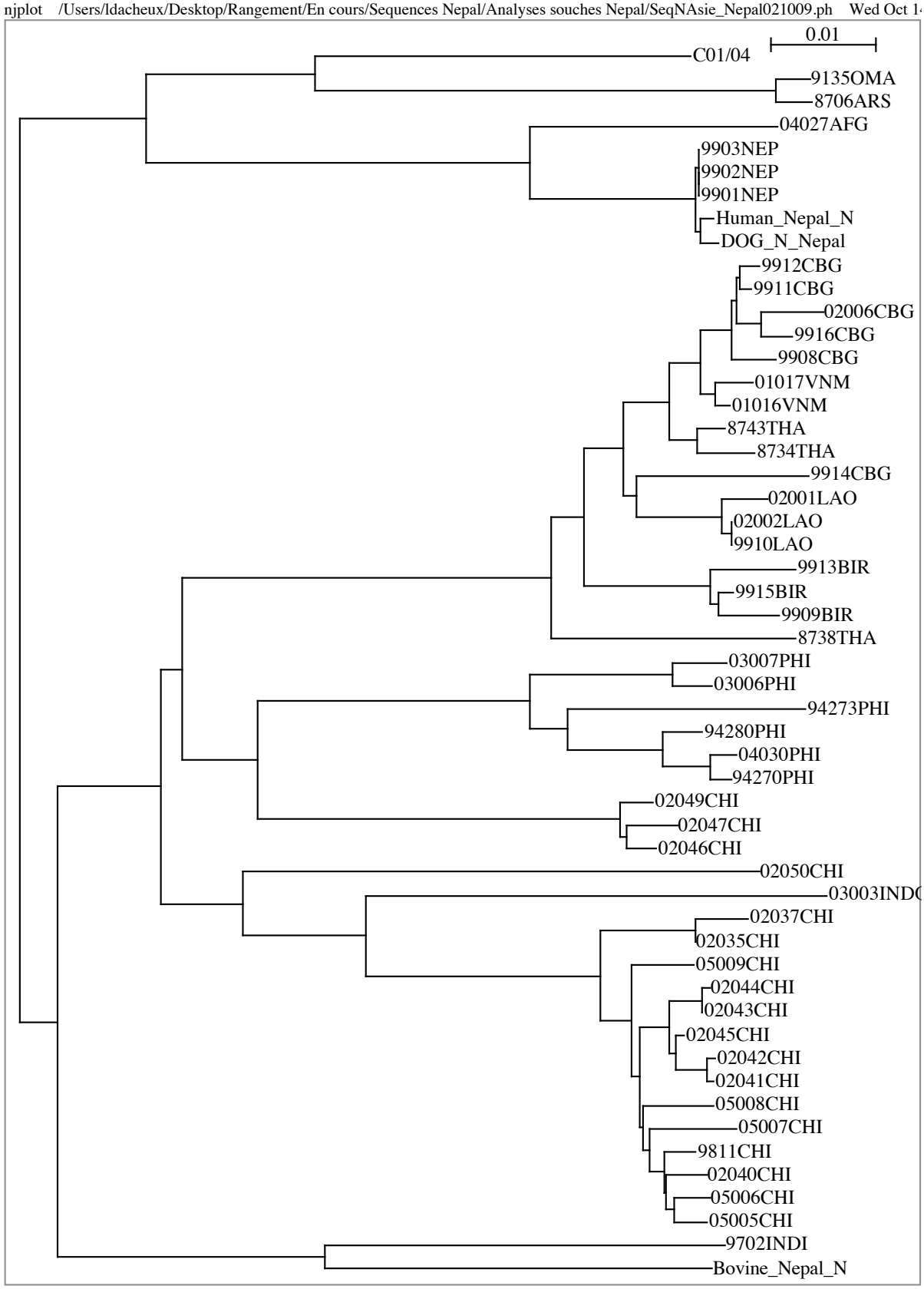
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9909BIR
8738THA
03007PHI
03006PHI
94273PHI
94280PHI
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EPIDEMIOLOGY OF FOOT AND MOUTH DISEASE IN NEPAL

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ABSTRACT

Foot and Mouth Disease (FMD) is endemic in Nepal. The details of epidemiology of FMD in Nepal were set out in this work. FMD was studied in different species of animal by seasons of the year, regions, ecozones, and virus serotypes. This study was carried out by using monthly epidemiological reports on the disease from 75 districts to Veterinary Epidemiology Centre, Directorate of Animal Health, Kathmandu, Nepal from 2000 to 2007. The results were processed and analyzed with the use of the computer program Microsoft Office. FMD was ranked first in terms of the number of outbreaks, the number of affected and dead animals in the structure of the major infectious and invasive diseases in Nepal. The predominant serotypes responsible for epidemic outbreaks of FMD in Nepal were O, A, and Asia-1 which were identical to other countries in South Asia. Cattle and buffaloes were the most susceptible animals for FMD in Nepal, whereas goats and sheep are relatively less susceptible. Hill and Terai (Plain) ecozones of Nepal are the most stressful areas and persistent disadvantage for the disease. Farwest and central regions were most vulnerable. Although the outbreak of FMD is reported all the year round, high incidence of FMD was noticed twice a year: in April-June and December (the movement of animals in previous religious activities). Vaccination was recommended to all susceptible animals at first in Far-western development region adding other regions in the next years using trivalent vaccine, containing virus serotypes O, A and Asia-1 to acquire the herd immunity for successful FMD, prevention and outbreak.

INTRODUCTION

The problem of foot and mouth disease (FMD) is very serious in livestock sector of the country. An infectious disease of animals, especially FMD is one of the major constraints to livestock development in Nepal. The damage from FMD in animals is a serious obstacle to the development of the national agricultural economy.

Economic loss due to FMD in terms of reduction of milk and meat production was estimated to be 66 million US dollars per year (Gongal, 2002). But the real economic damage could be much more higher if we take into consideration losses due to reduced reproduction of animals and the costs of towards treatment. Nepal is a member of the World Trade Organization (WTO), and the presence of FMD in the country is a barrier to international trade of livestock and animal (Thakuri, 2006). So, there is an urgent need to develop FMD control programs and effective measures to combat and prevent the disease.

MATERIALS AND METHODS

This study was performed at the Department of Veterinary Pathology of Peoples' Friendship University of Russia with continuous support from Veterinary Epidemiology Center (VEC), Directorate of Animal Health, Nepal.

The spread of FMD was studied among animals of different species by seasons of the year, regions, ecozones and virus serotypes. Monthly epidemiological reports were collected from 75 districts through VEC from 2000 to 2007. The results were processed and analyzed with the use of the computer program, Microsoft Office.

Analysis of data was held on the epizootic research methods (Makarov, 2001). Criterion validity was determined by the table of Student's (t_d). The difference in values were significant at * $p \leq 0, 05$, ** $p \leq 0, 01$ and *** $p \leq 0,001$ (Kulikov, *et al.*, 2006).

RESULTS

Distribution of FMD in 2000-2007

The results of analysis of FMD spread in Nepal, the number of outbreaks, the number of affected and dead animals in 2000-2007 found that the largest number were recorded in 2001 and 2003 (Table 1). In the preceding years, the number of outbreaks has decreased 2.0-4.3 times, the number of affected animals 2.0-7.8 times and the number of dead animals 2.4-12.0 times. Epizootical characters of disease like outbreak and mortality rate remain quite stable in years 2002, 2006 and 2007.

Table 1 Dynamics of the number of FMD outbreaks, the numbers of affected and dead animals in 2000-2007.

Number	Years								M**
	2000	2001	2002	2003	2004	2005	2006	2007	
Outbreaks	845	1903	546	2078	879	1042	710	481	1060
Affected animals	25841	51003	7261	57076	19525	19949	17389	13590	23961
Dead animals	527	861	118	1265	202	461	105	145	461
Index outbreak*	30.6	26.8	13.3	27.5	22.2	19.1	24.5	28.3	24.0
Mortality (%)	2.0	1.7	1.6	2.2	1.0	2.3	0.6	1.1	1.6

*Index outbreak –Number of affected animals in an outbreak.
**M- Mean

Structure of the major infectious and parasitic diseases in Nepal

The major infectious and parasitic animal diseases (on the List of the World Organization for Animal Health, 2005) in Nepal are FMD, plague and smallpox in small ruminants, classical swine fever, anthrax, rabies, paratuberculosis, babesiosis, brucellosis, hemorrhagic septicemia and theileriasis. The analysis found that in 2003 the largest number of outbreaks among animal diseases account for FMD. Thus, the number of outbreaks of FMD in that year was 54.45%. mortality with FMD was 83.79%, while mortality was 49.22% of the total number of affected and dead animals from all infectious and parasitic diseases. This is significantly higher compared with other diseases ($p \leq 0{,}001$).

The data of 2006 found that the largest number of outbreaks was due to FMD like in 2003. Number of outbreaks of FMD on that year was 30.59% of the total number of infectious and parasitic diseases, number of affected animals with FMD was 59.11%. However, in 2006 a smaller number of dead animals were recorded due to FMD. According to this indicator FMD was ranked fifth among 11 major infectious and parasitic diseases.

Study of serotypes of FMD virus in Nepal

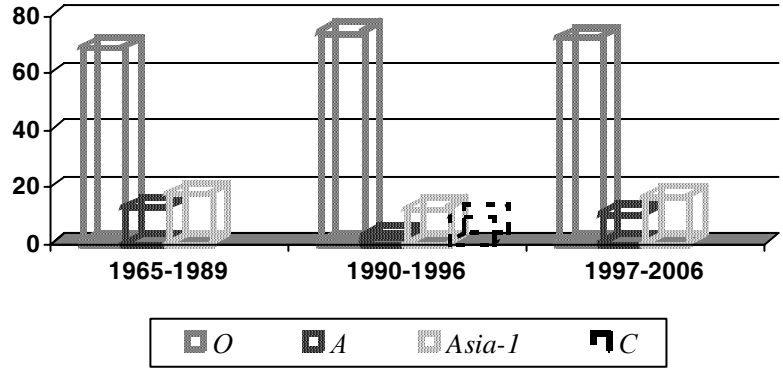


Figure 1. Serotypes of FMD in Nepal (Thakuri, 2006)

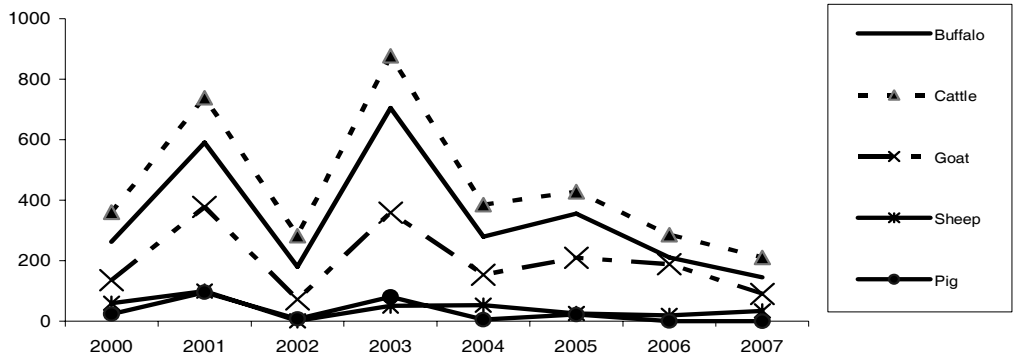
Followed by analysis of data for the period from 1965 to 2006 showed that serotype *O* was ranked serotype *Asia-1* and serotype *A*. Serotype *C* was recorded from 1990 till 1996 and after that it has not been recorded.

Dynamics of FMD in animals of different species

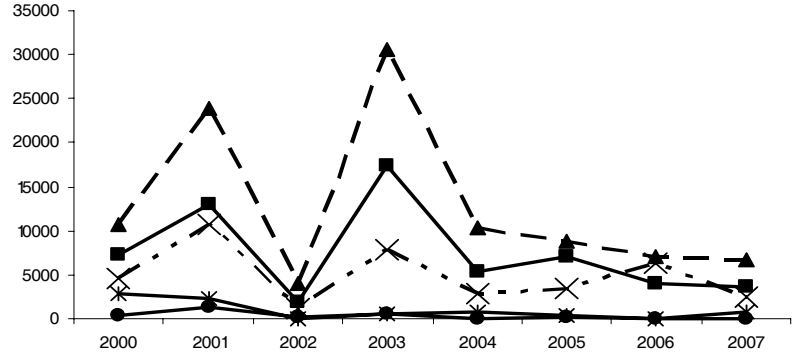
The greatest number of outbreaks has been noted in cattle during 2000 to 2007. The greatest number of outbreaks of FMD in this species was noted in 2001 and 2003 (respectively 739 and 877) (Figure 2/1).

During 2001 and 2003 the number of affected cattle was the largest compared to other animal species and amounted to 23877 and 30526, respectively (Figure 2/2). In analyzing the dynamics of FMD on the number of dead animals of different species, it was noted that this figure was the highest in years, the most pronounced epizootic trouble. The distribution of dead animals from foot and mouth disease by year marked the largest number of cases in cattle in 2003 (563 head). In the same year, the dead number of buffaloes 317 and goats 303 because of this disease, were virtually identical (Figure 2/3).

1.Outbreak



2. Number of affected animals



3. Number of dead animals

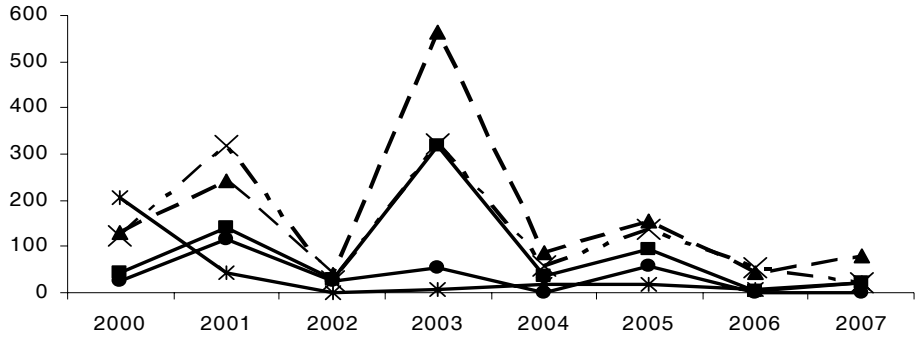
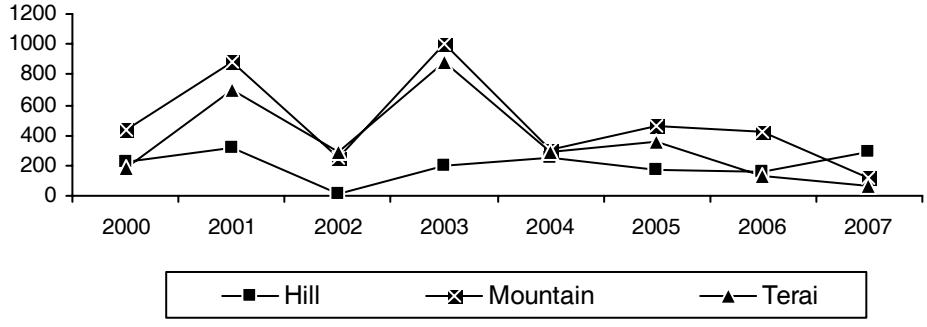


Figure 2. The dynamics of FMD on different animal species in the period 2000-2007

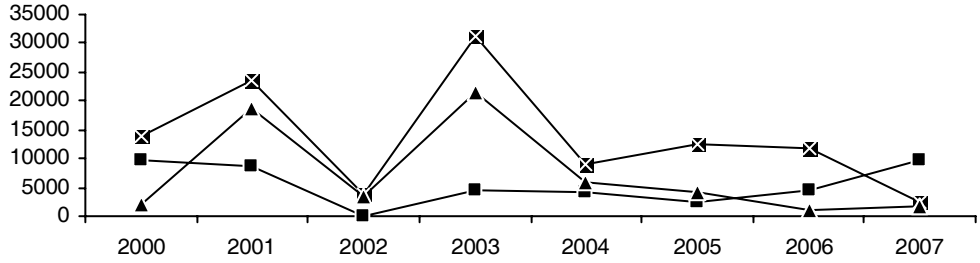
Dynamics of the spread of FMD in ecozones

In analyzing the dynamics of FMD on the number of outbreaks in different ecozones, it was found that the highest no (999 outbreaks) were noted in mountain in 2003, slightly less (884 outbreaks) in the same year were recorded in the terai and the lowest (195) in the hill (Figure 3/1).

