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EDITORIAL

Since the beginning of modern veterinary service five decades, and the organization of Nepal Veterinary Association some 35 years ago, Veterinarians in Nepal have been playing a vital role in promoting animal and human health through their contribution in increasing livestock production and productivity and in safeguarding public health through treatment, prevention and control of animal diseases of economic and/or zoonotic importance. A few noticeable successes have been achieved during this period in the field of veterinary profession and animal disease control in the country. Some noteworthy are: enactment of three legislations namely, Animal Health and Livestock Service Act, Nepal Veterinary Council Act and Slaughterhouse and Meat Inspection Act for regulation, modernization and advancement of veterinary service in Nepal. However, it has also been realised that there is an urgent need of Veterinary Drug Act and Veterinary Public Health Act to safeguard both human and animal population of the country. In addition, the continuous and dedicated efforts of Nepalese veterinarians and effective execution of programs at the national level, some dreadful animal diseases like 'Rinderpest' in cattle and buffaloes and 'Foot rot', in sheep and goat have been eradicated. Moreover, an effective vaccine has also been developed for the first time in the country against *Pesti des petis ruminants* (PPR) to control this devastating disease of small ruminants. These achievements bring the optimism and inspire us, as envisaged in the Agricultural Perspective Plan of the country, to exploit the tremendous potential that the nation possesses for the development of livestock sector. In the same manner, with the dedicated effort of Veterinarians, some remarkable achievements have been made in milk and poultry production in the country. Intensification of the production system also increases the chances of occurrence of diseases in these populations and the need of competent and efficient veterinary services in the country. Thus, both the government and private sectors have to recognize this necessity and potential for the promotion of veterinary profession in the country, the importance of which is going to become even more in the years to come after the entry of the country in the WTO network.

We must concede that many infectious diseases are enzootic among the populations of many domesticated and wild animal species of Nepal. These diseases are responsible for reduced productivity of our animals. Moreover, many of these diseases also pose public health risk either by being transmitted directly from animal to man or through food chain of animal origin. Studies on epidemiological, economic and public health significance of many of such diseases are extremely important to develop and execute measures on disease prevention and control. We need a national program on research and development to study important diseases and establish a scientifically authentic and reliable reference mechanism of animal diseases of the country, which is mandatory for national and international trade of animals, animal products and biologicals. The role of

research becomes prime in investigating and maintaining the scientific evidences for necessary references on animal diseases of the country. Therefore strengthening of national veterinary research and services system becomes essential to materialize our commitment to abiding WTO and OIE rules and regulations.

Veterinarians are not only concerned with increasing quantum of human food of animal origin but also with its quality and safety with regard to safeguarding public health. For this, veterinarians have to be aware of their responsibility and role in assuring consumers of risks associated with food and other products of animal origin. Nepal Veterinary Association expresses its commitment and dedication to strengthen the competences of its members. Meantime, it anticipates cordial cooperation from the government and all other sections of the society for the noble cause of safeguarding animal and human health in the country.

Many individuals contributed for the production and editing of this journal and the list is extensive to name all of them. The editorial team expresses its greatest admiration to all individuals who contributed directly or indirectly for the publication of this journal. We are also grateful to the contributors for their research and review papers, clinical reports or short communications, which will enhance the dissemination of information and technologies to a wider mass for the improvement in animal and human health of the country. We hope that this support will remain with us for the years to come.

Editors

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DETECTION OF NEUTRALISING ANTIBODY TITRE AGAINST RABIES IN DOGS BEFORE AND TWO WEEKS AFTER VACCINATION

D. D. Joshi¹, A. Wandeler², C. Glick³, B. Chhetri¹, K. Knowles²

ABSTRACT

Serum neutralisation tests carried out on 46 and 59 dogs of ward 19 and 20 respectively of Kathmandu municipality on the day of vaccination and after 14 days of vaccination with Biocan - R showed that protective levels of anti body (i.e. > 0.5 I.U./ ml) increased following vaccination. Protective antibody titres in dogs at the day of vaccination can be attributed to protective levels of antibody from preceding vaccination carried over to the day of vaccination. After 14 days of vaccination, 100% dogs from ward 19 had achieved protective levels of antibodies against rabies from the vaccination with the mean titre of 7.25 (range 0.98 – 15). Only 94 % of the vaccinated animals from ward 20 achieved protective antibody with a mean titre of 9.11 (range 2.76 – 11.03).³ Two dogs in ward 20 showed titres lower on day 14 than the base line titre, the reason for which is unclear. This study shows that most of the dogs developed high levels of neutralising antibody titres in 14 days post vaccination with Biocan-R vaccine. However, as the monitoring of neutralising antibody titres was not carried out for a longer period, the revaccination schedule for this vaccine could not be determined from this study.

INTRODUCTION

Rabies is endemic in Nepal and the disease occurs throughout the year with the death of 100 to 150 people annually. Dog is the principal animal reservoir of the disease to man and about 94 % of persons who seek antirabies treatment are cases of suspected rabid dog bites (Joshi, 1983). Both Semple type and tissue culture vaccine have been in use in Nepal though the total volume of nerve tissue origin vaccine produced by Rabies Control and Public Health Section meets only 50% of national demand Parajuli and Rai, (1999). Though the nerve tissue origin vaccine is the mainstay of rabies prophylaxis, the use of tissue culture vaccine is on the rise (Annon, 2002). One of the most important constraints in rabies control is the economic status of the dog owners and high cost of antirabies vaccine which compels dog owners to opt out of the usually followed annual vaccination scheme.

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Most of the tissue culture vaccines require revaccination every 2 to 3 years though the scheme is subject to levels of local infection present. However the usual practice is to revaccinate every year. No study has been done in past in Nepal under field conditions to establish the possible duration of immunity conferred by tissue culture vaccine neither has there been any attempt to demonstrate neutralising antibody in dogs before and after vaccination of dogs with commercial tissue culture vaccine. Satisfactory levels of neutralising antibodies have been observed in cattle vaccinated with tissue culture rabies vaccine (Ramanna *et al*, 1991). The present study aims to demonstrate antibody titre against rabies in dogs following vaccination with Biocan – R inactivated tissue culture vaccine.

MATERIALS AND METHODS

The study was carried out in the dog population of ward 19 and 20 of Kathmandu Municipality simulating field conditions. Two batches of blood samples from selected dogs were collected for seroanalysis two weeks apart i.e. on the day of vaccination and after 14 days of vaccination in each of the two wards from the same dog population.

A total of 46 and 59 blood samples, 5 ml each, were collected aseptically from the cephalic vein of the selected dogs. The blood samples were centrifuged and sera were preserved in dry ice and dispatched immediately to Dr. Wandeler's laboratory, Canada for Serum Neutralisation Test. Biocan R inactivated tissue culture anti rabies vaccine batch no. 145407 (antigenic value at least 1 IU/dose) manufactured by Bioveta Plc, Czech Republic was used for vaccination. All dogs aged above 3 months were vaccinated with 1 ml of the vaccine subcutaneously on the dorsal aspect of the neck. A second batch of 5 ml blood sample each from the same dogs were collected after 14 days of vaccination, the blood samples were centrifuged and sera samples were again sent to the earlier laboratory Canada for seroanalysis. The sera were analysed for the detection of neutralising antibodies against rabies in both batches by in vitro Serum Neutralisation Test (Smith, et al, 1973) according to the following protocol.

Test was conducted using 96 well microtest plate, MNA cells, and CVS virus. 20 ul of serum is added to 4 wells containing 60 ul of media, serial 4 fold dilutions of the serum to dilution of 1/256. CVS virus 100 TCID₅₀ in 60 ul was added to all wells and incubated for 60 minutes. Cells were added and plates were incubated for 2 days after which they were fixed and stained by direct immunofluorescence with an anti-rabies serum. Any serum with a titre > 0.5 I.U./ml was considered having protective levels of antibody against rabies.