1. Outbreak



2. Number of affected animals



3. Number of dead animals

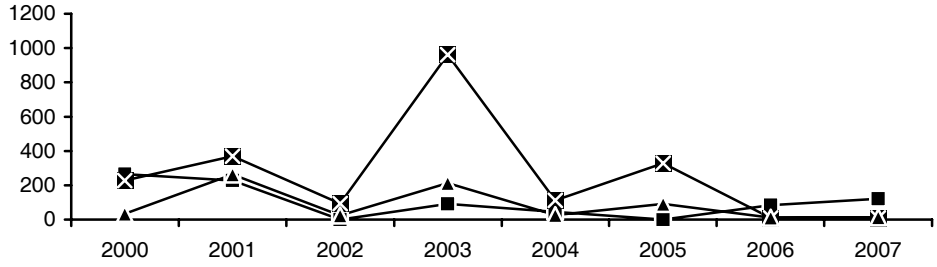


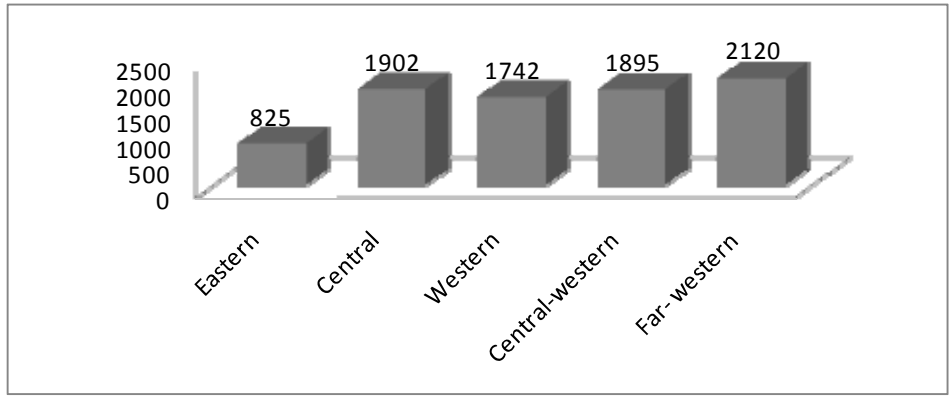
Figure 3. Dynamics of FMD in ecozones in 2000-2007

Characterizing the dynamics of FMD on the number of affected animals in ecozones, have found that this index correlated with those for outbreaks. Thus, in 2003 the number of affected animals in the mountain area was 31075, in the terai – 21409, and in the hill - 4592. A similar trend was observed in 2001 in the other years, this figure varied, but the trend of more affected animals in the terai and mountain was observed in almost all years (Figure 3/2). In the analysis of manifestations of FMD on the number of dead animals in various ecozones , it was found that the most pronounced during periods of epizootic trouble in Nepal (2001 and 2003), the largest number of dead animals mentioned in the Mountain and the Terai ecozones, especially in 2003. In other years, even at a higher level and the number of outbreaks and the number of affected animals, the rate of death of the animals was negligible (Figure 3/3).

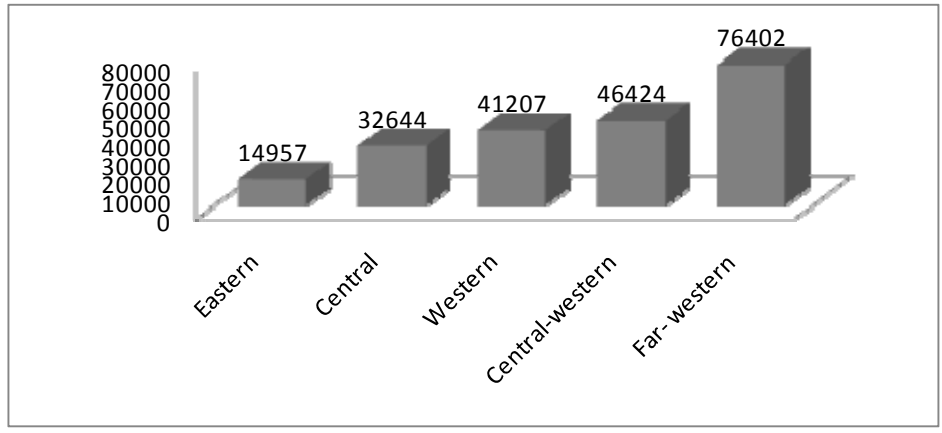
It should be noted that, despite some mixed trends in indicators of outbreaks of the disease, the number of affected and dead animals, higher values of these indicators are recorded in the ecozones, which are characterized by a large number of susceptible animals and the more developed animal husbandry - the plains and highlands.

Spread of FMD in different regions

1. Outbreak



2. Number of affected animals



3. Number of dead animals

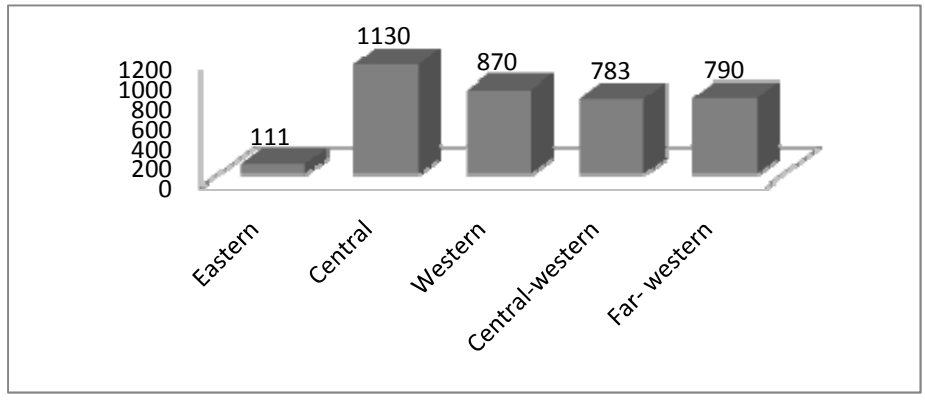


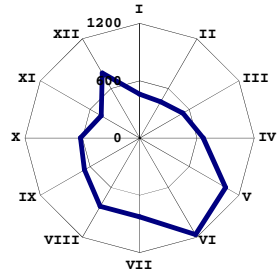
Figure 4. Distribution of FMD in regions for the period from 2000 to 2007

The total number of outbreaks of FMD for the entire period of research was greatest in the Far Western- 2120, while in the Central, Western, Central-western the number was almost the same (1742-1902). The smallest number of outbreaks of FMD (825) registered during the entire period of observation in the Eastern region (Figure 4/1). The total number of affected animals for the entire period from 2000 to 2007 in the Far-western region was 76402. Roughly the same was the figure in the Western (41207) and the Central-Western (46424) regions. Fewer affected animals (32644) for the entire period of observations noted in the Central region. Significantly fewer affected animals were registered (14957) in the Eastern region (Figure 4/2). A similar trend is set in the analysis of the number of dead animals from FMD in regions of Nepal. However, along with the relatively low number of affected animals in the Central region, especially compared to Far-western region, noted a higher rate of dead animals from the disease (1130) (Figure 4/3).

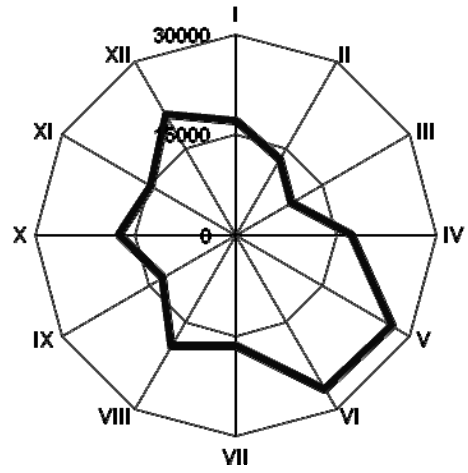
Seasonal distribution of FMD in 2000-2007

In analyzing the total number of outbreaks of FMD during the period , it was found that the most common disease in this indicator is in May and June with a gradual decrease in the number of outbreaks in July and August. It is also noted that an increase in the number of outbreaks in December, but by the time it was less prolonged (Figure 5/1).

1. Outbreak



2. Number of affected animals



3. Number of affected animals

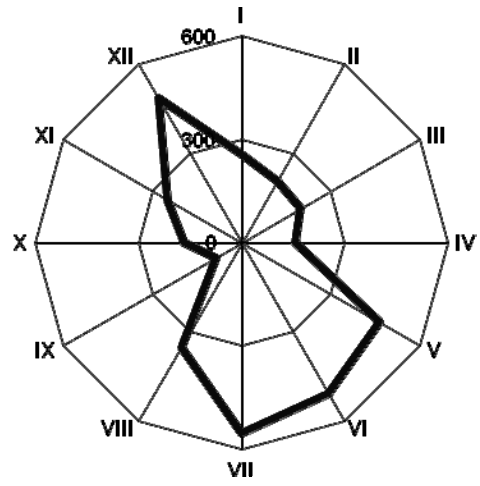


Figure 5. Seasonal dynamics of FMD during the period 2000-2007

While analyzing the total number of affected animals with FMD in the period, it was found that the increase begins in April, reaching a peak in May and June, with a gradual decline in July and August. It was also noted that an increase in the number of affected animals with FMD in December, with a gradual decrease in January (Figure 5/2). Analysis of the total number of dead animals from FMD for the period, showed that the greatest number of death occur in May, June, July and August with a sharp decline in September; and in December (Figure 5/3). Thus, the spread of FMD in Nepal is of perennial nature. The disease is recorded in all months and seasons. But along with those observed more pronounced manifestations of the disease occurs two times a year. The first is noted in pre-monsoon period (April, May and June) and may be associated with the free movement of animals in summer camps and the presence in this time favorable conditions for the spread of infection, because healthy animals are moving in the infected areas, and affected animals infect prosperous place. The second appears in December and is conditioned by previous intensive movement of livestock during religious events. Both these periods are characterized by increased in the number of outbreaks and the number of affected and dead animals of all types.

RESULTS AND DISCUSSION

FMD is endemic in Nepal since time immorable. The disease is characterized as hyper-enzootic. In the structure of the major infectious and parasitic diseases (on the List of the World Organization for Animal Health, 2005) in Nepal, FMD has the highest number of outbreaks, the number of affected and dead animals. Dominant in the occurrence of foot and mouth disease of animals in Nepal are the causative serotypes O, Asia 1 and A, which are identical to those highlighted in other South Asian countries and belonging to the Middle East – South Asia topotype. Most susceptible to FMD animals in Nepal are cattle and buffaloes. Goats and sheep are comparatively less susceptible. Mountain and Terai ecozones of Nepal are the areas with most intense and persistent disadvantage for FMD. The most unfavorable for FMD are regions of the Far-Western and Central. Least of all a manifestation of disease states in the Eastern region. Distribution of foot and mouth disease in Nepal is of perennial nature, but the highest incidence observed twice a year: in April-June (pre-monsoon period) and December (the movement of animals in previous religious activities).
Stamping-out, which is the most effective method to control and eradicate FMD, is impossible in Nepal for socio-cultural and religious reasons - cow in Nepal are sacred animals and forbidden to kill. Conducting systematic vaccination in the national level in Nepal is not possible due to the large number of susceptible animals, financial and technical difficulties (Gongal *et al*., 2000). In recent years, attempts were done to vaccinate high yielding dairy cows and ring vaccination with sporadic outbreaks in Nepal (Thakuri, 2006). But this approach does not give adequate results in the control of FMD. Currently in Nepal, two possible options are considered to eradicate FMD. The first option is proposed to commence a phase-wise vaccination against FMD trivalent vaccine (serotypes *A, O* and *Asia-1*) with the Eastern Terai region, the subsequent inclusion of new regions each year for 10 years. Vaccination should cover more than 80% of susceptible animals with an estimated frequency of vaccination 2 times a year (March-April and September-October) and organize expeditions to inform about vaccination (in February-March and August-September). The latter is proposed to commence a similar phased vaccination against the disease in the Far-western Terai region. Based on our analysis, both options have some drawbacks and cannot be effective because it is not possible to achieve success by 80% coverage of vaccination, the wrong time of vaccination, and expeditions, almost coinciding with the time of outbreaks.

We offer a program for controlling and eradication of FMD in Nepal to provide 100% planned two-time vaccination of all susceptible animals in the February - March and August-September, beginning with the Far-western region, followed by a phased vaccination and the involvement of frontal regions during the next 5 years. To ensure that the coverage of vaccination of susceptible animals - the necessary conditions for the control of FMD in their unique high population density in the country (more than 130 animals per square kilometer territory)-requires the organization of expeditions to inform the public about the activities held in January-February and July-August. In field outbreaks, it is recommended to provide unscheduled (emergency) ring vaccination of animals with 100% coverage of all susceptible livestock with the use of monovalent vaccines of the serotypes of the virus, conforming in diagnosis. Along with the specific prevention of foot and mouth disease, it is recommended that strict compliance with veterinary and sanitary rules should be maintained.

ACKNOWLEDGEMENT

The authors wish a deep sense of gratitude to Dr. G.N. Gongal, Technical Officer, Veterinary Public Health, World Health Organisation South East Asia Regional Office (WHO SEARO), New Delhi; Dr. K.C. Thakuri, Chief of Vetererinary Epidemiology Centre , Kathmandu and Dr. V.C. Jha, Chief of FMD and TADs Laboratory, Kathmandu for their help, support and guidance.

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PREVALENCE OF *SALMONELLA* SPP IN RETAIL MEAT SHOPS IN KATHMANDU

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ABSTRACT

A cross-sectional study was conducted from November 2008 to May 2009 to estimate the prevalence of Salmonella in retail meat shops in Kathmandu. The methods followed were ISO 18593:2004 for swab sample collection, ISO 6579:2002 for Salmonella isolation and manufacturer's instruction (SIFIN®, Germany) for serotype identification. A questionnaire was used to collect information on some of the risk factors of shops likely to be associated with Salmonella identification. A total of 492 environmental swab samples (164 chopping board samples, 164 knife samples and 164 table samples) from 82 retail meat shops were analyzed. The prevalence of Salmonella positive shops was 40.2% (95% CI: 29-51). The isolation rates of Salmonella from chopping boards (36.0%), knives (32.9%) and tables (25.0%) were not significantly different (p>0.05). Retail meat shops were 1.9 times more likely to yield Salmonella in the evening (38.2%) as compared to the morning (24.4%) (p=0.001). S. typhimurium (54.5%) was the most common serotype found in retail meat shops followed by S. enteritidis (16.9%), S. haifa (13.6%), S. virchow (10.4%) S. agona (3.9%) and S. enterica (0.6%). Among the risk factors examined, "hygiene status of shop", "type of shops", "number of person handling meats", "number of knives used", "number of kinds of meat sold" and "number of kinds of meat sold using different numbers of knives" were individually significantly (p<0.05) associated with Salmonella contamination in the retail meat shops. After univariate analysis of these risk factors, a final logistic regression model with Salmonella yes or no category of shops as outcome variable identified 4 significant predictors. Odds ratios, indicating the likelihood increase of a shop to achieve Salmonella positivity status were 10.17 for multiple persons rather than a single person involved, 7.66 for open rather than closed shops, 9.44 for use of several rather than one knife and 5.18 for single kind of meat using several knives.

The results of this investigation revealed that retail meat shops to a noticeable extent are Salmonella contaminated, with a considerable degree of cross-contamination between meats, personnel and equipment used during a day in processing of meats.

Keywords: *Salmonella*; retail meat shops; prevalence; swab samples; risk factors; Kathmandu

INTRODUCTION

Salmonella is one of the most widespread food borne pathogen and a growing public health problem both in developed and developing countries including Nepal. In the United States of America alone, the Center for Disease Control and Prevention (CDC) has estimated that out of 1.4 million cases , 16,430 hospitalizations, and 582 deaths are caused by salmonellosis annually (Mead et al., 1999). Each year, approximately 40,000 *Salmonella* infections are culture-confirmed, serotyped, and reported to the United States Center for Disease Control and Prevention (CDC). Of total cases, 96% are estimated to be caused by foods (Mead et al., 1999). In Europe, *Salmonella* was the second most reported cause of food-borne diseases in humans with 160,649 people suffering from *Salmonella* infections in 2006 approximately 35 people in every 100,000 (EFSA, 2007).

Kathmandu, the capital city, has an estimated population of 1.4 million (GeoNames geographical database) which is ever increasing due to tourists and immigrants. As a result of this, Kathmandu is continuously facing high demand for food. This has led to increase in food establishments, such as

food vendors, small shops, cold stores and butchers shops. The owners of these establishments have little knowledge or awareness of food hygiene and safety. Hence, a great majority of consumers buy meat from butcher's shops at which food hygiene and safety conditions are not assured. There is little information on the prevalence of *Salmonella* in meat in retail shops but no information particularly on the prevalence of *Salmonella* in retail meat shops in Nepal. Therefore, this study was conducted to determine i) the prevalence of *Salmonella* spp ii) the serotypes and iii) to know some of the risk factors associated with cross contamination of the retail meat in Kathmandu.

MATERIALS AND METHODS

This study was carried out from November 2008 to May 2009 in retail meat shops located in Kathmandu, Nepal. A total of 492 environmental swab samples (knives 164, chopping boards 164 and tables 164 each) were taken from randomly selected 82 retail meat shops located in 5 different divisions of Kathmandu. Three environmental samples in the morning and three environmental samples in the evening were collected from the same selected sites from sample shop.

For the collection of swab samples ISO 18593 2004 (E) was followed. The swab samples were kept in an icebox (4-5^{o} C) and were sent for analysis as soon as possible, but not more than 24 hours later, to the Central Veterinary Laboratory, Tripureshwor, Kathmandu, Nepal. The test tubes were incubated at 37 ± 1°C for 18 ±2 hrs. Before starting isolation, the test tubes were shaken vigorously. The microbial analysis was done using the methods for the detection of *Salmonella* following standard procedures from ISO 6579:2002 (E). After incubation on nutrient agar, pure colonies were picked up and inoculated into Triple Sugar Iron (TSI; Merck KGaA, Germany) slant, Voges- Proskauer (VP; Merck KGaA, Germany) broth, Motile-indole-lysine (Difco™ MIL Medium, Germany) broth and Urea (Urea; Merck KGaA, Germany) slant. All inoculated biochemical media were incubated at 37°C for 18-24 hrs and checked for confirmation.The serological confirmation of *Salmonella* antigens was performed by slide agglutination testing (appendix-D), according to the instructions of the manufacturer (SIFIN®, Germany).

The data collected from the field, laboratory investigation and the questionnaire were managed using Excel® version Microsoft Office® Excel 2003. The STATA version 10 (STATA Corp., College Station, Texas, USA) and the Statistical Package for Social Sciences (SPSS) version 16 were used for analysis of data. The significance level and confidence interval were considered to be 0.05 and 95% respectively. The prevalence of *Salmonella* was expressed by dividing the number of positive samples with the number of total samples tested (Thrusfield, 2005). A Chi-square Fisher exact test was used to compare the prevalence of *Salmonella* according to retailer shops, time, months and administrative divisions. McNemar's chi square test was used to determine significant differences between morning and evening isolation of *Salmonella* from different sample types. Data from the questionnaire were used to evaluate the association of the risk factors with *Salmonella* identification. A univariate analysis (Chi-square Fisher exact test) was conducted using the *Salmonella* status of the meat shops as the outcome variable. A multivariate analysis was performed to relate the potential risk factors (derived from the questionnaire responses) to *Salmonella* outcomes (present or not present) in samples and shops. All variables with a significant value $p \leq 0.05$ were selected for further analysis in a multivariate logistic model. A backward stepwise elimination process was used with a p-value for retention of a variable equal to 0.15 (Hosmer and Lemeshow, 2000). Interactions between variables were tested and retained with similar retention p-values. The Hosmer and Lemeshow goodness-of-fit test was carried out to assess the fitness of the model.

RESULTS

Out of the total of 82 shops sampled, 33 were positive for *Salmonella,* giving an overall shop prevalence of 40.2% (95% CI: 29.4-51.1). Numerically, division-specific *Salmonella* contaminations in their respective shops were different, with the highest rate of 45.8% recorded for the East Division and the lowest in the Center Division (27.8%). Statistically, no differences were established between the 5 divisions (p = 0.800) (Table 2).

Table 1: *Salmonella* positive meat shops in different divisions in Kathmandu

Division	Total	Positive	Percent	95% CI
Center	18	5	27.78	9.7-53.5
City Core	16	7	43.75	19.8-70.1
West	8	3	37.50	8.5-75.5
East	24	11	45.83	25.6-67.2
North	16	7	43.75	19.8-70.1

CI=Confidence Interval, *P-value=Fisher exact test

Out of a total of 492 samples collected from the retail meat shops, 154 samples were found positive giving an overall sample prevalence of 31.3% (95% CI: 27.2-35.6) (Table 1).
Morning and evening prevalences of *Salmonella* in the samples of retail meat shops were statistically significant (p = 0.001); prevalences were higher in the evening (38.21%) compared to the morning (24.39%). The proportions of *Salmonella* on chopping boards, knives and tables in the morning were 31.71%, 26.83% and 14.63%, while in the evening the proportions were 40.24%, 39.02% and 35.37%, respectively (Table 2).

Table 2: *Salmonella* in samples in mornings and evenings in meat shops

Time	Sample types	Total samples	Positive samples	Percent	95% CI
Morning	Knives	82	22	26.83	17.6-37.8
	Chopping boards	82	26	31.71	21.9-42.9
	Tables	82	12	14.63	7.8-24.2
	Total [a]	246	60	24.39	19.2-30.3
Evening	Knives	82	32	39.02	28.4-50.4
	Chopping boards	82	33	40.24	29.6-51.7
	Tables	82	29	35.37	25.1-46.7
	Total [b]	246	94	38.21	32.1-44.6
Grand Total		492	154	31.3	27.2-35.6

[a,b] = Statistically significantly different CI = Confidence Interval, *P-value= chi^2 test

Of the total 154 isolated samples, 5 serotypes were identified. The most frequent serotype identified in retail meat shop was *S.* typhimurium (54.5%) followed by S. enteritidis (16.9%), *S.* haifa (13.6%), *S.* virchow (10.4%), *S.* agona (3.9% %) and *S. enterica** (0.6%) (Table 3).

Table 3: *Salmonella* serotypes in each type of samples in meat shops

Serotpyes	No. of isolates (%) in different sample types			Total
	Chopping boards	Knives	Tables	
S. typhimurium	32 (54.2%)	30 (55.6%)	22 (53.7%)	84 (54.5%)
S. enteritidis	8 (13.6%)	10 (18.5%)	8 (19.5%)	26 (16.9%)
S. haifa	10 (16.9%)	7 (13.0%)	4 (9.8%)	21 (13.6%)
S. virchow	6 (10.2%)	4 (7.4%)	6 (14.6%)	16 (10.4%)
S. agona	2 (3.4%)	3 (5.6%)	1 (2.4%)	6 (3.9%)
*S. enterica**	1(1.7%)	-	-	1 (0.6%)
Total (%)	59 (38.3%)	54 (35.1%)	41 (26.6%)	154 (100.0%)

*O4,5,12:z10:-

The prevalence of *Salmonella* in shops with subjectively assessed poor hygiene was 55.81% (24 out of 43) in comparison to 23.08% (9 out of 39) in shops with good hygiene. The chance of getting

Salmonella in shops with poor hygiene was 5 times higher than in shops with good hygiene (OR = 4.21, p = 0.003 (Table 4 and 5).

Table 4: Prevalence of *Salmonella* in meat shops with different risk factors.

Factors	Level	Total	Positive	Percent	95% CI*	p-value*
Hygiene	Poor	43	24	55.81	49.1-79.0	0.003
	Good	39	9	23.08	11.1-39.3	
Type of shop	Open	44	24	54.55	38.8-69.6	0.004
	Closed	38	9	23.68	11.4-40.2	
Kind meat sold	1	37	9	24.32	11.8-41.2	0.008
	>1	45	24	53.33	37.9-68.3	
Knives used	1	30	5	16.67	5.6-34.7	0.001
	>1	52	28	53.85	39.5-67.8	
Persons handling meat	1	47	10	21.28	10.7-35.7	0.000
	>1	35	23	65.71	47.8-80.9	

*CI = Confidence Interval, *P-value = Pearson chi^2 test

Table 5: Potential risk factors associated with higher odds of *Salmonella* in retail meat shops in Kathmandu

Factor	Level	OR	95% CI	P-value
Hygiene	Poor Good	4.21	1.47-12.42	0.003
Type of shop	Open Closed	3.87	1.36-11.40	0.004
Meats sold	> 1 Single species	3.56	1.25-10.46	0.006
Knives used	>1 single knife	5.83	1.78-22.11	0.001
Persons handling meat	>1 Single	7.09	2.39-21.51	0.000

CI=Confidence Interval, *P-value=Pearson chi^2 test

The Chi-square univariate analysis indicates six variables with $p \leq 0.25$ which were further analyzed in a multivariate model. The final multivariate model does contain four risk factors for *Salmonella* contamination in retail meat shops in Kathmandu (Table 6).

Table 6: Final logistic regression model of risk factors associated with *Salmonella* isolations for 82 retail meat shops

Factor	Level	Odds ratio	P-value	95% CI
Type of shop	Open Closed	7.66	0.003	1.96-29.88
Knives	Multiple Single	9.44	0.005	1.94-45.89
Persons	Multiple Single	10.17	0.001	2.58-40.14
Species of meat sold and number of knives in use	Single species of meat using 2 or more knives	5.18	0.036	1.11-24.14
	Two species of meat using 3 or more knives	8.15	0.027	1.27-52.54

CI=Confidence Interval, *P-value=Pearson chi^2 test

The Hosmer and Lemeshow goodness-of-fit test indicated that the model did fit the data adequately (Hosmer and Lemeshow Chi-square (7) = 6.53, p = 0.479). In the final logistic regression model risk factors associated with higher likelihood of *Salmonella* were open shops (OR = 7.66, p = 0.003), multiple knives used (OR = 9.44, p = 0.005) and more persons involved (OR = 10.17, p = 0.001) (Table 6).

DISCUSSION AND CONCLUSIONS

The study was conducted in retail meat shops of all the five administrative divisions of Kathmandu metropolitan city. Study units were butcher's shops located in the city, which are the major meat retail outlets in the city. Most of the shops do receive meat from the cold store in Kathmandu, with a few exceptions when the shops had facilities themselves for evisceration (for e.g. goat meat). The samples were collected from a shop at 7-8 am in the morning and 5-6 pm of the same day. These two times are the peak meat trade hours in Kathmandu. Washing of all utensils used in the shops, typically was done on a daily basis, particularly at the end of the day, before closure of the shops. Dry scrapping of chopping boards was done 5 to 6 times a day, depending on how much meat trimmings and fat had accumulated on them. Likewise, knives were mostly wiped off by a piece of cloth and washed with water. All the shops used wooden chopping boards. The use of protective clothes was not seen in any of the shops. The owners of the shops were not much aware of foodborne pathogens and diseases.

Investigations of environmental swab samples do provide an estimate of the prevalence of *Salmonella* in retail meat shops. A shop was considered as positive for *Salmonella* if one out of six samples were confirmed *Salmonella*. The eventual prevalence of 40.2% of *Salmonella* in retail meat shops was found for much higher than the prevalence of 16.4% reported by Bhandare *et al*., (2007) in their study from retail meat shops in India. The present finding of 31.3% of *Salmonella* in different swab samples was also high compared to 11.4% reported by Maharjan *et al* (2005) in their study from retail meat shops in Kathmandu. These differences may be the result of different sample types (Hurd *et al*., 2004), or different methods for the detection of *Salmonella* (Pangloli *et al*., 2003). Identical serotypes present on knives, tables and chopping boards of the same shops can be linked to widespread cross-contamination of the bacteria in the shops in Kathmandu. Higher prevalences in the shops in all likelihood are related to the poor infrastructure of shops such as lack of dressing facilities, drainage, differentiation between clean and unclean operations, and a general lack of basic maintenance of hygiene and sanitation. It is suggested that contamination levels further are increased due to excessive handling of carcasses, by too many people, by keeping more than two kinds of meat in a shop without proper separation of meat areas in the shops and by a constant flow of contamination from to the unsuitable floors of the shops.

The contamination rate of 36.0% on chopping boards and 33.0% on knives can be compared to results of study carried out by Sanguankiat *et al*. (2005) in a pork processing plant in Thailand; Likewise the authors also found that chopping boards (55.0%) compared to knives (30.0%) were more often contaminated with *Salmonella*. A higher level of *Salmonella* contamination on chopping boards (36.0%) was found, as compared to only 18.8% in retail meat shops in another study in India by Thiruppathi *et al*., (2004). In thier study, the chopping boards were also found highly contaminated, followed by knives which can be compared to our study. Also in the Netherlands contaminated chopping blocks made up about two thirds of all cross contaminations that occurred during meat processing (Edel *et al*., 1977).
High contamination of chopping boards, knives and tables in this study indicated improper and ineffective cleaning and disinfection. The rough, porous wooden surface of the boards does play a role in harboring and multiplying the organism better than with the other two sources. In fact, cleaning and disinfection of the wooden chopping boards is not possible for the shops personnel. Almost all chopping boards in this study contained remnants of meat, meat juice and bones, and were rough from immeasurable knife cuts.

The higher proportions of *Salmonella* in samples of retail meat shops in the evening compared to those in the morning (p = 0.001) reflects the spread of contamination throughout the day within the shops. The proportions of *Salmonella* in the mornings and evenings samples from chopping boards, knives and tables were statistically significantl reflecting poor hygienic practices resulted to a widespread accumulated contamination at the end of the day.