RESULTS

Out of 46 and 59 sera tested in the first batch from ward 19 and 20, 68% and 58 % did not have protective levels of antibody (i.e. > 0.5 I.U./ ml) (Table 1). The batch showed a mean titre of 0.78 I.U. (range 0.06 to 6.56 I.U.) and 1.15 I.U (range 0.06 to 11.03 I.U.) for dog population of ward 19 and 20 respectively.

In the sera of 14 day post vaccination, 100% of the dogs from ward 19 had achieved protective levels of antibodies with a mean titre was 7.25 I.U. (range 0.98 – 15.6). In dogs of ward 20, 94 % achieved protective levels of antibody with a mean titre of 9.11 I.U. (range 2.76 – 11.03). Two dogs in ward 20 showed titres less than the base line titre on 14 day post vaccination (Table 1). While 46 and 59 sera samples were available in the first batch of testing i.e. from blood collected on the day of vaccination, only 31 and 34 samples could be tested in the second batch as 80 ul of serum sample with less than was omitted for test.

Table 1 Antibody titre against rabies before and after antirabies vaccination in dogs of ward 19 and 20 of KMC

Site	Unit	Day- 0	Protected population	Day- 14	Protected population
Ward 19	Mean Titre (I.U./ml)	0.78	32.6 (15/46)	7.25	100 (31/31)
	Range(I.U./ml)	0.06 - 6.56		0.98 - >15.60	
Ward 20	Mean titre* (I.U./ml)	1.15	42.37(25/59)	9.11	94 (36/38)
	Titre range(I.U./ml)	0.06 – 11.03		2.76 – 11.03	

* Base line titre = 0. 50 I.U./ml

All male and female dogs in ward 19 developed high level of antibody titres on 14 days post vaccination (6.8 and 9.3 respectively) and the protected population increased to 100 percent from 38.2 percent male and 16.6 percent female on the day of vaccination (Table 2). Similarly, 95.4% and 94% dogs from ward 20 showed neutralising antibody titres above the base line on day 14 post vaccination with a mean titre of 8.37 and 9.38 respectively, while one male and a female dog failed to develop sufficient neutralising antibody titres.

Table 2 Pre and post vaccination antibody titre against rabies in dogs according to sex

Site	Sex	Unit	Day-0	Protected population	Day-14	Protected population
Ward 19	Male	Mean titre (I.U./ml)	0.78	38.2 (13/34)	6.8	100 (21/21)
		range	0.03 – 6.56		0.98– 15.60	
	Female	Mean titre (I.U./ml)	8.58	16.6(2/12)	9.3	100 (10/10)
		range(I.U./ml)	0.03 – 6.56		1.16– 15. 60	
Ward 20	Male	Mean titre (I.U./ml)	1.63	45.9(17/37)	8.37	95.4(21/22)
		range(I.U./ml)	0.06 – 11.03		2.76 – 11.03	
	Female	Mean titre (I.U./ml)	0.8	36.3(8/22)	9.38	94 (15/16)
		range(I.U./ml)	0.06 – 3.9		2.76 – 11.03	

On age wise analysis, sera from dogs with ages <1 year in ward 19 had a neutralising antibody titre (range 0. 03 to 0. 82) before vaccination but achieved complete protection on 14 days after vaccination (Table 3). Two dogs below 1 year had protective levels of immunity even before vaccination (10.14%) while 48.14% of the dogs aged above 1 year had protective levels of antibody before vaccination and after 14 days the selected population achieved complete protection (100%). Similarly, at the second site (ward 20), 25% and 49% dogs below 1 year and above 1 year respectively had above baseline titre before vaccination. The mean titres before vaccination were above the baseline titres in both age categories. High neutralising titres were achieved after 14 days of vaccination in both age categories (Table No. 3).

Table 3 Pre and post vaccination antibody titre against rabies in dogs according to age

Ward	Age	Unit	Day-0	Protected population	Day-14	Protected population
Ward 19	<1 year	Mean titre (I.U./ml)	0.22	10.14(2/19)	6.87	100 (19/19)
		Range (I.U./ml)	0.03 - 0.82		1.38 – 15.60	
	>1 year	Mean titre (I.U./ml)	1.24	48.14(13/27)	10.98	100 (12/12)
		Range (I.U./ml)	0.03 – 6.56		0.98 – 15.60	
Ward 20	<1 year	Mean titre (I.U./ml)	0.69	25(4/16)	8.73	90 (10/11)
		Range (I.U./ml)	0.60-3.9		3.90 – 11.03	
	>1 year	Mean titre (I.U./ml)	1.51	48.88(22/43)	8.14	96 (26/27)
		Range (I.U./ml)	0.60–11.03		2.76 – 11.03	

DISCUSSION AND CONCLUSIONS

The two batches of collected sera from dogs vaccinated with Biocan – R showed high seroconversion rate within 14 days of vaccination. Only two of the vaccinated dogs failed to develop protective levels of antibody probably due to errors in vaccination that are warranted in the mass vaccination campaign or due to inherent physiology of the dogs. It cannot be ruled out that these animals might have developed protective levels of neutralising antibody titres after 14 days. On the vaccination day, mean titres in dogs of both wards were higher than the baseline titres, though the protected population was only 32 and 42 % in ward 19 and 20 respectively. The high titres of the protected population seem to influence the mean titres in both wards. Protective antibody titres on the day of vaccination can be attributed to the antibodies developed from preceding vaccination and/or maternal antibodies carried over till date. Owners either revaccinated their dogs before the usual scheme of 1 year or the antibody titres from preceding vaccination still holds at the protective levels. Most of the manufacturers recommend revaccination after two or three years of the first shot, though scheme is subject to modification depending on local infection status. This study showed that most of the dogs developed high levels of neutralising antibody titres on 14 days post vaccination with Biocan-R vaccine, however, the persistence of this level could not be ascertained. Hence, assessment of neutralising antibody titres must be carried out regularly so that the duration of immunity could be determined and a suitable revaccination scheme could be ascertained to cut out the extra cost of unwarranted vaccination.

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A STUDY ON THE EFFECT OF IRON SUPPLEMENTATION IN PRE-WEANING PIGLETS

S. Singh¹, S.K. Karna, R.K. Bhattarai, S. Bhandari

ABSTRACT

This study was conducted at IAAS livestock farm Rampur, Chitwan during August to November 2000 to determine the hematological response and response of iron supplementation to prevent piglet anemia in Yorkshire×Landrace piglets. In the experimental period of 56 days hematological parameters e.g. Hb, RBC count and PCV in Imferon injected group (G₁); and in oral supplemented group (G₂) were significantly higher than control group (G₃). Result of present study revealed that administration of iron both orally and parentally was effective to prevent piglet anaemia and other secondary complication to minimize farm economic loss. Iron supplementation in piglet should be in the routine practice to check piglet anaemia and other secondary complications. Administration of Imferon @ 2 ml on the 4th and 14th day intramuscularly in hind leg and oral iron preparation 1.8% ferrous sulfate @ 4 ml per piglet daily for seven days from 24 hours of their birth were found to be very useful in preventing piglet anaemia.

INTRODUCTION

Swine husbandry is considered to be most economical due to high feed conversion ratio (FCR) of the animals. Pig raising is highly profitable as pigs are famous for their voracious eating habit and are efficient convert or of the agro waste into highly nutritious meat with high dressing percentage of 65-70 percent. Pigs have most efficient feed conversion ratio of 3-3.5: 1 i.e., 3-3.5 kg feed /kg live wt. gain (Chakravarti, 1998).