S. typhimurium is a common cause of human salmonellosis in many countries (Tavechio *et al*., 1996, Leegaard *et al*., 2000, Esaki *et al*., 2004, Martinez-Urtaza *et al*., 2004, Gorman & Adley 2004). Isoalation from human and animal sources also shows S. typhimurium as the common serotype in the United States (Bender *et al*., 2001, Rabatsky *et al*., 2004) and the second in the United Kingdom (Martinez-Urtaza *et al*., 2004). The predominant serotype was *S*. typhimurium (54.5%) followed by *S*. enteritidis (16.9%), *S*. haifa (13.6%), *S*. virchow (10.4%) *S*. agona (3.9%) and *S. enterica** (0.6%) in our study. In a previous study with meat of different species in retail meat shops in Kathmandu, predominant serotypes reported were *S*. pullorum, *S*. typhi, *S*. gallinarum and *S*. choleraesuis (Maharjan *et al*., 2006). The result obtained by Bhatta *et al*. (2007) can be comparable with our study where they also found the predominant serotype as S. typhimurium from urban water supply system in Nepal. The butchers' shops used water derived from various sources due to scarcity of water in the capital city that can be contaminated out or inside shops. Another study in human blood samples in Kathmandu (Pokharel *et al*., 2006 and Maskey *et al*., 2008) revealed that S. typhi and S. paratyhi as the common serotypes responsible for enteric fever in human. However, none of the isolates from retail shops characterized as *S*. typhi and *S*. paratyphi in this study which reflects these two as highly confined serotypes in human in Kathmandu.

S. agona, *S*. haifa and *S*. virchow have been found first time in this study which has never been reported earlier in Nepal. *S*. agona had been isolated from asymptomatic children in Mexico (Zaidi *et al*., 2006), retail chicken meat in Vietnam (Huong *et al*., 2006) and *S*. Haifa from faeces of 3 year old child having enteritis in Israel (Sapira and Hirsch, 1950), fecal samples of a old person having food intoxication in Japan (Kaibu *et al*., 2005) and chicken samples in Ethiopia (Molla *et al*., 2003). These findings clearly indicate the zoonotic importance of these two serotypes.

In open shops (p=0.004) the free movement of persons and the touching of meat by different customers with unclean hands as well as dust from the roads are likely to add further hazards from outside. Inside a shop, cross-contamination of meat is likely due to manipulations and use of utensils on the meat itself. The butchers usually wash the carcasses or parts of it with only small amounts of water, usually in a bucket, and the same water is used for washing knives, hands and even the offal and carcasses/parts. In the closed shops, in contrast, the water used is potable and special provisions exist for washing and cleaning inside the shops. Generally, departmental stores buy ready-to-sale meat from cold store; no evisceration and other steps of meat processing are carried out consequently in such closed shops, in contrast to open shops.
Our study found that only 29.7% of the shops sold one kind of meat compared to 60.0% selling several kinds of meeting. Meat may become contaminated by improper handling during transport and storage or display. Keeping and selling different kinds of meat from the same counter in all likelihood did increase further contamination. If stored meat comes in contact with other contaminated meat or with contaminated equipment, cross-contamination is very likely. In this case, the contamination rate will increase with an increasing number of kinds of meat sold in the same shop. There might be other factors in shops which likely to enhance further cross-contamination.

The use of several knives over a single knife in the shops did increase the prevalence of *Salmonella* in the shops (p = 0.001). This result contradicts with the finding of Huong *et al*. (2005) they found higher prevalence of salmonella in shops that used single knives in Vietnam. Use of several knives in the shops though does increase the prevalence since handling persons have opportunity to switch between different knives during peak trading hours, with leaving used knives for some time unattended and un-cleaned. There is no destruction of bacterial cell from such knives, growing of bacterial cell is increased during the day. Such knives play a role to transfer the bacterial cell to the other surfaces as well. Moreover, meat handlers keep their knives above the chopping boards and cover the chopping boards with a cloth. It is not possible to periodically hand-dip in chlorinated water, to wear gloves, or periodically clean and disinfect utensils as it is done in the processing plants. No respective standard operating procedures are prescribed for these retail shops. They removed blood and meat from the surface of the knives at their will; how finely and frequently dirt material is removed from the surface of knives remains an open question. Cross-contamination may occur when microorganisms are transferred from one surface to another, possibly leading to contamination of otherwise safe meat or clean equipment. Cross-contamination can occur between equipment, meat, the environment, and even employees.

Differences in *Salmonella* prevalences were significant between shops where multiple persons did handle the meat compared to shops that only had a single person (p=0.000); more persons lead to higher *Salmonella* prevalence. Without doubt knowledge of handling meat may not be the same in every person working in the retail shops. More likely, there may be free use of knives to cut meat of all kinds. Dirty or unwashed hands of workers will contaminate meat and equipment. Employees who perform many different tasks in retail meat shops without proper hand washing in between, or who fail to use appropriate utensils (knife used to cut chicken meat might be used to cut goat meat, too) will contaminate meat and equipment. The widespread presence of *Salmonella* in the retail meat shops' environment is clear evidence.

The use of a separate knife for one kind of meat each was a normal practice found in the retail shops in Kathmandu. Four types of shops with respect to the kinds of meat handling with different classified numbers of knives can be for Kathmandu, as:

i. Two species of meat were sold using two knives
ii. One species of meat was sold using 2 or more than 2 knives
iii. Two species of meat were sold using 3 or more knives
iv. Two species of meat were sold using single knife

The prevalence of *Salmonella* in those shops that sold two species of meat using 2 knives was lower than in the shops that sold two species of meat with 3 or more knives in use (p = 0.023). Increased prevalence may be indirectly due to the spread of *Salmonella* onto meats through the knives themselves, more likely due to their contact with tables and chopping boards.

The calculation of odds ratios assisted to quantify the relative importance of risk factors. The dimensions of the odds ratio point to particular and pressing risk factors.

The logistic regression process served to identify the likelihood of a positive *Salmonella* classification of a retail meat shop by a combination of predictor variables. The risk of a shop to achieve a S*almonella* positive status, when the four variables were present, thus was about 10 times increased when many people rather than a single person were working in the shop and about 7 times increased when the shop was of the open rather than the closed type. The variables 'knives' and 'species of meat sold and numbers of knives in use' in all likelihood are not independent of 'persons'. Logic tells that more persons will use more knives and more persons will work, the higher the number of meats is in a shop. For this, persons' and 'type' in combination are the most significant predictors (risk factors) for a *Salmonella* positive status of a retail meat shop.

CONCLUSIONS

Sell of meat of different species, use of multiple knives and involvement of several persons handling the meat in open and dirty premises in Kathmandu were identified as risk factors for *Salmonella* contamination. Implementation and maintenance of a package of integrated hygienic measures will lower the probability of *Salmonella* contamination in the shops. Incoming meat at the shops may be one source of contamination. Shops are not organized and never are cleaned and disinfected thoroughly; residual contamination on utensils, floors and hands does propel multiplication and spread of organisms during a normal shop day.

It is certain that *Salmonella* or other bacteria are present in the environment of the retail meat shops in considerable numbers and that may they appear on a regular basis. There is need to comply with meat inspection act immediately. All steps in the food chain must be considered while developing control strategy of *Salmonella.* Joining forces of meat handlers, trade associations, academics and government is necessary to minimize the prevalence of *Salmonella* in retail shops.

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STUDY ON EFFECT OF DIFFERENT FAT LEVELS ON THE QUALITY OF *CHHURPI* PREPARED FROM COW AND BUFFALO MILK

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ABSTRACT

A study was carried out to standardize the method of manufacturing of Chhurpi on the basis of fat percentage of cow and buffalo milk. On the basis of market survey, three different combinations of recipes for cow milk fat (0.20%, 0.50% and 0.80%) and for buffalo milk (0.20%, 0.50% and 0.80%) were selected for optimizing the process for preparation of Chhurpi.

The Chhurpi preparation included skimming of milk, pasteurization, cooling to 82°c, inoculation of citric acid using 2% and coagulation occur until clear whey obtained after 15-20 minutes. The 1/3 rd whey was drained after coagulation and filtered using a muslin cloth. The curd was cooked for 15 minutes, wrapped in muslin cloth. The curd were pressed over night and dried in a room temperature on shade for 14 days. The process optimized product was selected on the basis best sensory scores by using 9-point Hedonic rank sum method. The Chhurpi having 0.8 % fat content in cow and buffalo skim milk Chhurpi highly acceptable product in which gumminess of cow milk with significant ($p<0.05$) improvement in sensory attributes.

The experimentally prepared, process optimized Chhurpi were analyzed in laboratory and compared its quality with control product. Parameters used to monitor to the quality of Chhurpi were chemical (moisture%, fat%, protein%, total ash%, lactose%, acidity %) and microbial quality (yeast and moulds). The physicochemical parameters showed that there was significant ($p<0.05$) variation in moisture, fat, protein and total ash and lactose were non-significant ($p<0.05$). The yeast and mold count in process optimized product and control product of cow and buffalo milk Chhurpi did not showed significant ($p>0.05$) difference in the initial and final count of yeast and molds. The product yield obtained was higher in more fat concentration i.e. 3.98% and 4.05% of cow and buffalo milk prepared Chhurpi, respectively. The higher percentage yield occurred to the cow and buffalo milk having high percent of fat. The average selling price of process optimized Chhurpi was found Rs.206.56 per kg and average market price of Chhurpi was found Rs.210 per kg in the study area.

Key words: *Chhurpi,* Citric acid, Fat effect, Sensory evaluation, Composition, Product, Quality.

INTRODUCTION

Milk is considered as an excellent food for human being. It is the only source of first class proteins especially for those who are vegetarian. It is an essential item for the newly born young ones and equally important to the expectant mothers for supply of most essential elements like calcium and phosphorus along with numerous other essential major and minor substances. For each aged groups people milk is essential to maintain its health. Milk is broadly classified to contain milk fat (along with fat soluble vitamins and pigments) and solid not fat. Consumption of milk and dairy products is a part of Nepalese culture. Dahi ghee, *Chhurpi*, khoa and khoa-based sweets are the main traditional commercial dairy products (Pradhan *et al.,* 2003).

Chhurpi is casein based traditional dried milk product. It is often called hard cheese. It is good source of Protein and mineral-Calcium. The casein contain in milk is coagulated with acid and/or previous day whey. The coagulated casein is pressed with heavy load overnight which expel the moisture to a minimum level. Then it is dried for a long time to reduce moisture to an acceptable level. It is use as a nutritious masticatory milk item (Karki, 1986).

Chhurpi is called *durukowa* or *durukho* in Bhutan. *Chhurpi* is made from the milk of a yak or chauri, and cow and buffaloes in different regions of Nepal. It is prepared in a local dairy or at home from a material extracted out of buttermilk called *sergem*. The *sergem* is wrapped in cloth, usually jute bags, and pressed hard to get rid of water. Then, it dries out and becomes similar to cheese. Finally, in this cheese-like stage; it is cut into pieces, and hung over the smoke to make it stone hard. It is used during trek of high hill by people to moistens the mouth

Chhurpi is a traditional and smoke dried cheese. It is very popular in Himalayan region. *Chhurpi* is a highly nutritious and shelf stable product with characteristic flavour and pronounced chewiness and gumminess. The production of *Chhurpi* is confined to traditional families and is manufactured in crude manner, which results in variation in its quality attributes. With advancement of the technology and increasing demand for this product, there is a need to standardize the manufacturing methods and packaging of the product (Pal *et. al.*1996).

Chhurpi is used fresh or can be used after certain period, ranging between days to months. It is used as chewing like nut. Now a day consumption of Chhurpi has been increased in the urban areas However, long storage causes natural fermentation, giving off typical flavour which is liked by Jirel and Sherpas. In China, excellent examples of diversification exist, including the processing of yak milk into powder. It is very popular among trekkers and campers because of their advantages of being light weight, easy to found and pack to eat (FAO, 1990).

Growth of yeast and mold is a common problem and exposure to environment during drying that may be one of the reasons for deterioration of quality.

Problem statement

The *Chhurpi* technology has been very useful to the remote and mountainous milk producers who do not have access to raw milk market. The producers are able to convert perishable milk into long life products like *Chhurpi,* butter and ghee. The technology has been quite useful to generate cash income and local employment at the rural level. There is no cheese factory, so milk is made into *Chhurpi* and butter, where contractors sell in Kathmandu; the herders have no marketing problems. The demand of *Chhurpi* only in Kathmandu valley estimated is about 40 MT. The consumer consciousness low on health benefit of *Chhurpi,* government policies focus on pasteurize milk/ Yoghurt/Ghee/Ice-cream etc. only and *Chhurpi* yet not to include into Food law provision. These are the problems of the *Chhurpi* production.

Farmers produce milk for their families' consumption and about 87% of Nepal's total milk production is for home consumption. The large milk production is not yet considered an important commercial venture by most farmers. However in areas where market and market infrastructure existing commercial production of milk is common and growing phenomenon. Marketing horizontal and concept of marketing of the whole have expanded in areas where accessibility is not a problem.

METHODOLOGY

Market survey

Primary data was collected from a survey. A set of questionnaire was prepared. The survey work was carried out to assess the marketing and socio-economic status; to prepare recipes and production of traditional technology of the *Chhurpi in* 3 districts. For this, almost all of the producer of *Chhurpi* in the targeted areas were listed and interviewed to obtain primary information. Secondary level information was obtained from different publication sources. The data obtained from survey were compiled and analyzed.

The materials for the preparation of *Chhurpi* were mainly fresh cow and buffalo milk skimmed and standardized skim milk of 3 recipe of were prepared based on fat percentage. The recipes were prepared for cow milk (0.20%, 0.50% and 0.80%) and for buffalo milk (0.20% 0.50% and 0.80%).

The collected product of *Chhurpi* from Baglung of buffalo milk *Chhurpi* and Ilam of cow milk *Chhurpi* packed in LDPE was purchased from shop as a control product.

The preparation of *Chhurpi* manufacture was done on the fat basis by which different recipe were prepared. On the basis of these recipes different types of *Chhurpi* were prepared by using different levels of treatments with 2% citric acid. The tables, cloths, equipments, hands were cleaned properly by using the hot water and ethanol. The milk was tested for fat for standardization for skim milk i.e.0.5 per cent fat and 8.7 per cent SNF. The standardized and filtered milk was heated to 90°C and cooling the milk at 82°C and then inoculation of cogulants(2% citric acid). Coagulation occurs until clear whey was obtained at 15-20 minutes and then whey was drained. The coagulum now called green curd was cooked in an open pan over water bath for 15 minutes. The hot cooking curd wraps in muslin cloth and pressed in a wooden hoop at 9 kg /cm^2 pressure for over night. The mass was cut into small square shape and dry at room temperature (28° - 32° C) on shade or air for 14 days. Then *Chhurpi* was packed in polyethylene.

The sensory attributes of 3 experimental products of cow milk and one control product (purchase from Ilam ,mangalbare) and similarly 3 experimental products of buffalo milk *Chhurpi* and one control product (purchase from Baglung Cooperative) were evaluated by conducting sensory evaluation with the help of panel of 10 trained judges. The parameters for evaluation were gumminess, chewiness and overall acceptability assessed by using 9-point Hedonic scale (Rangana, 2001) after 14 days drying of the product. The scoring data obtained from different panelists were statistically analysed. The chemical analysis of the product was made by the standard methods given.

Physico-chemical analysis of prepared product of both cow and buffalo milk and respective control product of Chhurpi was analyzed by Ranganna (2001).

RESULTS AND DISCUSSIONS

Formulation of *Chhurpi*

The preparation of *Chhurpi* is based on the combination of different fat percent of cow and buffalo milk. The required combinations of fat of cow and buffalo milk are Recipe No.1= 0.2, Recipe N0.2= 0.5 and Recipe No.3=0.8 respectively. All these 3 experimental product of *Chhurpi* were prepared as per the standardized flow process.

The *Chhurpi* prepared with different combination of fat were evaluated for their sensory quality by conducting sensory evaluation using trained panelist. Three experimentally prepared *Chhurpi* and one control product cow and buffalo milk *Chhurpi* were evaluated with the help of panel of 10 trained judges. The gumminess, chewiness and overall acceptability of the product were analyzed by using 9-point Hedonic rank sum method (Table 4.1).

Sensory evaluation of *Chhurpi*

The combination type 3 was found to be superior to other combinations and control product (Table 1, Fig.1). The combination 3, which getting higher acceptability of sensory characteristics was selected as a best product and corresponding procedure and level of basic ingredients used *Chhurpi* preparation. The cow milk produces *Chhurpi* with moist surface, light yellow colour, too soft body and sooth texture. Cow milk was more suitable for *Chhurpi* preparation than the buffalo milk *Chhurpi,* the latter being hard in body and coarse in texture, besides whitish colour

Table 1 Mean (±S.E.) value of sensory evaluation of prepared and control product *Chhurpi,*

Products	Gumminess		Chewiness		Overall acceptability	
	Cow	Buffalo	Cow	Buffalo	Cow	Buffalo
CC	5.6±0.54 (18)	5.7±0.21 (29.5)	6.1±0.45 (19.5)	6.3±0.36 (21)	6.7±0.33 (17.5)	6.6±0.30 (24.5)
ER-1	5±0.29 (31)	5.6±0.40 (28)	5±0.39 (32)	5.3±0.42 (31.5)	4.9±0.17 (35)	5.9±0.40 (31)
ER-2	5.7±0.26 (26.5)	5.8±0.32 (27)	5.5±0.40 (28.5)	5.6±0.33 (28)	5.6±0.33 (30.5)	6.5±0.26 (26)
ER-3	6.7±0.26 (15)	6.6±0.26 (15.5)	6.9±0.37 (17.5)	6.6±0.37 (18.5)	7±0.11 (15.5)	7.1±0.17 (18.5)

CC- Control *Chhurpi* ER-Experimental Recipe

The number within the parenthesis are ranks sum-Lower the sum better will be the product and vice versa. Since, cow milk *Chhurpi* in each column carried lower rank sum was regarded better than buffalo milk *Chhurpi.*

According to sensory evaluation, significant difference ($p<0.05$) was observed in the different *Chhurpi* prepared from cow and buffalo milk in respect to gumminess, chewiness and overall acceptability.

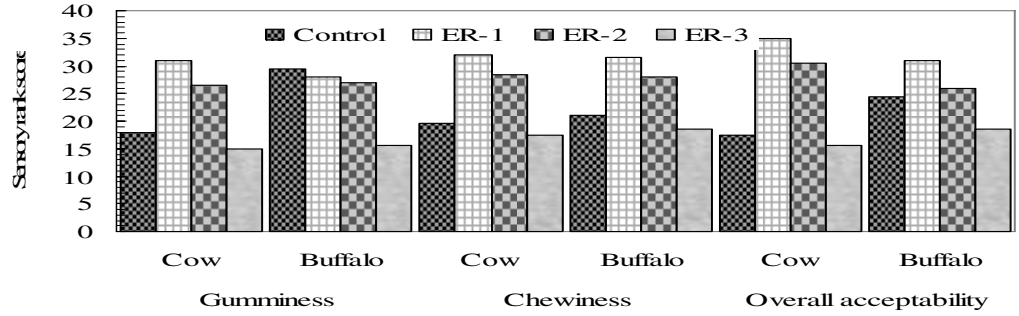

Fig. 1 Sensory analysis of three experimental and one control product of *Chhurpi*

Physico-chemical qualities

The mean ± standard error (S.E.) values of moisture content, fat content, protein content, total ash, acidity, gross energy and calcium of all qualities of prepared and control product of *Chhurpi* is presented in Table 2.

Moisture content

The moisture content of prepared and control product of cow and buffalo milk *Chhurpi* is presented in Table 4.3. The average with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 8.18±0.26, 11.57±0.44, 9.19±0.35 and 10.04±0.23, respectively. The water activity of that product was between 0.35 and 0.50, the aw at this level was suitable to prevent the microbial growth. Lawire (1979) revealed that rancidity did not develop when the moisture content reduced to 1.5%. The high moisture content of Chhurpi has poor mouth-feel, low shelf-life. In this study, the moisture content was ranging from the lowest value 8.18% and highest value 11.57%, it increased the storage period and longer would be the shelf life of the Chhurpi.

This study showed that the moisture content of almost all the samples of *Chhurpi* remained less than 11.57%, in which 8.18% and 9.19% of cow and buffalo prepared milk *Chhurpi* than control product, which was optimum to prevent the microbial growth during three months of storage at room temperature.

Fat content

The average fat content (with standard error) of prepared and control product of cow and buffalo milk *Chhurpi* were 5.13±0.03%, 6.26±0.11%, 5.08±0.10% and 5.38±0.38% respectively.

Table 4.3 Physico-chemical parameters of prepared and control product of *Chhurpi*

S. N.	Physico-chemical parameters	*Chhurpi* type			
		Prepared product		Control product	
		Cow milk	Buffalo milk	Cow milk	Buffalo milk
1	Moisture (%)	8.18±0.26	9.19±0.35	11.57±0.44	10.04±0.23
2	Fat (%)	5.13±0.03	5.08±0.10	6.26±0.11	5.38±0.38
3	Protein (%)	77.49±0.37	77.74±0.23	73.77±0.01	74.92±0.29
4	Total ash (%)	5.40±0.40	4.72±0.09	5.16±0.28	6.11±0.04

5	Lactose (%)	3.10±0.05	3.08±0.03	3.02±0.02	3.1±0.03
6	Acidity (%)	0.22±0.01	0.16±0.01	0.21±0.01	0.18±0.01
7	Gross energy (Kcal/kg)	5492±28.70	5530±17.32	5610±19.49	5540±55.49
8	Calcium (%)	4.61±0.01	4.16±0.01	2.22±0.15	3.10±0.20

The climatic conditions (temperature, humidity etc.) can affect the quality of *Chhurpi,* comparatively hill and mountain high fat content signify the good quality whereas in Terai low fat is desirable, high fat develop rancid taste and flavour and its mean poor quality.

Chhurpi prepared having fat content of 0.8% in cow milk and in buffalo milk was to obtain with satisfactory body and texture. Lower than 0.8% fat leads to hard body and texture in *Chhurpi* while higher fat level *Chhurpi* results not dry easily, oozing out ghee drop by drops, do not store long time and moulds was develop.

The value of fat was 5.13% of prepared and 6.26% of control cow milk *Chhurpi* and value of fat was 5.08% of prepared and 5.38% of control buffalo milk *Chhurpi*. The prepared cow and buffalo milk *Chhurpi* was found a high calorie value of 46.17 and 45.72 kcal/100g, respectively.This variable values may be due to species, breed, stage of lactation, seasonal variations, length of interval between milking, feed etc.

Crude Protein

The average protein content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 77.49±0.37, 73.77±0.01, 77.74±.23 and 74.92±0.29, respectively.

The protein content of *Chhurpi* was found to be higher in control product of cow milk *Chhurpi* than the prepared cow milk *Chhurpi* and high in prepared buffalo milk *Chhurpi* in comparisons to control product of buffalo milk *Chhurpi*.

This protein content fluctuation is may be due to the factors such as species, breed, nutrition or milking interval. The proteins contain of prepared cow and buffalo milk *Chhurpi* was 77.49% and 77.74%, respectively. This value was greater in comparison of the control products. Proteins are used as a fuel by the body. Each gram pf protein yields 4 calories energy. The prepared cow and buffalo milk *Chhurpi* was found a high calorie value of 309.96 and 310.96 kcal/100g, respectively. This study indicated that the estimated protein content was 78.5% and remaining 19% drain out in whey which was given by Walstra *et al.,* 2005.

Total ash content

The average total ash content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 5.72±0.40, 5.16±0.28, 4.72±0.09 and 6.11±0.04, respectively. This value indicates that milk uses for *Chhurpi* making do not affect final ash content of *Chhurpi*.

Ash content generally indicates the minerals and inorganic matter present in the product. Ash is a complicated mixture containing a number of metallic in dry milk product.

Lactose content

The average lactose content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 3.1±0.05, 3.02±0.02, 3.08±0.03 and 3.1±0.03 respectively. This value of lactose is not so differences. Acharya and Gupta (1998) who found the lactose content in the mixed milk Chhurpi was 3.1%.

When milk is used for *Chhurpi* making, most of the lactose follows the whey. Each of these products contains a relatively high proportion of lactose (Eckles *et al.,* 2001).Lactose content of the *Chhurpi* was found 3.1% which is 64.6% lactose remained in the products. The remaining out of 35.4% drain out in the whey. The prepared cow and buffalo milk *Chhurpi* was found an energy value of 12.4 and 9.24 kcal/100g, respectively. This variable value may be due to individuals within the breed and breed

itself is a small factor. The main flavour compounds of milk and milk product *Chhurpi* are lactose and the dissolved salts, which cause a sweet and salty taste, respectively.

Titrable acidity

It is essentially the measure of lactic acid produced in the product and the acidity reflects the conditions of preparation and microbial activity (Arbuckle, 1986). The mean acidity values with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 0.22±0.01, 0.21±0.01, 0.16±0.01 and 0.18±0.01, respectively. The acidity of the product varies considerably depending on species, breed individuality, stage of lactation and health of animal, etc.

Yield of *Chhurpi* on fat basis

The yield mainly depends upon the type of milk used, higher heating temperature, casein content, and coagulant, genetic variants of milk protein especially those of ß-lactoglobulin and other aspects which are known to vary to a great extent (Bhattacharya et al., 1971).

Whey proteins are denatured by high-temperature pasteurization, which makes them less soluble and cause them to precipitate with casein during acid precipitation. Low-temperature pasteurization has little effect on the proteins. This means *Chhurpi* making from high temperature pasteurized milk gives a higher yield because a greater part of the milk proteins goes into the *Chhurpi.*

The higher percentage yield occurred to the cow and buffalo milk was 3.98% and 4.06% (Table 3). The yield of *Chhurpi* obtained in the study is comparable with the published reports, which were in the range of 3.40 to 4.27 per cent (Pal *et al.,* 1996).The yield of *Chhurpi* varies from place to place of different factors like composition of milk and environmental conditions of those regions. Schedev and Singh (1987) reported that loss of total solid in whey was 6.4 %, when coagulated with 2 % citric acid. Total solid loss in whey is relatively higher so adoption of technological innovation for utilization of remaining constituents of whey is necessary. The table pertaining to yield of *Chhurpi* revealed that increased the yield of *Chhurpi* as increases the percentage of fat in both cow and buffalo milk prepared *Chhurpi.*

Table 3 Effect of fat content of milk on the yield of *Chhurpi*

Parameter of 4 L skim milk with fat percent	Coagulant	Coagulant (ml) /L milk	Total yield ,g	
	Citric acid %			
			Mean±SE	%
Cow milk (0.2%)	2	12 ml	153.58±1.15	3.83
Cow milk (0.5%)	2	12 ml	153.74±0.20	3.84
Cow milk (0.8%)	2	12 ml	159.52±0.24	3.98
Buffalo milk (0.2 %)	2	12 ml	154.30±1.18	3.85
Buffalo milk (0.5 %)	2	12 ml	155.41±0.95	3.88
Buffalo milk (0.8%)	2	12 ml	162.54±0.95	4.06

Cost of production

The production cost of process prepared *Chhurpi* was calculated by considering the variable cost (cost of raw materials, fuel and labour etc.).The average selling price of prepared *Chhurpi* was found Rs.206.56 per kg and average market price of *Chhurpi* was found different from place to place Rs.210 to Rs.300 per kg in the study area.

CONCLUSION

Chhurpi is casein based Nepalese indigenous dried milk product, made by coagulation with acid. The coagulated casein is pressed with heavy load overnight which expel the moisture to a minimum level. Then it is dried for a long time to reduce moisture to an acceptable level. The *Chhurpi* having 0.8 % fat

content in cow and buffalo skim milk was highly acceptable in quality having gumminess and chewiness. The product yield obtained was higher in more fat concentration i.e. 3.98% and 4.05% of cow and buffalo milk as acceptable product. *Chhurpi* is main source of protein, fat, lactose and minerals. *Chhurpi* is solid hard casein containing 77 % protein and 5% fat in cow and buffalo and 9% in yak milk.

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EXPERIENCE SHARING AND LESSONS LEARNED FROM THE OUTBREAK OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN POKHARA

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ABSTRACT

Following the history of mass mortality and morbidity in ducks, six samples of dead birds submitted to the Regional Veterinary laboratory, Pokhara showed positive for rapid flu A test on 26 January 2010 which was later confirmed as H5N1 at Central Veterinary Laboratory and Veterinary Analytical Laboratory, UK on 3 February, 2010. Government of Nepal declared Gharipatan of Pokhara as bird flu infected zone and a bird flu control room was established for the stamping out operation, followed by cleaning, mopping and disinfection in the same fixed boundaries of infected zone.

In 1287 households, total of 11,129 birds, 144.5 kg poultry meat, 1902 eggs and 1516 kgs of feed were destroyed. Due to delay in declaration of bird flu infected zone, the disease was spread in other parts of city as well as in neighboring district, Tanhun. Ten places were declared as hot spots in which 1276 birds, 107.5 kg meat, 669 eggs, 385 kg of feeds were destroyed in 246 households. The probable hypothesis for the spread of disease is thought to be through wild migratory birds. Total number of rapid response team (RRT)/prompt response team (PRT) employed were 5 and total numbers of human resources were 165 in which 47 were labourers. Total of 478 personal protective equipment (PPE) and 24300 kgs lime, 98450 gm Virkon were used for complete operation. Total number of backyard birds/farm observed by surveillance team was 2269 and 455 samples were collected from 157 farms.

Major strengths, constraints, post operative activities and lesson learned were discussed in this article for further facilitation of operation for future control strategy of bird flu control in Nepal. Late declaration of infected zone, unpractical specification of procedure, budgetary constraints and monetary compensation were found to be main constraints where multidisciplinary coordination and responsible media were major strengths of Pokhara operation.

INTRODUCTION

Influenza A virus subtype H5N1, also known as "bird flu" virus or simply H5N1, is a subtype of the Influenza A virus which can cause illness in humans and many other animal species. A bird-adapted strain of "highly pathogenic avian influenza (HPAI) virus of type A of subtype H5N1" is the causative agent of "avian influenza" or "bird flu" (FAO, 2008). It is enzootic in many bird populations, especially in Southeast Asia. One strain of HPAI, a (H5N1) is spreading globally after its first appearance in Asia. It is epizootic (an epidemic in nonhumans) and panzootic (affecting animals of many species, especially over a wide area), killing tens of millions of birds.