Among various diseases of the swine, one of the most common problem is piglet anaemia, which is caused by low level of iron in the piglet diet i.e. either sows milk or creep ration. The manifestation of this condition is lean carcass, loss of appetite, rough body coat, pale visible mucous membrane of the piglets. This is the most common finding in almost all rural farms where no additional supplementations is provided to piglets.

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The present study is undertaken to evaluate the usefulness of iron supplementation in piglets to prevent piglet anaemia so that the technology could be recommended to pig raisers of the country.

MATERIALS AND METHODS

The study was conducted at Livestock farm, IAAS, Rampur. Eighteen crossbred piglets (Yorkshire × Landrace) were selected from sows producing 6-8 piglets from IAAS livestock farm, Rampur. The experimental animals were divided into three groups G₁, G₂ and G₃ with 6 piglets of either sex in each group. Piglets were marked with Silver Nitrate solution on the white belt area of the body. Animals were maintained under similar feeding and management conditions during the experimental period of 8 weeks. All the piglets were allowed to suckle mother's milk *ad libitum* regularly from birth and creep feed were provided from 14 days onward up to weaning age of 56 days. The piglets of G₁ group were injected with 2 ml Imferon® [Rallis], each ml containing Iron Dextran, providing 50 mg. of elemental iron, I/M in the hind leg on the 4th and 14th day. The piglets of G₂ group were fed orally on iron preparation (1.8 % Ferrous Sulfate syrup) at the dose rate of 4 ml/piglet daily for 7 days from first 12 hrs. of their life. The piglets of group G₃ were not given any iron supplement and kept as control animals. All piglets were clinically examined and observed daily for general health conditions such as change in mucous membrane, appetite, coat color and growth rate throughout the experimental period. Body temperature and pulse rate were recorded at weekly interval. Blood sample were collected directly from heart at birth, on 4th day, on 7th day, and thereafter at weekly intervals up to 8 weeks of age and were analyzed for hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), by standard methods.

RESULTS AND DISCUSSION

The general body health condition such as appetite, defecation and body coat were observed to be normal with pink normal conjunctiva and mucous membrane of piglets in G₁ and G₂ groups, which were given iron parentally and orally respectively. In control group₂ piglets died showing anorexia, pale conjunctiva, and mucous membrane.

Effect on body temperature and respiration:

No significant differences were observed among the groups from 0 to 56 days of experimental period in body temperature but significant differences were observed in respiration rate among groups G₁, G₂ and G₃. The temperature (°C) and respiration rate (per minute) of three different groups of piglets is presented in Table 1.

Table 1 Mean Values of Temperature ($^{\circ}\text{C}$) and Respiration (per minute) in three groups of piglet during the experimental period.

DAYS	Temperature ($^{\circ}\text{C}$)			Respiration (per minute)		
	G ₁	G ₂	G ₃	G ₁	G ₂	G ₃
0	39.52	39.41	39.52	45.69	46.02	46.02
4	39.52	39.36	39.54	34.19	34.36	34.69
7	39.48	39.59	39.54	32.86	33.69	34.86
14	39.49	39.48	39.53	34.36	34.36	35.03
21	39.49	39.44	39.53	34.69	35.03	39.36
28	39.54	39.49	39.49	34.69	34.69	51.53
35	39.54	39.46	39.51	34.69	34.69	51.53
42	39.48	39.49	39.46	34.86	34.69	60.11
49	39.49	39.54	39.51	34.86	34.69	56.86
56	39.51	39.51	39.51	34.86	34.86	56.86
Overall Mean	39.506	39.477	39.514	35.575	35.708	46.685

The respiration rate was found to be significantly higher between 21 to 56 days in G₃ groups than in other groups. The respiration rate increased in all experimental piglets at birth but reduced subsequently to normal in G₁ and G₂ groups. labored breathing observed in piglets of G₃ group might be due to hypoxia resulting from low hemoglobin level.

The occurrence of diarrhea might be due to marked impairment of gastric secretion of acid and chloride and changes in gastrointestinal flora, which ultimately contributed to the increased susceptibility to diarrhea. Similar results were also interpreted by *Sarmah et al.* (1999).

These normal symptoms and appearance could be due to the normal hemoglobin level in G₁ (10.82 gm. /100 ml) and G₂ (10.66 gm./100 ml). The Hb deficient piglets of G₃ (7.05 gm./100 ml) showed signs as pallor mucous membrane, anorexia, rough body coat, unthriftiness, anaemic, yellow color diarrhea and labored breathing on 5th week as has also been reported by *Sarmah et al.* (1999). Anaemia and paleness of the mucous membrane observed on 35th days might be due to decreased hemoglobin level (5.31 gm. /100 ml) and clinical signs appeared when hemoglobin level fell below 50 % of normal value (Blood *et al*, 1994).

Table 2 Mean Values of Hemoglobin (gm. /100 ml) in three groups of piglet during the experimental period.

DAYS	G ₁	G ₂	G ₃
0	10.79	10.81	10.84
4	7.78	10.86	7.91
7	10.86	10.82	7.74
14	11.48	10.83	7.49
21	11.88	10.76	6.56
28	11.71	10.69	5.92
35	10.89	10.52	5.31
42	10.92	10.61	5.73
49	10.98	10.32	6.56
56	10.96	10.38	6.52
Overall Mean	10.82	10.66	7.05

The mean values of hemoglobin at 0 day ranged from 10.79 gm./100 ml to 10.84 g/100 ml in G₁, G₂, and G₃ groups. The level of hemoglobin decreased significantly during first four days in G₁ (7.78 gm. /100 ml) and G₃ (7.91 gm. /100 ml.) groups, which might be due to rapid destruction of fetal hemoglobin. However, the higher level of the hemoglobin in G₂ group of piglets (10.86 gm. /100 ml.) might be due to oral administration of iron preparation within 12 hours after birth as a result of efficient absorption of iron through the neonatal intestinal mucosa. During the entire experiment, the weekly average values of Hb in G₁, G₂, and G₃ were 10.82, 10.66 and 7.05 gm. /100 ml. respectively. No significant differences was observed between G₁ and G₂ but the control group was significantly lower than the other two groups, which might be due to dietary iron deficiency.

Mean value of TEC (total erythrocytes count (10^6 /cmm.) were almost similar at 0 day i.e., 6.69, 6.54 and 6.61(10^6 /cmm.) in all groups but the mean values decreased in G₃ as the age progressed. The overall total erythrocyte counts during the experimental period are as Table 3.

Table 3 Mean Values of Total Erythrocytes count (10^6 /cmm.) in three groups of piglet during the experimental period.

DAYS	G ₁	G ₂	G ₃
0	6.69	6.54	6.61
4	5.19	5.89	5.21
7	5.80	5.69	4.76
14	5.91	5.67	4.11
21	5.99	5.59	3.93
28	5.89	5.53	3.62
35	5.92	5.53	3.35
42	5.83	5.42	3.47
49	5.69	5.53	3.76
56	5.67	5.52	3.76
Overall Mean	5.86	5.69	4.26

Mean Packed Cell Volume (PCV) on 7th days in G₁, G₂ and G₃ groups ranged from 34.83, 34.16 and 28.56 percent respectively. The overall mean weekly mean values of PCV in G₁ and G₂ were significantly higher than control animals. The mean PCV of piglets in different groups is presented as figure 1.

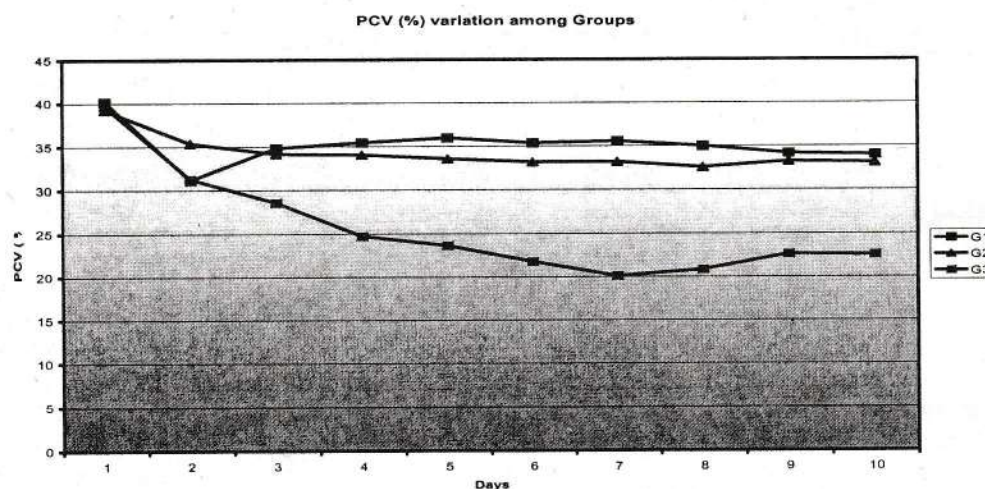


Figure 1. PCV percent in different groups of piglets

The significantly higher level of PCV in G₁ and G₂ groups might be due to rapid utilization of iron and due to increased level of hemoglobin as well as total erythrocyte count in piglets. *Swennson* (1977) stated that packed cell volume was directly related to the level of erythrocyte count and Hb content.

The level of Hb, TEC and PCV decreased significantly during the first four days in all groups. The reason for fall in Hb levels might be due to rapid destruction of foetal Hb (*Sarmah et al.*, 1999a,b) and for TEC could be due to reduced release of erythrocytes count into the circulation. *Swennson* (1977) stated that PCV was directly related to the level of erythrocyte count and Hb content. So the fall in PCV could be due to decreased Hb and erythrocyte levels.

CONCLUSION

Piglet anaemia is more common in pre-weaning piglet due to low level of iron in sow milk and faster growth rate in piglets. Iron supplementation in piglets has major role in pig farming because it prevents the piglet anaemia and other secondary problems causing severe economic losses. Special attention should be paid by swine producers for iron supplementation in routine practice to prevent piglet anaemia and other

secondary complication to minimize farm economic loss and ascertain best swine performance. Administration of Imferon® @ 2 ml. on the 4th and 14th day intramuscularly in hind leg or oral preparation of 1.8% Ferrous sulfate @ 4 ml. per piglet daily for 7 days from 24 hours of their life should be practiced. Traditional pig farmer belonging to lower strata and villages should be made aware about piglet anaemia and their successful prevention with iron supplementation.

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PREVALENCE OF BRUCELLOSIS IN YAKS AND OTHER CATTLE OF LANGTANG VALLEY

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ABSTRACT

Brucellosis is endemic in Nepal and is a disease of significant economic and public health importance in the dairy production systems of the country. The epidemiology and prevalence of brucellosis in the national herd has not been evaluated in the country and the government has not yet formulated the national strategy for brucellosis control. In this present study the prevalence of brucellosis in Yak (Naks), Chauries and hilly cattle of Langtang valley has been studied. A total of 74 animals (18 in Chandanbari and 56 in Kyanjing gumpa) were sampled, of which 13 were either moderately or strongly positive for brucellosis, giving an overall prevalence of 17.6% of this disease in the tested population.

INTRODUCTION

Agriculture dominates the economy of Nepal, with livestock revenues accounting for 30% of the Agriculture Gross Domestic Product (AGDP). In the mountain communities, livestock diseases have a large impact due to the heavy economic dependence on farming and the scarcity of veterinary care.

Brucellosis is a disease that affects humans and livestock. In humans, brucellosis is termed "undulant fever" due to the recurrent fever and fatigue that typify the disease. In cows, brucellosis is characterized by late gestation abortion and decreased milk production. In livestock, there is no economically feasible cure for brucellosis. Due to the contagious nature of the disease, infected animals must be separated from the herd to prevent endemic spread.

Brucellosis is also known as undulant fever in human beings because of the waxing and waning of the fever. It is also called Malta, or Mediterranean fever. Its insidious onset and nonspecific clinical features make the disease difficult to recognize for the physicians. The commonest complications are osteoarticular (20 to 60 percent of cases) and genito-urinary (2 to 20 percent). If untreated, the case fatality rate is up to 2 percent, usually from endocarditis, but specific antimicrobial chemotherapy is available.

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There are several *Brucella* species, which are often associated with a particular reservoir species, although the bacterium is not confined to those species. *B. abortus* is usually associated with cattle, *B. suis* with pigs, and *B. melitensis* with goats.

Brucellosis can also be acquired from consumption of unpasteurized/unboiled milk. Brucellosis is caused by bacteria of the genus *Brucella* and in animals is characterized by abortion, retained placenta, and occasionally orchitis and infection of the accessory reproductive glands in males. The disease is present in many countries in the world. It primarily affects cattle, buffalo, bison, pigs, sheep, goats, dogs, elk, and sometimes horses. The disease in humans is a serious public health concern.

Raw materials such as milk may transmit the disease to humans. When infected, droplets of the fluids involved in the birth process splash into the eyes, the mouth, or open cuts, people assisting those animals may develop brucellosis. The organism infecting goats presents the most serious disease in people (Stone and Belton, 2002).

While the prevalence of brucellosis has been studied in many species in Nepal, no current information regarding the prevalence of antibodies to *Brucella abortus* in naks (female yaks), chauries (cross breed between yak and local cattle) or cows in the high mountain valleys like Langtang Valley has been published. The objective of this study was to determine the prevalence of antibodies to *B. abortus* among migratory yaks, chauries and cows used for milk production in Langtang Valley, Nepal.

MATERIALS AND METHODS

Yaks and chauries in Langtang Valley are migratory. During the summer months, they are moved to high altitude pastures where they graze freely. The husbandry practices vary slightly between different regions.

Two different regions within the Langtang National Park were tested in this study. The first was Chandanbari (Gumba), elevation 3500m. In Chandanbari, two farmers participated in the study, one with 12 lactating chauries and one with 6 lactating chauries. The chauries were free roaming and brought in twice daily for milking.

The second region in this study was Kyanjin Gompa (Jangbu), approximate elevation 5000m. There were over 100 yaks, chauries, and cows at this location. Fifty-six of these animals were tested for brucellosis during this study. The animals in this herd were owned by nine different farmers, though they lived and grazed on communal pasture. Each farmer knew which animals were his, and knew the basic medical and ownership history of each animal.

A convenience sampling technique was used based on association with the yak cheese factories as well as accessibility of the herds by foot during the summer monsoon season.

A sterile milk-collecting vial was given to the farmer and a milk sample was collected. Care was taken not to touch the vial to the teat of the animal. An equal aliquot of milk was taken from each quarter, and the milk was thoroughly mixed. This process was repeated for the majority of lactating animals within the herd. The milk was stored at 5 to 20 degrees centigrade for 24 to 48 hours, depending on the availability of refrigeration. After 24 to 48 hours, the milk was brought to room temperature and the Milk Ring Test was performed.

For each yak, chauri or cow sampled, her name, owner's name, approximate age, medical history, date of last parturition, and past incidence of abortion were recorded. A negative vaccination history was confirmed for all of the animals tested.

Milk Ring Test Procedure

The Milk Ring Test (MRT) was used to detect antibodies to *Brucella abortus*. This is an accepted standard for brucellosis testing of cattle in the United States. The MRT reagents were obtained from the National Veterinary Services Laboratory, USDA, Ames, Iowa.