According to the FAO, Avian Influenza Disease Emergency Situation Update, H5N1 pathogenicity is continuing to gradually rise in endemic areas but the avian influenza disease situation in farmed birds is being held in check by vaccination. Eleven outbreaks of H5N1 were reported worldwide in June 2008 in five countries (China, Egypt, Indonesia, Pakistan and Vietnam) compared to 65 outbreaks in June 2006 and 55 in June 2007. The "global HPAI situation can be said to have improved markedly in the first half of 2008 but cases of HPAI are still underestimated and underreported in many countries because of limitations in country disease surveillance systems" On December 21, 2009 the WHO announced a total of 447 human cases with 263 deaths.

BACKGROUND

Kaski district is one of the avian influenza high risk district of Nepal. This district possesses 70,000 commercial layers (Bhattrai farm is the single of comprising more than 40,000 birds in a farm) and more than 300 broilers farms which produce 400,000 of broilers round the year. Per capita egg consumption is 1, 50,000 in which 42,000 are supplied by local producers and 108,000 were usually imported from other parts of the country. Per day broiler meat consumption is 15000 kgs which is high meat consumption in the country (DLSO Bulletin, 2008). Other high risk predisposing factors are: transit route of meat and meat products to other mountains districts. Pokhara is also famous for lakes, ponds and Seti Rivers which are the habitats of wild birds. High demand of poultry products are also due to tourism. This also gives the high potentiality of demand of value added diversified products of indigenous ducks, pigeons and back yard poultry.

The aim of this paper is to share some of the experience gained during the outbreak of Highly Pathogenic Avian Influenza (HPAI) in Pokhara city of Nepal. The data and results available from the office of Bird flu Control Room, Regional Veterinary Laboratory, Pokhara, Central Veterinary Laboratory, Tripureshwor and VAL, UK were collected and analyzed. The authors themselves were involved in bird flu control room and in the process of stamping out, disposal, mopping, cleaning and disinfection and in surveillance activities. The daily reports recorded were finally analyzed to share the experiences of HPAI outbreak control. The operation was conducted on the basis of SOP of Government of Nepal.

Reporting of Outbreak Investigation

Deaths of ducks were reported from Gharipatan during regular surveillance program and six samples (dead birds) were collected and submitted to Regional Veterinary Laboratory, Pokhara. The rapid flu A test showed positive indication and the post mortem examination revealed hyperemic lungs, edematous visceral, meningeal hemorrhages and head swelling (Figures 1-3). Samples collected were dispatched to CVL, Kathmandu which was confirmed positive for H5N1 and the same samples were then dispatched to VAL, UK for further confirmation.
Early Disease Investigation Team (EDIT) arrived in pokhara from Kathmandu and surveillance intensified around Gharipatan on 29 January 2010.

Early Preparedness

Emergency meeting in presence of the secretary, Ministry of Agriculture and Cooperative (MOAC) was held on 30 January with local officers of Department of Livestock Services, Chief District Officer (CDO) and other security personnel. The meeting decided to intensify surveillance, cleaning and disinfection in and around epicenter with lime and Virkon. Poultry population for destruction was estimated around 12,000. Proposed allotment of responsibilities, fixation of boundaries of infected zone, check points were thoroughly discussed.

On 31 January, another meeting was held with CDO, Superintendent of Police and Public Health Officer for proper allocation of resources. The chief of District Livestock Services Office, Kaski declared the ban on the export of poultry and poultry products from Kaski and regulated the imports on the basis of Bird flu Control order, 2009 Clause 4.3.

From February 1st to 3rd, processes of early preparedness were adapted. There was special meeting with Regional Director of Department of Livestock Services Director of Western Region, surveillance officers, Rapid Response Team (RRT) members, check points' members, disposal sites responsible persons. The meeting of Regional Avian Influenza Control Technical Committee (RAICTC) was held in the chairmanship of Regional Administrator of western region which decided the coordination with public health and security personnel.

Declaration of Bird Flu Outbreak

Government of Nepal declared the bird flu in Pokhara on 3 February, 2010.evening by cabinet decision at 7:30 P.M. and a control room was established on 4 February. Control room in charge, communication officer, sector officer and logistic officer were appointed by Director General of Department of Livestock services. Control room in charge was given the authority of Director General. RRT was mobilized (Figure 2). DAICTC meeting was held to propose the boundaries of infected

zones, which was later endorsed by the Ministry of Agriculture and Cooperative. The boundaries were fixed: Seti River in the east, Sangrila junction (Chowk) up to eye hospital in the west and Gharipatan Chowk in the north up to Sahara School and Hanuman Tole in the south. Six check points were established at Sahara School, Sitaram Bridge, Ghari patan Chowk, Eye Hospital Chowk, Tallo Rato Pahira and Sangrila Chowk. Check point teams comprising 27 members were mobilized. Then, search of disposal team started their activities and media briefing was arranged shortly.

Opposition on disposal site

Before the start of stamping out operation, there was a strong opposition on disposal site and alternate site was chosen but opposition continued for three consecutive sites and finally, the stamping out operation could be started on 4 February. CDO and control room in charge visited many places for selection of suitable sites and faced many objections and finally decided to use the government owned land of Tutunga. Despite of strong opposition to this new site, the disposal was managed in the night hours (9 pm onwards) with the help of heavy security forces. The situation was tense thereafter even during daytime and local leaders asked for a meeting the following day to settle the demands of local people. Their demands were construction of paved road to reach Tutunga, construction of a concrete disposal pit and on the sickness and one million rupees compensation on the event of human death.

Stamping Out

Coordination meeting with local multi party representatives was held at CDO's office and the problem of disposal site was resolved. Three RRT of thirty members were mobilized from different sites.

i ii iii

Figure 1. Showing torticolis (i) in the duck; severe brain hemorrhage (ii) and positive in rapid flu test

Four surveillance teams of 20 members and check points team of 27 members were also mobilized. The excavator was used to dig 19 pits and simultaneously the stamping out operation was initiated. Review meeting was held at every morning and evening. Office remained opened from 7 am to 10 pm.

Hot Spots

Sampling and submission of dead birds were carried out through surveillance team as well as through personal approach to farmers. A front desk information center was made functional to receive the message, and for verification of rumors Rapid flu a test was carried out and post mortem examination was done by using PPE. This is how hot spots were detected. From 7 February onward, infection was also traced outside the infected zone. Kaski district had 7 hot spots viz. Khapaundi, Baudha chowk (fresh house), Amar singh chowk (Supplier), armed force camp, Birauta, Lampatan, Gagan Gaudda. Neighboring Tanhun district had three hot spots viz. Dule gaunda, Damauli and Bhimad. Two Problem Response Teams (PRT) comprising of 10 members, led by a veterinary officer were mobilized to address hot spots from 7 February onward. The hot spot stamping out, cleaning and disinfection processes were performed during the night. Being cautious to disclose the message gave panic to the public. Rumor of disease spread was everywhere. Review of vigilance on movements of poultry and poultry products was necessary to control the disease spread to adjacent districts and accordingly request was made to Regional Administrator. Regional coordination meeting with Tanhun, Gorkha, Lamjung, Parbat, Banglung, Myagdi, Syangja districts was arranged in presence of respective chief of district livestock services offices. The Director General addressed and guided the meeting. They were requested to submit the surveillance report daily by fax to control room. Hot spot operation included stamping out, cleaning, disposal, disinfection and sealing of poultry premises. Coordination meeting with poultry entrepreneurs, suppliers, fresh house owners, poultry and meat producers' associations was held almost everyday during stamping out. There was action group from poultry entrepreneurs

which helped in communication and facilitation for easier operation. In hot spot operation, 246 households were visited in which 191 ducks, 844 backyard poultry, 241 broilers, 107.5 kgs of meat, 669 eggs, 385 kgs of feeds were destroyed.

Compensation
The monetary compensation was not convincing most of the people as to the cost of the property. This caused the delay in stamping out operation. However, totally, Rs. 11, 28,556 was distributed on spot.

Mopping, Cleaning and Disinfection
Cleaning and disinfection were continued in the back yard farm along with stamping out operation. However, revisit of the farms were made to check the cheating, hiding and restocking and on finding of the same, stamping out was done followed by cleaning and disinfection. These activities were carried out from 12 February for 3 consecutive days. In this operation, 602 households were cleaned but 2033 houses were visited; 403 PPE, 1004 kgs lime, 29050 gm Virkon were used in the operation.

Figure 2. RRT on stamping out operation

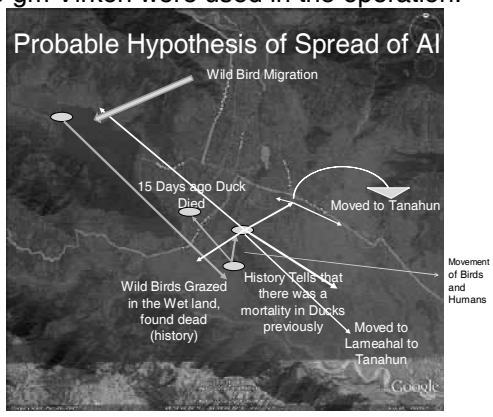

Figure 3. Probable route of spread of AI

Surveillance
Surveillance was the major element to apply into the control strategy of avian influenza. Initially, a planned surveillance was established and 4 teams were mobilized to trace the disease. Surveillance team was also mobilized in verification of rumor of disease spread. From 4 February to 16 February, 4 teams comprising 252 members were mobilized in 5 municipalities, 51 VDCs, 165 wards covering 472 households. They collected 438 samples, used 130 kgs of lime and 5150 gm of Virkon.

Check points
Six check points were fixed as mentioned above and 37239 vehicles were disinfected at check points and only 1 kg meat was seized at the check point.

Communication
Several means of communication were used to disseminate the proper information to public. Only the communication officer was allowed for media briefing. Medias were quite responsible and they helped in preventing the spread of wrong message. The miking was done for 8 days within the infected and surveillance zone of 10 km radius. Regular talk programs were given on 5 FMs radios and one television. Press appeal in local print media for 2 days in 10 daily and weekly newspapers was given. Daily press releases at 16:00 o'clock were done as well as printed materials i.e. posters, pamphlets, folders were distributed. Mass communication, personal communication, door to door communication through mobilization of facilitators, vets, local representatives were provided. Every day briefing meeting was held with CDO and Regional Administrator.

Sanitary Certification
Sanitary certification was given by a special sanitary investigation team (SSIT) led by program director of animal health directorate. The team visited the commercial and backyard farms to inspect the level of sanitation. A satisfactory certificate was delivered to complete the process.

Letter of Appreciation and Chicken Festival

Letters of appreciation were awarded to all 165 persons and 10 institutions for their devoted contributions to control HPAI in Pokhara. The award was offered by the Secretary of the Ministry of Agriculture and Cooperatives. This was followed by chicken festival from the promoter of poultry business.

Over all Summary

From Feb 4 to Feb 17, 2010

S.N.	Particulars	Total
1	Total No. Of birds	11129
2	Eggs	1902
3	Meat (Kg)	144.5
4	Feed(kg)	1516
5	Total No. of RRT/PRT deployed	5
6.	Total No. of Labor	47
7.	Total no. Of human resources	165
8.	PPE used	478
9.	Lime used (20 kg bag)	1215
10.	Virkon used (gm)	98450
11.	Total VDC/Muni/Wards visited by surveillance/Households	45/5/144/320
12.	Total sample collected	455
13.	Total No. of back yard birds/Farms observed by surveillance team	Back yard 2269/ Farms 157

Major Strengths

- Regional headquarter provided the manpower and resources.
- Outbreak in sub metropolitan Pokhara city made easier in procurement of logistics.
- Responsible media made the operation easier.
- There was a good coordination with administration, security, public health, entrepreneurs and other agencies.

Major Constraints

- Lengthy process of declaring outbreak.
- Epicenter of outbreak in middle and busy part of the city created a difficulty in preventing the transportation of poultry and poultry products.
- Large no. of backyard poultry needed long time of stamping out.
- Disposal within infected zone was difficult to find.
- Less compensation in comparison to market price.
- High standard of SOP increased the overall cost of operation.
- Unequal allowances and facilities for Government employees created conflict.
- Government financial and procurement rules were difficult to follow during emergency.

Post Operative Activities

Post Operative activities were performed from 17 February to 25 March by Service center, district level and RVL. The post operative activities were conducted at infected zone, hot spots areas and in whole district. The interval was twice a week. The sites were 4 in Kaski and 3 in Tanhun. The composition of post operative surveillance team included a district level officer, 2 technicians and a driver. The team performed rumor registration; mortality recording, regular visit, awareness campaign, supervision and monitoring, reporting, investigation were done by regional laboratory team. They also compiled reports and prepared for final reports.

Lessons Learned

- It is difficult to follow SOP in stamping out operation in the case of backyard poultry.
- Due to budget constraint, PRT with small control staffing could be most feasible.
- R-EDIT is required.
- Compensation in terms of money is not convincing.
- Government financial rules do not harmonize the bird flu control order.
- Multidisciplinary coordination made the operation easier.

Crisis Management Committee (CMC) team Visit

CMC team comprising 4 foreign delegates from OIE visited the infected zone, Gharipatan, hot spots, disposal sites and discussed the operation procedure with all stakeholders like concerned officers, poultry entrepreneurs, CDO, Security personals, local citizens and other high level authorities and expressed satisfactory progress. The team also investigated the probable route of disease spread (Figure 4).

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PROGESTERONE CONCENTRATIONS IN BLOOD SERA AND MILK WHEY OF MURRAH BUFFALOES FOR THE FIRST FOUR WEEKS OF PREGNANCY IN NEPAL

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ABSTRACT

The progesterone (P_4) concentration of buffaloes in Nepal was investigated. Monitoring six buffaloes showed that the mean of the P_4 concentration in sera was lowest on day 0, gradually increasing to a peak on day 14 from 0.41 ng/ml to 1.62 ng/ml, and that the mean of the P_4 concentration in milk whey was lowest on day 0, gradually increasing to a peak on day 14 from 0.03 ng/ml to 0.46 ng/ml, respectively, for the first four weeks of gestation.

INTRODUCTION

Reproductive disorders such as delayed puberty, slow recovery of postpartum estrus, and anestrus occur in many of buffaloes. Hormone assays like progesterone may be useful as diagnostic aids, but there have been only a few reports (Avenell et al., 1985, Batra et al., 1979 and Kanai and Shimizu, 1984) on the progesterone values in serum, and little information is available on milk whey yet. The objective of this study was to obtain P_4 concentrations in serum and milk whey for the four weeks of pregnancy.

MATERIALS AND METHODS

This study was conducted at five traditional farms located in the Siraha district (Grid co-ordinate: 26°93′ N, 86°53′ E) of Nepal during February and April 2003. Day length ranged approximately between 11 and 13 h. A total of six lactating Murrah buffaloes aged 6.3 (range 3 to 10) years whose open days after parturition ranged from 120 to 395 days were used.

Cyclic changes of the ovaries such as follicles and corpora lutea were diagnosed by rectal palpation along with external clinical signs at 7-day intervals. The six normally cycling buffaloes in estrus had been artificially inseminated and diagnosed pregnancy. They calved at between 314 and 316 days of conception.

Blood and milk were collected in the morning at a time when rectal palpation and AI were done, and the collections of blood and milk were continued at 7-day intervals up to 28 days. These specimens were kept in an ice chest and brought to the laboratory for serum and milk whey separation within two hours after collection. Blood was collected into unanticoagulated vacuum tubes from the jugular vein, centrifuged at 1900×g for 10 min at room temperature and stored in 2 ml sterile tubes at –20 ºc until assay.