Thirty ul of antigen was added to each 13 x 100 mm glass test tube. Milk samples were inverted and mixed, and 1 ml of milk was added to each of the test tubes. Due to the high fat content of yak milk (6.5%), no additional cream was added. The samples were again thoroughly mixed, and then incubated at 37 degrees Celsius for 1 hour. The incubation procedure was dependent on availability of electricity. Samples collected in Chandanbari were incubated using a Styrofoam vaccine box and a low voltage light source. Temperature within the incubator was monitored closely using an atmospheric thermometer and the box was vented appropriately to keep the temperature at 37 degrees. In Kyanjin Gompa, samples were incubated in a small Styrofoam and aluminum incubator, using lantern candles as a heat source. The incubator was heated to 37 degrees, at which time the samples were placed inside the incubator. The box was vented as necessary to hold the temperature of incubation between 35 and 40 degrees Celsius. Following incubation, Milk Ring Test results were read and recorded. All questionable milk samples were retested using the Milk Ring Test. Positive and negative controls were run with each batch that was incubated to assure that all reagents and equipment were working properly.

The Milk Ring Test results were classified as negative, weak positive, moderate positive, or strong positive. In calculating prevalence, only those tests reading as moderate or strong positive were counted. Animals testing weakly positive for antibodies to *B. abortus* were not included in calculations of prevalence.

RESULTS

All 74 animals (18 from Chandanbari and 56 from Kyanjin Gompa) tested for antibodies to *B. abortus* in this study, no animal had been vaccinated for brucellosis. Of the 18 chauries in Chandanbari, the prevalence of antibodies to *B. abortus* was 17% (Table 1). Two of the chauries had a prior history of abortion, one of which had aborted during past three successive years, and tested strongly positive for antibodies to *B. abortus*. The other chauri with a positive abortion history tested negative for antibodies to *B. abortus*. Of the 56 naks, chauries and cows tested in Kyanjin Gompa, the prevalence of antibodies to *B. abortus* was 18% (Table 2). None of the animals within this herd had a positive history of abortion.

Table 1 Prevalence of Brucellosis in Chandanbari

Animal Category	Number	Negative	Weak Positive	Moderate Positive	Strong Positive	Prevalence*
Chauries	18 (100%)	13 (72.5%)	2 (11%)	2 (11%)	1 (5.5%)	17%

* Only animals testing moderately or strongly positive are included in prevalence

Table 2 Prevalence of Brucellosis in Kyanjin Gompa

Animal category	Number	Negative	Weak Positive	Moderate Positive	Strong Positive	Prevalence*
Chauries	48 (86%)	34 (71%)	6 (12.5%)	5 (10%)	3 (6%)	17%
Naks	5 (9%)	3 (60%)	0 (0%)	2 (40%)	0 (0%)	40%
Cows	3 (5%)	3 (100%)	0 (0%)	0 (0%)	0 (0%)	0%
Total	56 (100%)	40 (71%)	6 (11%)	7 (13%)	3 (5%)	18%

• Only animals testing moderately or strongly positive are included in prevalence

In total, 74 animals were sampled, of which 13 tested either moderately or strongly positive, giving an overall prevalence of 17.6%.

DISCUSSION

Within Nepal, brucellosis testing has been conducted on many different species by several researchers. The prevalence of brucellosis varies significantly between different reports, leaving questions as to the true prevalence of the disease.

While many researchers have tested for brucellosis in yaks, cows and buffalo and found either no or low disease prevalence (Punel *et al*, 1999; Xue and Wang, 1998), a moderate to high prevalence was found in this study. Similarly, other studies conducted in specific regions of both Nepal and China show equal or greater disease prevalence (Joshi, 1999, 2000; Xue and Wang, 1998). A study done among yaks on the Baieaopo pasture in China revealed a much higher prevalence of 47.19% (Xue and Wang, 1998), thus demonstrating the range of reported statistics in different locations among yak herds.

It appears that endemic pockets of brucellosis are located throughout Nepal, possibly explaining the range in disease prevalence reported by different studies. This theory can be accounted for by local husbandry practices that encourage endemic spread of brucellosis. The national Hindu religion of Nepal prohibits slaughter of yaks, chauries and cows. The migratory and free-roaming nature of the herds makes it impractical to separate infected animals from non-infected animals, thus necessitating intermixing between non-infected and infected individuals. This problem is exaggerated by the fact that bulls are co-housed with cows, increasing the chance of sexual transmission of the disease. Furthermore, in Chandanbari it is customary to put salt on recently aborted fetuses as a means of encouraging the lactating females within the herd to lick the fetus. It is believed that this practice will increase milk production. As *Brucella* organisms are spread from aborted fetus or placenta to non-infected individuals that come in contact with it, this practice likely plays a key role in endemic disease spread.

Because herds of migratory yaks and chauries are grazed separately, there is little or no inter-herd contact. Thus, disease is not easily spread from one endemic pocket to another. Therefore it is possible that the overall prevalence of Brucellosis within a large geographical location might be low, but specific herds within that region might have a high prevalence of disease.

The occurrence of abortion among the animals studied in the Langtang Valley is lower than expected given the prevalence of brucellosis. There was only one association between a brucellosis positive chauri and an abortion incident. Further research is needed on the symptoms of brucellosis in chauries, as this observation is inconsistent with the usual pathology associated with brucellosis in cattle.

This preliminary study provides a foundation for further research. The study design was limited by monetary and time constraints. It could be enhanced by a larger sample size of multiple herds distributed over a larger geographic location, use of a random sampling technique, and incorporation of both the Milk Ring Test as well as serology for antibody detection.

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HYPERIMMUNE SERA PRODUCTION IN DOMESTIC AND LABORATORY ANIMALS AGAINST TOXOIDS OF *C. PERFRINGENS*

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ABSTRACT

Hyperimmune sera against Clostridium perfringens beta & epsilon toxoids was produced in animals and the level of antibody production was evaluated in these animals. Highest antibody titre of $\log^{10} \times 2.10 \pm 0.03$ was recorded in sheep on 21st day post inoculation of toxoid, while antibody titre of $\log^{10} \times 0.60 \pm 0.05$ was recorded in guineapig on the above date. Significant rise of antibody titre was observed from 14th day to 21st day post inoculation of toxoid. Antibody production did not vary significantly due to interaction between day of inoculation and toxoid. Higher level of immunity was produced in domestic animals than that of laboratory animals.

INTRODUCTION

Enterotoxaemia is an acute toxemia with intestinal infection by *Clostridium perfringens* (Hornitzky *et al*, 1989). *C. perfringens* is considered to be the most widely occurring pathogenic bacterium and has been isolated from enterotoxaemia in cattle of Bangladesh (Rahman *et al*, 1997). The organism is capable of liberating different toxins (McClane, 1992) of which beta and epsilon are the most lethal (El-Idressi and Ward, 1992). Hyperimmune sera is the only method of treatment of enterotoxaemia caused by *Clostridium perfringens* (Radositits *et al*, 1994) which is not available locally and need to be imported from foreign countries costing considerable foreign currency. Therefore, an attempt has been made to prepare the hyperimmune sera in animals using *Clostridium perfringens* beta and epsilon toxoids under local conditions of Bangladesh.

MATERIALS AND METHODS

The international reference preparations of *Clostridium perfringens* beta and epsilon toxoids obtained from International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England were reconstituted in 1.0 ml of distilled water. The entire contents of ampoule were completely resuspended in 18.0 ml of physiological saline containing 0.01% concentration of thiomersalate and 5.0 ml of sterile aluminium hydroxide gel (2%). These were thoroughly mixed and the reconstituted reference toxoid was transferred to 23.0 ml of diluted aluminium hydroxide, making a total volume of 25.0 ml. The trace of toxoid remaining in the ampoule was washed by transferring volume of the mixture to and from the ampoule several times using a Pasture pipette and mixing

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thoroughly after each transfer. The toxoid was allowed to absorb at room temperature for three days, shaking at intervals to ensure a homogenous suspension. The reference toxoid was further diluted in five-fold steps i.e. 1/5, 1/25, 1/125 in a diluent consisting of 1 part of aluminium hydroxide gel (2%) and 4 parts of physiological saline. This diluent was used as a constant proportion of adjuvant. The reconstituted toxoid was tested for safety and sterility as per the procedure of Annon (1992). Apparently healthy (disease free) 5-6 month old 12 sheep, 12 goat and 3-4 months old 12 rabbits and 12 guineapigs were used in this experiment. The animals (sheep, goats, rabbits, guineapigs) were allocated in 4 equal groups. An 2.5 ml of toxoid was inoculated subcutaneously in each of sheep and goat and 2.0 ml each to each rabbit and guineapig subcutaneously. Beta toxoid was inoculated in one group of sheep, goats, rabbits and guineapigs and epsilon toxoid was inoculated to second group of sheep, goats, rabbits and guineapig. Both beta and epsilon (1:1) toxoids combinely were inoculated to third group of sheep, goats, rabbits and guineapigs. Fourth group of sheep, goats, rabbits and guineapigs were maintained as control. Animals were maintained in separate cages in a well ventilated room and given sufficient balanced feed and fresh drinking water. Blood samples were collected on day 0, 14th and 21st day of toxoid inoculation. The serum collected from blood samples were stored at -25^o C. The antibody titres of sera samples were tested in mice by toxin antitoxin neutralization test as described by Rahman *et al.* (1996).

Statistical analysis of variance was computed according to Gupta (1983) to determine the difference among the intervals of days, toxoid and groups.

RESULTS

The antibody titre of hyperimmune sera in different animals produced by *Clostridium perfringens* beta and epsilon toxoids are shown in Table 1.

Table 1 Antibody titre of Hyperimmune sera in different animals produced by *Clostridium perfringens* beta and epsilon toxoids (n=3)

Animal Species	Day of observations	Toxoids inoculated	Antibody titre in $\log_1 0$	
			Mean	$\pm$ SE
Sheep	14 th day	Beta	1.80	0.12
		Epsilon	1.80	0.12
		Beta + Epsilon	1.50	0.12
	21 st day	Beta	2.10	0.03
		Epsilon	2.10	0.03
		Beta + Epsilon	1.80	0.03
Goat	14 th day	Beta	1.50	0.06
		Epsilon	1.50	0.06
		Beta + Epsilon	1.20	0.06
	21 st day	Beta	1.80	0.06
		Epsilon	1.80	0.06
		Beta + Epsilon	1.50	0.06

Animal Species	Day of observations	Toxoids inoculated	Antibody titre in $\log_{10}$	
			Mean	$\pm$ SE
Rabbit	14 th day	Beta	1.20	0.06
		Epsilon	1.20	0.06
		Beta + Epsilon	0.90	0.06
	21 st day	Beta	1.80	0.06
		Epsilon	1.80	0.06
		Beta + Epsilon	1.50	0.06
Guineapig	14 th day	Beta	0.90	0.05
		Epsilon	0.90	0.05
		Beta + Epsilon	0.60	0.05
	21 st day	Beta	1.20	0.06
		Epsilon	1.20	0.06
		Beta + Epsilon	0.90	0.06

N= No of animals in each toxoid inoculated group, SE= Standard error,
Significant at 1% level

Statistically significant ($P < 0.01$) rise in antibody production was recorded between 14th day and 21st day of inoculation. It was evident that the antibody titre of beta and epsilon toxoid were statistically similar independently but their mixture reduced the titre significantly. The antibody production did not vary significantly due to interaction between day of inoculation and toxoid used. Detectable antibody titre was not observed in preimmunising sera collected on day 0 and in control animals.

DISCUSSION

Bacterin has been used for longer period of time to prevent clostridial diseases in animals with minimum satisfactory result. Oxer (1971) reported that toxoids prepared from the potent *Clostridium perfringens* toxins induced better protection against clostridial diseases than bacterin. The results of the present study revealed that single dose with beta and epsilon toxoids induced satisfactory ($P < 0.01$) level of immune responses on 14th day and 21st day of inoculation although level of antibody was not satisfactory when both beta and epsilon toxoids were inoculated combinely. This might be due to antigenic competition of two toxoids used in combination. *Clostridium perfringens* induce better immunity in domestic animals than that of laboratory animals. Cameron (1980) and Dholakia *et al.* (1980) immunized sheep with *Clostridium perfringens* toxoid with satisfactory results. Tripathi *et al.* (1992) used neutralization test for antibody titre in animals. The immune response against *Clostridium perfringens* is a controversial subject and different workers have different claim (Ryskulov, 19983; Udovic *et al.*, 1983; Nilo and Cho, 1985), besides the fact that inactivated cultures and toxoids posses weak immunogenic properties (Ispolatovskaya, 1971). Ripley (1983)

used neutralization test in guineapig for antibody titre against *Clostridium perfringens* beta toxoid. Komkova (1944); Larina and Ispolatovskaya (1966); Vartholomew and Stringer (1983) successfully immunized rabbits and guinepigs. Verma (1986) injected subcutaneously aluminium hydroxide gel adsorbed toxoid of *Clostridium perfringens* type A and observed stronger immunity for longer duration. Corea and Taylor (1989) used *Clostridium perfringens* enterotoxin subcutaneously to rabbits for the production of hyperimmune sera. They successfully detected hyperimmune sera on 21st day of inoculation. The results of the present study are in agreement with these authors. Verma (1987) observed immune serum on 4th week of inoculation in rabbit with *Clostridium perfringens* type A.I. Pal *et al.*, (1990) used toxoid of *Clostridium perfringens* and the antibody titre of beta and epsilon toxoids were 5 and 3.3 IU/ml respectively on day 20. Hepple *et al.*, (1960) observed immune response in sheep at 4th week of vaccination. The difference in the results of the present study might be due to different factors like composition, pH of the medium, time of incubation, pathogenicity of the organism, intervals of the immunizing doses. (William and Chase, 1967; Macario and Macario, 1975; Gonzelez, 1984). Further work on the protective levels of antibody with virulent challenges of *Clostridium perfringens* needs to be carried out to investigate on these various aspects of hyperimmune sera production against *Clostridium perfringens* toxoids.

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ECONOMIC IMPACT OF CLINICAL MASTITIS IN BUFFALOES DURING LACTATION

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ABSTRACT

This study was conducted during 1998-99 to evaluate the efficacy of systemic vs. intramammary therapy against clinical mastitis and to analyse the expenditure on treatment, cost for extra labor and determination of milk losses due to Mastitis. In this study, 46% of the buffaloes were found to be affected with clinical mastitis with higher prevalence in first two months of lactations. Highest proportions of animal were affected with Coliform mastitis (65%) followed by Staphylococcal and Streptococcal mastitis (26%). Systemic therapy was found better in comparison to intramammary therapy. In present study, highest proportion of losses resulted from decreased milk production (Rs. 4287 or USD 63) per buffalo per lactation. Milk loss was found to be about 11% of the total lactation yield. Nearly 68% of the losses resulted from drop in milk production. Expenditure on medicine accounts for 1.4% of the total cost and nearly 6.5% of the total cost is needed for the Veterinarians fee. Salaries for the extra labor accounted for 12% of the total treatment and management cost. About 13% of the animals suffering from clinical mastitis were culled due to failure of treatment.