Fore milk after discarding a few streams of initial milking was collected into sterile tubes from a quarter of the udder, and centrifuged at 1900×g for 20 min at room temperature. A hole was made in the wall of each tube at around the bottom by heated wires so that skim milk in the lower part dropped into a 10 ml clean tube by gravity. A 2% HCl solution was added to the skim milk drop by drop to reach the pH of 4.6 for centrifugation at 1900×g for 10 min, which produced milk whey. Two milliliters of milk whey was stored in 2 ml sterile tubes at –20ºC until assay.

Concentrations of progesterone (P_4) in serum and milk whey were measured by radioimmunoassay (RIA) at Azabu University, Japan using commercial kits purchased from Diagnostic Products Corp. (Los Angeles, CA, USA). This kit employs a solid-phase radioimmunoassay designed for the direct, quantitative measurement of progesterone in serum and requires neither extraction nor pre-dilution. All procedures followed the manufacturer's instructions. In measuring the control sera containing known amounts of steroids, the recovery was 80~120%. The cross-reactivities of the anti-P_4 antibody for progesterone, 5α-pregnan-3, 20-dione, 17α-hydroxyprogesterone, 5β-pregnan-3, 20-dione, 20α-dihydroprogesterone, testosterone, 5β-pregnan-3α-ol-20-one, androstenediol and estradiol-17β were 100, 9, 3.4, 3.2, 0.2, 0.1, 0.05, <0.05 and <0.05 %, respectively. The intra-assay and inter-assay coefficients of variation of these assays were <8.8% and <9.7%, respectively.

RESULTS

The P_4 concentrations in serum and milk whey were determined at 7-day intervals for four weeks and the results are shown in Table 1 and Table 2. Mean P_4 concentrations in serum rose from a basal level of 0.41 ng/ml to a peak of 1.62 ng/ml and returned to approximately the basal level on day 21 even when the buffalo became pregnant. Mean P_4 concentrations in milk whey rose significantly ($P<0.05$) from the mean basal level of 0.03 ng/ml to a peak of 0.46 ng/ml on day 14, and returned to approximately the basal level on day 21 even when the buffalo became pregnant. In an attempt to determine the relationships of P_4 concentrations between serum and milk whey for the first four weeks of gestation the correlation coefficient was 0.629, with statistical significance (F-test, $P<0.01$).

Table 1. Changes of 95% confidence intervals of P4 concentrations (ng/ml) in sera of six cow buffaloes

Determined on day	n	Mean	95% confidence interval		
0	6	0.41	0.00	~	1.06
7	6	0.89	0.18	~	1.60
14	6	1.62	0.48	~	2.75
21	6	0.75	0.00	~	1.71
28	6	1.11	0.08	~	2.14

Table 2. Changes of 95% confidence intervals of P4 concentrations (ng/ml) in milk whey of six cow buffaloes

Determined Day	n	Mean	95% confidence interval		
0	6	0.03	0.01	~	0.06
7	6	0.14	0.02	~	0.31
14	6	0.46	0.10	~	0.97
21	6	0.08	0.00	~	0.21
28	6	0.19	0.00	~	0.52

DISCUSSION

The fluctuation pattern of the P_4 concentrations in serum during the estrous cycle nearly coincided with those of previous reports (Avenell *et al.,* 1985, Batra *et al.,* 1979 and Kanai and Shimizu, 1984), even though the P_4 values in the present study were approximately half of the reported ones.

There has been little information reported on the P_4 concentration in milk whey of cow buffaloes. The P_4 concentrations in milk whey were three or four times lower than that in serum, but the correlation coefficient between the serum and milk whey indicated that the fluctuation pattern of P_4 concentrations in milk whey was similar to those in serum. Thus the milk whey was also considered to be a useful specimen to monitor the P_4 concentration during the estrous cycle of the buffalo.

The P_4 concentrations on the estrus day excluded functional corpora lutea existing and the P_4 concentrations on day 14 in the luteal phase clearly indicated the corpora lutea existed. Therefore, the P_4 concentrations on these occasions may be used to detect estrous and luteal phases of ovaries of buffaloes as a diagnostic aid supplementary to rectal palpation and external clinical signs. The low P_4 on the estrus day and its return to the basal level on day 21 even in gestation need further study in the future.

In conclusion, the present study presented the P_4 concentrations in serum and milk whey of the six Murrah buffaloes for the first four weeks of gestation for further study.

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Short communication

CLINICAL-LABORATORY INVESTIGATION OF MOULDY MAIZE AND FODDER POISONING IN GOAT IN KATHMANDU VALLEY AN INVESTIGATION REPORT.

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ABSTRACT

An outbreak of a syndrome of unknown etiology associated with the feeding of moldy maize grain and green fodder occurred to the male goats in a flock of 2000 brought for Dashahara festival during October-2008 in Kathmandu valley A total of 52 goats suddenly became ill with symptoms of anorexia, apathy, diarrhea and ruminal stasis. On clinical examination tantative diagnosis was made as moldy corn poisoning. Clinical pathological findings included mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum. Liver had shrunken appearance, pale to yellowish discoloration with distended gall bladder, petechial hemorrhage in the kidneys, small intestine and with excessive mucus. Laboratory examination of tissue samples of maize fodder revealed Penicillium spp and E. coli.

Key words: Moldy maize, green fodder, Penicillium, Fungus, Male Goat, Kathmandu valley.

BACKGROUND:

During the Dashahara festival of year 2008, 2000 male goats were procured from the mid-western region of Nepal by Nepal Food Corporation. In lairage, goats were fed exclusively with whole maize grains and green fodder leaves. Based on history and clinical examination of those ailing goats, a provisional diagnosis of moldy corn/fodder poisoning was made and have treated with liquid Toxol, bio-sel-e and liquid zist of which 34 male dead goats were presented to Central Veterinary Laboratory Tripureshwor, for futher investigation.

CLINICAL AND LABORATORY EXAMINATION

1: Clinical examination of goats in lairage.
On clinical examination, all goats present in lairage of Nepal Food Corporation had symptoms of Anorexia, Ataxia, Diarrhea, Dullness, Dysmetria, Generalized weakness. [(similar to reported by Dr. Maurice E. White 2008, , and ruminal stasis, Schneider D. J., Marasas W. F., Collett, M. G., van der Westhuizen G. C., 1985. R. W. Medd, G. M. Murray and D. I. Pickering, 2008. : L. W. Whitlow and W. M. Hagler, Jr.2008.)].

2: Post-Mortem Examination of dead goats.
Post mortem examination of all 6 male dead presented to Central Veterinary Laboratory Tripureshwor ,Kathmandu revealed the lesions of mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum. Liver with shrunken appearance pale to yellowish discoloration with bile filled distended bladder pin point hemorrhage in kidney, small intestine with excessive mucus.

3: Microbial/Mycological Culture examination of Post-Mortem Tissue samples.
Mycological and microbiological examination of tissue samples from post-mortem of dead goat revealed the growth of fungal pathogens like Aspergillus and Penicillium spp with E.coli. similar to the findings of Karki *et.al.* (2008) C. Wendell Horne (2008). Where as all nasal and rectal swabs from sick and dead animals tested for PPR with penside test turn out to be negative.

Since warm and humid climate of tropics and subtropics favors growth of mold and fungus in feed grains and fodder especially after heavy monsoon rain, feeding exclusively of such grain to livestock and poultry seems to cause the detrimental effect in the health these animals. Clinical signs of anorexia, apathy, diarrhea and ruminal stasis were noticed and Clinical pathological findings included mild focal erosions to severe, diffuse, coagulative necrosis of the mucosa in the rumen, reticulum and omasum and congestion and hemorrhages in the abomasum. The other lesions seen were liver with shrunken appearance pale to yellowish discoloration with bile filled distended bladder pin point hemorrhage in kidney, small intestine with excessive mucus. Circumstantial evidence that feeding of moldy maize grain and green fodder leaves infected by Penicillium and Aspergillus spp may have caused this outbreaks of a systemic Mycosis in these goats need to be thoroughly investigated in field areas from where these goats were bought.

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ACKNOWLEDGMENT

We would like to thank Dr.Samjhana Panday of Central Veterinary Hospital Tripureshwor, Kathmandu for providining early indication of problem. Thanks are due to Mr. Bal Bahadur Kunwar Mr. Tek Bahadur Air Senior Vet. Technician and Mr.Bhimsen Adhikari Vet. Technician of Microbioly Unit,Mr Purna Maharajan Vet Technician of Central Veterinary Laboratory for doing the microbiology and post-mortem works and office assistant Mr. Chandra Bahadur Rana for his tireless effort in handling the carcass during post-mortem work .

PREVALENCE OF HELMINTH PARASITES OF PIGLET IN PERI-URBAN AREAS OF KATHMANDU VALLEY

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ABSTRACT

A study was conducted to find out the prevalence of helminth parasites of piglet in peri urban areas of Kathmandu valley. Altogether 148 faecal samples were brought from different areas of Kathmandu and were examined for helminth parasites in the Parasitology Laboratory of Animal Health Research Division, NARC. Results of faecal examination in different areas of Kathmandu showed mainly two helminths parasites: Ascaris (29) and Strongylides (14) in piglets below three months of age. The overall prevalence as detected by faecal egg output in swine was (29%). Piglets from Jadibuti area showed the highest prevalence (44.44%) while Nayapati area showed a lowest (27.27%) prevalence. Similarly, piglets below 60 days showed highest prevalence of Ascaris while animals between 60-90 days showed higher prevalence of Strongyloides. The prevalence of Ascaris below 60 days age group was highest. From the questionnaire survey, it was known that most of the farmers were familiar about the helminthes parasite of swine. It was reported that their pig are treated with Ivermectin in every 3-5 months interval and Ivermectin was the drug of choice. Albendazole, Ivermectin, Fenbendazole and Levamisole are most commonly used anthelmintic drugs.

INTRODUCTION

Agriculture sector is a dominant player of the Nepalese economy. Livestock farming is an indispensable part of socioeconomic life of most of the Nepalese farmers. The contribution of livestock in AGDP is 31%, which is expected to reach up to 45% by the end of 12th plan (APP, 1995). It has been estimated that there are 0.94 million with 15.6 thousand metric ton of pig meat production during 2001/2002 (ABPSD,2005). Pig farming is an important component of hill farming system especially in Rai, Limbu, Tamang, Magar and other occupational castes (Tailor, Blacksmith, Shoemaker and Dom, etc) communities who traditionally raise pigs and is major source of animal. The pig population has increased from 0.76 million to 0.98 million where as pig meat has increased from 13.0 thousand metric ton to 16.0 metric ton in 10 years period. So, both pig population and pig meat production is in increasing trend (CBS, 2007).

Among the various constraints of pig production, disease is considered to be one of the important limiting factors, not only to increase productivity but they are also important from zoonotic point of view. The major disease affecting the pig production is internal parasites (Thakuri, 1996). The role of parasitism is important in village reared pigs throughout the territory and contributes substantially to the high mortality and retarded growth commonly observed in weaner animals.

MATERIALS AND METHOD

The study was conducted in five different places (Jadibuti, Gothatar, Jorpati, Nayapati and Balambu) of Kathmandu district. These areas were selected as representatives of pig farming (Peri-Urban) in Kathmandu district from August to November. Epidemiological informations were carried through designing of questionnaire format in the study area which incorporated informations about source of forages, feeding management/methods of feeding, types of housing, drenching schedule, etc. A total of 148 samples were collected. Animals were selected randomly from the population covering different farmers. The faecal samples thus collected were placed in a plastic bag with grips. 2-3 drops of 3% of formalin was put into each bag. Each sample was labeled clearly with animal identification, date and place of collection. A questionnaire survey was carried out during September to November from the different pharmacies of Kathmandu valley to get the information on the types of Antihelminthics drugs used for swine in sampled areas. The Meteorological information were collected from the Government of Nepal, Department of Hydrology and Meteorology, Babarmahal. The result obtained was analyzed statistically using simple statistical tools (Microsoft Excel 2003).

RESULTS AND DISCUSSIONS

Percentage of positive and negative cases

Among 148 samples, 43 samples showed positive for helminth parasites, representing 29% prevalence. The prevalence rate estimated in piglets of peri-urban areas of Kathmandu Valley were (29.05), which is very low to those reported by few researchers in the eastern hills of Nepal. Thakuri, (1996) reported 57% of prevalence in the eastern hills recorded over eight years of period. Likewise Thakur (1996) also reported 70-90 percent prevalence in different altitudes of eastern Nepal. Some international research on different places of the tropics (Papua New Guinea) also showed higher prevalence. A drastic difference in number of samples, coverage of area, longevity of the time period, husbandry system, comparably hygienic pens, knowledge of the farmers on deworming of pigs etc might have played a significant role.

Area wise prevalence of the disease

Jadibuti showed the highest prevalence (44.44%). It may be due to long deworming interval i.e. once or twice in a year or irregular drenching, serving uncooked food, ignorance in pig health management by the farmers etc. These factors might be the reasons for high prevalence in this area. Comparatively, Nayapati showed lowest number positive cases (27.27%). This may be due to less number of samples, serving of boiled feed, provision of regular deworming practices, etc. This indicates that the piglets which were fed boiled feed and sows, provided with regular deworming have less parasitic burden as compare to the pigs fed uncooked feed and lacked deworming or were irregularly dewormed.

Parasitic infestation according to types

Out of total number positive cases, 29 were of *Ascaris* and remaining 14 were *Strongyloids*. The result obtained showed the distribution of *Ascaris* and *Strongyloids*. The findings of the study are not comparable with those done by many researchers in the eastern hills of Nepal and many other tropical countries. Thakur (1996) reported the prevalence of six to seven species of parasite of pig in the eastern hills of Nepal and the result of Talbot, (1970) in Papua New Guinea showed 25 different species of parasites in pig. A significant difference in the duration of data recording, number of samples and age group of the piglets may be the important factors for recovering only two species of parasites.

Age-wise prevalence of parasites

Animals below 60 days showed higher prevalence of *Ascaris* while animals between 60-90 days showed higher prevalence of *Strongyloids* parasites besides older piglets showing the prevalence of *Ascaris* too. The results obtained are comparable with the findings of researcher in the eastern hills of Nepal. Thakur, (1996) stated that age of the piglets have significant effect on the level of infection especially for *Ascaris* while other parasites are less affected by the age of pigs. It has been reported that as the age of the piglets increases the prevalence of *Ascaris* decreases. This is mainly due to age related immunity development in the piglets. Increased immunity of the animals with increase in age might be the cause for lower prevalence rate.

Month-wise distribution of the disease

The parasitic incidence was highest in the month of September representing 26 (17%) positive cases while October showed lower occurrence of parasites representing 6 (4.05%) positive cases. The temperature, relative humidity and rainfall during these three months varied. September exhibited higher temperature and rainfall with 28.1°C and 332.5mm respectively while October had faced comparatively lower temperature and rainfall i.e. 28.0°C and 18.5mm respectively. The difference in amount of rainfall and temperature might be stated for the higher incidence of positive cases during September and lower happening of positive cases during October. This result is similar with the epidemiological study by Martin *et al.,* 1988. He stated, warm wet conditions provide a very suitable environment for the survival and multiplication of insects/parasites that can serve as a vector of disease. In this study also, month of September revealed warm and wet conditions in comparison to month of October. Despite these facts, the difference in number of samples during these consecutive three months might have played a noteworthy role. The difference in relative humidity during these

three consecutive months had no role in the incidence for positive cases. However, to interpret the relation between Meterology and Parasites the study is insufficient.