INTRODUCTION

Mastitis is considered as the most serious disease of buffaloes in Nepal. Mastitis in buffaloes causes severe economic losses resulting from decreased milk production and increased management costs.

Mastitis causes significant losses in milk production and changes the chemical composition, physical properties and microbiological quality of the milk. National Mastitis council (NMC) estimated the losses from reduced milk production alone amount to over 1 billion dollars in the United States annually with the total losses amount to almost 2 billion dollars. The cost to the average dairy farmer is approximately 180 dollars per cow per year. Recent research has revealed that mastitis accounted for 26% of the total cost of all dairy cattle diseases. Losses from mastitis were twice as high as losses from infertility and reproductive diseases. NMC has also confirmed that 70 to 80% of all losses are associated with sub-clinical mastitis while only 20 to 30% are due to clinical mastitis (Philpot and Nickerson, 1991). Most estimates in bovine shows that

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on an average an affected quarter suffers 30% reduction in productivity and an affected cow is estimated to lose 15% of its production. Quarters found to be infected in the late lactation causes 11% depression of milk yield after calving (Blood and Henderson, 1989).

In Nepal, Dhakal and Kharel, (1988) recorded that mastitis was one of the most important bacterial diseases recorded at Chitwan. Jha, *et al.*, (1993) reported that 53% of buffaloes were affected with clinical mastitis in eastern Nepal. Dhakal, (1996) reported that 61% buffaloes and 42% quarters were affected with clinical mastitis in Chitwan valley of Nepal. However, despite being an important disease problem, the economic analysis of the both sub-clinical and clinical mastitis has not been carried out so far in the country. Hence, the present study was undertaken to evaluate the losses caused by this disease in the lactating buffaloes in Chitwan valleys of Nepal.

MATERIALS AND METHODS

Study population

Hundred buffaloes kept at different villages of Chitwan district were identified for this study. History and relevant information such as age, lactation number, date of calving and date of collection of samples was recorded. The animals brought at Veterinary Teaching Hospital of Institute of Animal Science (IAAS), Rampur and District Veterinary Hospital, Chitwan and their sub-centers were the site for getting the samples for the study.

In addition, a questionnaire was developed for getting the information on mastitis regarding losses in milk production, rate of premature culling, cost of medicine and veterinary service for treatment, amount and cost of discarded milk, loss in ghee and butter production, cure rate of the animals following treatment and various other control measures adopted by the farmers.

Mastitis diagnostic tests

Mastitis was diagnosed using California mastitis test using the procedure adopted by Pandit and Mehta (1969). The reagent was prepared locally. The reaction was classified as negative, trace 1, 2 and 3 as strong positive.

Bacteriological examinations

Aseptically collected milk samples were streaked in blood agar plates and Mac Conkey agar plates. The resulting growth from the respective plates of the media was purified and identified on the basis of Gram's reaction, morphology and colony characteristics and some biochemical tests.

Clinical examinations:

The animals were examined clinically for the presence of clinical mastitis. Appearance of milk (colour and consistency) and absence or presence of other local sign of inflammation were examined as the signs of clinical mastitis.

Treatment of clinical mastitis

Buffaloes with positive samples were treated with the following drugs on the basis of the results obtained from the *in vitro* drug sensitivity test.

- a. Tilox (Ampicillin 75 mg and Cloxacillin 200mg) -1 tube intramammary daily for 5 days
- b. Gentim injection (Gentamicin sulphate 800 mg)-800 mg I/M bid in the first day followed by 800 mg I/M daily for next 4 days

The animals treated with above preparations were reexamined clinically and bacteriologically after 21 days of the last treatment day. The quarter was considered cured if it yielded no pathogens. A non-cure quarter was recorded if post treated milk samples yielded the infective organisms.

Economic analysis of mastitis

The data obtained from the questionnaire were processed to evaluate the economic impact of mastitis. Age, breed, lactation number, past history of mastitis, stage of lactation for mastitis, milk colour, milk yield/day before and yield/day after the cure of mastitis, amount of milk loss per lactation, etc. were recorded on the basis of the farmer interview. After getting the information or records from individual animals affected with mastitis the amount of milk losses per buffalo was calculated. The cost of the milk losses was determined. Veterinary services (fees) and treatment cost was noted. The requirement of extra labor because of mastitis was identified and their cost was recorded. Premature culling of the animals due to mastitis was also determined in this study. Among of ghee/butter production in mastitis buffaloes was identified in some of the cases.

RESULTS

Prevalence of clinical mastitis in buffaloes is presented in Table 1. In this study, 46% of the animals and 61% of the quarters were found to be affected with clinical mastitis.

Table 1 Prevalence of clinical mastitis in buffalo.

Parameters	Numbers tested	Numbers positive	Percentage
Mastitis buffaloes	100	46	46.00
Mastitis quarter	196	120	61.22

The survey showed that the prevalence of clinical mastitis was highest during the first lactation, which gradually decreases in the subsequent lactations (Table 2).

Table 2 Prevalence of clinical mastitis at different lactation

Lactation number	Positive number	Percentage
First	47	47
Second	21	21
Third	8	8
Fourth	8	8
Fifth and above	16	16
	100	100

It was also noticed that 57% of the buffaloes were affected with clinical mastitis during first two months of lactation, while between 2 to 4 months, the prevalence decreased to 21% (Table 3).

Table 3 Prevalence of clinical mastitis at different stage of lactation.

Months after calving	Animals affected	Percentage
Up to 2 months	57	57
2 to 4 months	21	21
4 to 6 months	9	9
above 6 months	13	13
	100	100

In this study, maximum number of buffaloes were affected with coliform mastitis (65%) followed by *Staphylococcal* and *Streptococcal* mastitis.

Therapeutic responses in clinical mastitis

Therapeutic responses against clinical mastitis was evaluated by using systemic or intramammary infusions. Systemic versus local therapy against mastitis pathogens is presented in Table 4. Maximum number of Coliform mastitis cases were cured by systemic administration of the drugs (73.33%) in comparison to intramammary therapy (26.66%). Similarly, 66.66% of the *Staphylococcal* and *Streptococcal* mastitis were cured by parental therapy. However, 33.33% of the cases were cured by intramammary therapy.

Table 4 Systemic therapy vs intramammary therapy against mastitis pathogens

S.N	Mastitis	Buff. No.	Cure rate by systemic therapy		Cure rate by intramammary therapy	
			No.	%	No.	%
1.	Coliform	30	22	73.33	8	26.66
2.	<i>Staphylococcal</i> & <i>Streptococcal</i>	12	8	66.66	4	33.33

Economic effects of mastitis:

The amount of milk loss due to mastitis was calculated on the basis of the data obtained from the farmers. The loss in milk production in different lactation is presented in Table 5. Maximum amount of the milk was lost in the fifth lactation (24.54%) buffaloes followed by second lactation (13.32%) and first lactation (8.68%).

Table 5 Milk production losses in mastitis buffaloes in different lactations.

Lactation number	Average milk loss (in litres)	Percentage milk loss per lactation
First	167.54	8.68
Second	256.90	13.32
Third	26.00	1.34
Fourth	82.00	4.25
Fifth	437.25	24.54

Analysis of expenditure on the treatment and management of clinical mastitis is presented in Table 6. Average cost of medicine for the treatment of clinical mastitis in a buffalo was estimated to be Rs. 872/- (USD 13) and veterinarian fee per cases was Rs. 420/- (USD 6). Similarly extra cost for the labor due to mastitis problem was found Rs. 750/- (USD 11).

Table 6 Analysis of expenditure on the treatment and management of clinical mastitis.