Epidemiological findings

The epidemiological findings revealed that most of the farmers were small holders having 40-50 pigs of different age group. Among the study areas, Jorpati, possessed highest number of pigs. All piglets were raised in pens. The feed provided to them consisted kitchen wastes, rice, different vegetables and poultry intestine. Some farmers were practicing the service of semi or completely boiled feeds and forages while others were lacking this type of practice. The areas in and around the farm was not managed properly. For example, the hygiene maintenance was poor and biosecurity was completely lacking etc. Some farmers were involved in the farming of pig cum fish, pig cum fish and duck. The pens were floored with concrete and fenced with wood, bricks or both and blocks. Some pens were divided into small chambers in order to separate the piglets from their mothers. Almost all pens were roofless. The facility of drainage was seen in almost all the pens. Most of the farmers were familiar with the helminthes parasite of swine. They said that they heard about helminth parasites from the different technicians (Veterinary Health Workers) involved in the treatment of their pigs. Most of the farmers were aware of the diseases but very few adopt the advance technology and follow the improved methods of pig keeping. They have had the practice of drenching their animals in every 3-5 months interval. They also reported that they used to vaccinate their animals against swine fever and HS. According to them the new born piglets were injected with “inferon” the iron tonics. However, they were having moderate to heavy loss of piglet mortality.

Drug sales findings

The survey on sales of anthelmintic drugs for swine revealed that sales of Ivermectin was highest followed by Albendazole, Fenbendazole and Levamisole.

ACKNOWLEDGEMENTS

The authors are thankful to all the working staffs of Animal Health Research Division,NARC,HICAST, Department of Hydrology and Meterology, Babarmahal, all the pharmacies of kathmandu and all the farmers ,those assisting in the study.

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Recommendations

नवौं राष्ट्रिय भेटरिनरी सम्मेलन
२०६७ बैशाख ९ देखि ११ बाट पारित
सुझाव तथा कार्यक्रमहरु

क. गरिवि निवारण तथ ग्रामिण विकास

१. राष्ट्रिय तथा अन्तर्राष्ट्रिय संघ संस्था तथा नेपाल सरकारबाट संचालित गरिवि न्यूनिकरण कार्यक्रमहरूमा प्रवेश बिन्दुको रूपमा पशुपालन व्यवसायलाई अंगिकार गरिनुपर्ने ।

२. गरिवि न्यूनिकरण कार्यक्रमहरूमा मूल्य मान्यतामा आधारित (Value Based) तालिम प्रदान गरी समाज परिचालन गर्ने गराउने व्यवस्थामा Veterinarianलाई अगुवाको भुमिका खेल्नु पर्ने पृष्ठभूमि तयार हुनु पर्ने ।

३. विश्वभरी नै खाद्यान्नको चर्किदों मूल्यलाई नियन्त्रण तथा खाद्य सम्प्रभुतामा पहुँच बढाउन बैकल्पिक श्रोतको रूपमा घाँसमा आधारित उपयुक्त प्रविधिको छनौट गरी पशुहरूको उत्पादकत्व बढाउने कार्यक्रमहरूलाई अभियानको रूपमा संचालन गरिनुपर्ने । खाद्य सम्प्रभुतामा खाद्यान्न वालि मात्र रहेको हुँदा सोमा दुध, फुल र मासलाई पनि समावेश गरि सोही अनुसार कार्यक्रम तय हुनु पर्ने ।

ख. भेटेरिनरी शिक्षा तथा जनशक्ति विकास

४. पशुपालन क्षेत्रमा देखा परेको मानव संशाधनको कमीलाई परिपूर्ति गर्न Public Private Partnerxhip नीति अनुरूप मध्यम स्तरीय उच्चस्तरीय जनशक्तिको विकास गर्न सबै विकास क्षेत्रमा पशुपालक सहकारी संस्थाहरू सितको सहकार्यमा अनुषन्धान तथा शिक्षण प्रतिष्ठानहरू स्थापना हुनु पर्ने र भएका संस्थाहरूलाई संस्थागत विकासको लागि अनुदान रकम उपलब्ध संस्थाहरूलाई सवल र सक्षम बनाईनु पर्ने । यस आवश्यकतालाई परिपूर्ति गर्न बहुचर्चित तथा बहुप्रतिस्थित कृषि तथा पशु विज्ञान विश्वविद्यालय स्थापना कार्य अविलम्ब थालनी हुनुपर्ने । साथै शिक्षा, अनुसन्धान र प्रसारलाई सवल बनाउन छुट्टै भेटेरिनरी Institute को साथै Veterinary Deemed University को स्थापनालाई अगाडि बढाउनु पर्ने ।

५. पशुपंक्षीको उत्पादन तथा उत्पादकत्व वृद्धि गर्नको लागि आवश्यक विकास तथा अनुसन्धान (Research and Development)का लागि आवश्यक श्रोत र साधन उपलब्ध गराई नीजि, विश्वविद्यालय तथा सरकारी क्षेत्रबाट एकमुष्ठ रूपमा परिचालन हुने व्यवस्था गरिनु पर्ने ।

ग. पशु स्वास्थ्य सेवा सुदृढिकरण

६. विश्व पशु स्वास्थ्य संगठन (OIE) को मापदण्ड अनुसार National Veterinary Service लाई सुदृढिकरण (Strengthen) गर्न तत्काल राष्ट्रिय योजना आयोगबाट प्राथमिकताका साथ श्रोत र साधनको व्यवस्था गराईनुपर्ने । हाल उपलब्ध भेटेरिनरी जनशक्ति, संगठनात्मक संरचना, श्रोत साधनहरूको मूल्याङ्कन गरी सवल र सक्षमता तिर अग्रसर गराईनु पर्ने ।

७. निर्वाहमुखी पशुपालनलाई व्यवसायीकरण गर्न हाल जिल्ला सदरमुकामसम्म सीमित भेटेरिनरियनहरूको पहुंच ग्रामीण तहसम्म पुऱ्याउन सेवा केन्दहरूको स्तर वृद्धि गरी दरवन्दी सिर्जना गरिनु पर्ने ।

८. पशुपालन क्षेत्रमा देखापरेको मानव संसाधनको कमीलाई परिपूर्ति गर्न तत्कालको विद्यमान शैक्षिक प्रतिष्ठानहरूको क्षमता अभिवृद्धि गरी विद्यार्थी भर्ना संख्या वृद्धि गर्ने र दीर्घकालिन रूपमा भेटेरिनरी विश्वविद्यालयको रूपमा विकास गरी सोही अन्तरगत हरेक विकास क्षेत्रमा PPP को model मा पशु सेवा विभागको क्षेत्रीय पशु सेवा तालिम केन्द्रहरूलाई सुदृढ गरि भेटेरिनरी स्नातक तयार गर्ने एक एक वटा भेटेरिनरी कलेजहरू स्थापना गरिनु पर्ने ।

९. नेपालको वर्तमान कुखुरापालन व्यवसाय तथा उद्योगलाई तत्कालै टेवा दिन र समस्या न्यूनिकरण गर्न राष्ट्रिय पोल्ट्रिनीति अविलम्ब स्विकृत गरी कार्यान्वयनमा ल्याउनु पर्ने ।

१०. वर्ड फ्लु देशमा भित्रि सकेको अवस्थामा यसको नकारात्मक असर बढ्न नदिन र पुनः संक्रमण हुन नदिन राष्ट्रिय तथा अन्तराष्ट्रिय दात्री संघ संस्थाहरूको सहयोग लिई एकिकृतरूपमा प्राथमिकताका साथ परिचालन गरिनु पर्ने ।

११. पशुपालन क्षेत्रको महत्व, उपलब्धता अन्तर्राष्ट्रिय स्तरमा प्रतिस्पर्धा र सम्भावनालाई अंगिकार गर्दै यस क्षेत्रको द्रुततर विकास तथा खाद्य सुरक्षाको लागि व्यवसायि क्षेत्रबाट समेत माग भई आए अनुसार छुट्टै पशुपालन मन्त्रालयको स्थापना हुनुपर्ने ।

१२. विचाराधिन रहेको भेटेरिनरी औषधि ऐन र पशु कल्याण ऐन संसदबाट पारित गराई अबिलम्ब लागू हुनुपर्ने । विद्यमान पशु सेवा ऐन कानूनलाई समय सापेक्ष परिमार्जन गरिनु पर्ने ।

१३. ग्रामिण भेगको वहुसंख्यक पशुपालकहरूमा पशु सेवाको पहुँच बढाउन **PPP** नीति अन्तरगत हरेक निर्वाचन क्षेत्रमा कम्तीमा एक पशु सेवा कार्यालय/पशु चिकित्सालयको स्थापना विस्तारका साथै सेवा रोजगारीको अवसरमा वृद्धि गराईनु पर्ने ।

१४. बर्ड फ्ल्यू, रेविज रोग तथा अन्य यस्तै जोखिम पूर्ण जुनोटिक रोग सम्बन्धि निदान तथा नियन्त्रणको काममा संलग्न पशु स्वास्थ्यकर्मीहरूको लागि विषेश जोखिम भत्ता र रू.२० लाखसम्मको जीवन वीमा गर्ने व्यवस्था अविलम्ब लागू गरिनु पर्ने ।

१५. आम नागरिकलाई सुरक्षित खाद्य (food safety) उपलब्ध गराउनु राष्ट्रको दायित्व भित्र पर्ने हुँदा सम्माननीत अदालतबाट पनि मासु जाँच ऐन लागु गर्ने परमादेश जारी भैसकेको हुँदा यसको पुर्वाधारको लागि प्राथमिकताको साथ स्थानिय नगरपालिका उप–नगरपालिका तथा महानगरपालिकाहरूमा क्रमशः कम्तिमा एक, तीन र सात जना मासु निरिक्षक र पशु सेवा विभागले मासु सुपरिवेक्षकको व्यवस्था गरि पशु वधशाला तथा मासु जांच ऐन, २०५५ लाई तत्काल लागु गराईनु पर्ने । साथै पशुवधशाला र पशुवध स्थलको निर्माणमा स्थानिय निकायबाट प्राथमिकतामा पारी कार्य संचालन गराईनु पर्ने ।

१६. व्यवसायिक रूपमा अगाडि वढिरहेको पोल्ट्री व्यवसायलाई थप टेवा स्वायत्त National Poultry Development Board अविलम्व गठन गरिनुपर्ने । साथै देशमा वर्ड फ्लु भित्रिसकेको अवस्थामा संकटग्रस्त पोल्ट्री व्यवसाय तथा पीडित कृषकहरूलाई राहतको प्याकेज ल्याईनुपर्ने र जैविक सुरक्षाका उपायहरू अपनाई नयां Poultry Zone हरूको पहिचान गरी विकास गरिनु पर्ने ।

१७. वर्ड फ्लु, रेविजजस्ता खतरनाक रोगहरूविरूद्ध दिनरात खट्नुपर्ने भेटेरिनरी सेवालाई Emergency Service को रूपमा सूचिकृत गरिनु पर्ने । जोखिम विश्लेषण गरी विमाको कानूनी व्यवस्था गरिनु पर्ने । भेटेरिनरी सेवाको विशिष्टतालाई मध्यनजर राख्दै विशेषज्ञताका आधारमा सरकारी सेवामा सेवा, समूह तथा उपसमूहहरू अविलम्व गरिनु पर्ने । हालको संरचनामा रहेका पशु रोग निदान, उपचार, अनुसन्धान तथा नियमन कार्य गर्ने निकायहरूमा विषेशज्ञताको सेवा उपलब्ध गराउन सक्षम र सवल जनशक्ति र आधारभूत आवश्यकता परिपुर्ति गर्न विद्यमान सरकारी सेवा समुहलाई परिमार्जज गरी समयसापेक्ष गराईनु पर्ने ।

१८. वन्यजन्तुहरू तथा Animal Origin का खाद्यतत्वहरूबाट विभिन्न जुनोटिक रोगहरू सर्नसक्ने सम्भावनालाई मध्यनजर राख्दै हरेक राष्ट्रिय निकुञ्जहरू तथा वन्यजन्तु संरक्षणसम्वन्धी अन्य निकायहरूमा तथा खाद्य गुण नियन्त्रण लगायतका भेटेरिनरियनहरूको यथेष्ठ दरवन्दी अविलम्व सिर्जना गरिनुपर्ने

१९. राष्ट्रिय पशु चिकित्सा सेवालाई अत्यावश्यक सेवा (Emergency service) को रूपमा अन्य अत्यावश्यकमा वर्गिकरण गरिनु पर्ने ।

घ. पब्लिक प्राइभेट सहकार्य कार्यक्रम

२०. Livestock Industry को लागि आवश्यक पर्ने दाना सामाग्रीहरू उत्पादन गर्न सहकारी, करार तथा करर्पोरेट खेती प्रणालीको शुरूवात गर्ने नीति कृषि तथा सहकारी मन्त्रालयबाट व्यवस्था गराई अविल्म्व लागु गरिनु पर्ने ।

२१. APP नीति अन्तरगत छिमेकी देशहरूबाट आयात गर्ने प्यारेन्टस्टक लाई स्वदेशभित्रै उत्पादन गर्न Grand Parent Stock को फार्म स्थापना गर्न आवश्यक श्रोत साधनको सहयोग उपलब्ध गराईनु पर्ने ।

२२. पशु तथा पंक्षीजन्य उद्योग तथा व्यवसायका लागि आवश्यक पर्ने सम्पूर्ण Input तथा यस्ता उद्योग तथा व्यवसायहरूबाट उत्पादित पदार्थहरूलाई प्रतिस्पर्धि बनाउन छिमेकी मुलुकहरूमा भएको व्यवस्था अनुरूप तथा विश्व व्यापार संगठनको प्रावधान अनुसार एकरूपता ल्याउन र व्यवसायिकरण गर्न प्रोत्साहन गर्न लागि Value Added Tax को प्रथा पुनर्विचार गरी वर्गिकरण गरी लागू गरिनु पर्ने ।

२३. पशु पंक्षीजन्य उत्पादनको लागि आवश्यक पर्ने गुणस्तरीय उत्पादन सामाग्री तथा उत्पादित वस्तुहरूको आपूर्तिका लागि गुणस्तर कायम गर्ने व्यवस्था प्राथमिकताका साथ निजी तथा सरकारी क्षेत्रका प्रयोगशालाहरूलाई राष्ट्रिय तथा अन्तर्राष्ट्रिय संस्थाहरूबाट सम्बन्धन (Accreditation) गराई क्रियाशिल गरिनु पर्ने ।

२४) दक्ष भेटेरिनरी सेवा–केन्द्रबाट जिल्ला सदरमुकामसम्म सिमित रहेकोले, ग्रामिण पशुपालकहरू दक्ष भेटेरिनरी सेवाबाट वञ्चित छन् । निर्वाहमुखि पशुपालन पेशालाई व्यवसायिकरण गर्न र विश्व व्यापार संगठन र OIE को मापदण्ड अनुसार पशुजन्य खाद्य सुरक्षा एवं जुनोटिक रोगको नियन्त्रण हुनु पर्ने भएकोले सेवा केन्द्र/उपकेन्द्र र उत्पादन सञ्जालको केन्द्रसम्म दक्ष भेटेरिनरी सेवा उपलब्ध गराउनु पर्ने ।

धन्यवाद