1.	Cost of Medicine/animal	Rs. 872.91	USD. 13
2.	Veterinarian fee/animal	Rs. 420.58	USD. 6
3.	Salary for the extra labor at the time of mastitis	Rs. 750.00	USD. 11

Similarly, loss in milk yield due to clinical mastitis has been found to be about 214.35 litres per lactation per buffalo, which is equivalent to Rs 4287/- (Us 63). In addition, it was also recorded that nearly 29% of the fat was reduced due to clinical mastitis in buffaloes. The losses in milk production due to mastitis has been presented as Table 8.

Table 8 Effect of clinical mastitis on milk production.

1.	Average milk yield of normal Murrah buffaloes	1928 litre/lactation*
2.	Average reduction of milk yield per day during treatment	0.79 litre
3.	Total milk losses per buffalo	214.35 litre
4.	Economic loss due to milk reduction/ buffalo/lactation	4287 (USD. 63)
5.	Premature culling rate	13.04%
6.	Reduction of the fat due to clinical mastitis	29.16%

*Source: Kolachhapati et al. 1990

About 13.04% of the total animals suffered from clinical mastitis have been culled due to the failure of treatment. Mastitis may result in culling due to repeated cases, peracute cases and lower production.

Effect of clinical mastitis upon fat percent of milk

Percentage fat reduction has been noticed in 29.16% of the clinical cases in buffaloes and the overall fat is reduced by an average of 42.72%. According to the result of most investigators, the fat content decreased by less than 10% in the cows. The fat composition however, changes considerably lowering the quality of milk products. The total amount of fatty acids remains unchanged but the quantity of free fatty acids increases. The quantity of unsaturated long chain fatty acid is however higher in mastitis milk than in normal milk.

DISCUSSION

This study is only focused on the evaluation of the drugs by systemic vs. intramammary therapy and some economic analysis of the milk production losses due to clinical mastitis.

In this study, 46% of the buffaloes and 61% of the quarters were affected with clinical mastitis in Chitwan valley of Nepal. Lower infection rate were recorded at western Nepal by Joshi and Shrestha, (1995). Prevalence of clinical mastitis was highest at first lactation buffaloes and most common in the first two months of lactation. Similar reports were made by Joshi and Shrestha, (1995).

There is no standard treatment for mastitis, each infection requires a specific treatment. The advantage of systemic treatment is efficient distribution of the drugs in the infected udder. In acute mastitis, absorption of intramammary drugs is ineffective as a result of swelling and secretions blocking the milk duct. Intramammary treatment can also disturb phagocytosis in the udder. In the present study, systemic therapy was found better for the treatment of Coliform, *Staphylococcal* and *Streptococcal* mastitis in comparison to local intramammary therapy. The disadvantage of local application of antibiotic is the slow and uneven distribution of certain drugs in the infected udder.

Intramammary treatment is most common method of treating bovine mastitis, however such therapy usually results in clinical cure but bacteriological cure rate is low (Plommet and Lelaedec, 1975). In acute mastitis, there is a failure of intramammary antibiotic therapy due to poor or uneven distribution of the drugs throughout the swollen udder parenchyma and blockage of milk duct system by inflammatory products. In *Staphylococcal* mastitis, *Staphylococci* are invading the tissues so the intramammary therapy is unable to gain access to organisms. For systemic mastitis therapy, effective passage of the drugs from blood into and near the foci of infection must be achieved.

Passage of the drugs across the blood to milk barrier takes place by passive diffusion (Miller *et al.* 1967).

Mastitis in buffalo causes severe economic losses mainly from decreased milk production but also from increasing management costs. In this study, largest proportion of losses i.e. Rs. 4,287/- (USD 63) per buffalo per lactation results from decreased milk production. The milk loss was found 11.11% of the average total lactation yield in a period of 270 days. Nearly 68% of the total losses results from drop in milk production. Most of the milk losses occur due to reduction in secretion of milk and part of milk losses occurred due to withdrawal of milk during mastitis. However most of the farmers are not aware of the withdrawal of the milk during and after treatments of mastitis. There is no system of evaluating milk quality, drug residues and withdrawal period of the milk after local or parental therapy. Singh and Myllys (1995) reported lower reduction milk yield (50%) and economic loss due to clinical mastitis results, Rs. 2345/- I.C. (USD 56)/buffalo/lactation. However, in cows in abroad, milk yield losses accounts approximately 70 to 85% of the total loss in most of the studies (Lighner *et al.*, 1988).

The expenditure on medicine accounts for about 34.23% of the total treatment and management cost. Nearly 14.26% of the total cost was needed for the veterinarian's fee. Similarly, Sharma *et al.* (1987) in India reported that 35.34% of the total cost was involved in medicine for the mastitis in dairy cattle. However, Morin *et.al.* (1993) in USA indicated that mastitis treatment cost and veterinarian's fees accounted 2 to 12%.

Salaries for extra labor for the time of mastitis accounts for 49.19% of the total treatment and management expenditure. Higher percentage (62.48%) of the cost for labor was reported by Sharma *et al.* (1987) in dairy cattle in India.

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Research article

HEMORRHAGIC SEPTICEMIA IN BOVINES: STUDIES ON EPIDEMIOLOGY &
CONTROL STRATEGIES IN THE WESTERN HILLS OF NEPAL

B.R. Joshi¹ and H.D. Joshi²

ABSTRACT

Haemorrhagic Septicemia (HS) has been regarded as one of the important diseases of bovines in Nepal causing considerable economic loss. Outbreaks of this disease has been recorded in all seasons in the western hills of Nepal with relatively higher frequencies during the summer season. Mortality in the affected cases was recorded to be about 20 percent. Pasturella multocida of serotype B:2 was isolated from all investigated outbreaks. The protective immunity of locally produced alum precipitated vaccine was found to be for three months, thus grazing animals will need to be vaccinated at three monthly intervals. The strategic period for vaccination would be at the beginning of the summer and autumn seasons and oil adjuvant vaccine would be better than alum precipitated vaccine for preventive vaccination due to its longer immunity.

INTRODUCTION

Haemorrhagic Septicemia (HS) is an acute, highly fatal disease of cattle and buffaloes caused by *Pasteurella multocida* (De Alwis, 1984). In Nepal, HS has been reported from all parts of the country throughout the year. A conservative estimate shows that HS causes an annual loss of Rs. 40.8 million to Nepalese livestock industry (Lohani and Rasali, 93/1995). Economically, HS is regarded as one of the important diseases of farm livestock and is a serious cause of concern for farmers as the infected animals generally succumb to death. In Nepal, outbreak reporting are based on clinical symptoms resembling of HS shown by animals and not followed with confirmatory diagnosis of the disease but with ring vaccination of the animals around the outbreak area. Presumptive diagnosis of HS is often made on the presence of clinical signs of throat and neck swelling and high temperature. Similarly, isolation of *P. multocida* from animals with typical clinical signs of HS has not been attempted or been successful in the past, hence some workers have doubted the presence of this disease in the hill regions of the country. As the disease has been suspected only on clinical symptoms without isolating the causative organism, it was necessary to identify the disease properly and characterize the causative agents for this disease. Moreover, it was

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also important to study and understand the epidemiology of this disease in the hill environment so that preventive strategy for its control could be developed and implemented. Hence, this study was conducted to understand the clinical significance and epidemiology of HS in the hill districts of western development region of the country between the year 1998 to 2001.

MATERIALS AND METHODS

This study was conducted under following three components.

1. Outbreak investigation of HS in the 11 hill districts of western development region.
2. Isolation and serotyping of the isolated organisms involved in the outbreaks.
3. Evaluation of the responses of buffalo calves vaccinated with alum precipitated vaccine.

This vaccine is produced within the country and used extensively for the prevention of HS.

Outbreak Investigation

The number of HS outbreaks reported in 11 hill districts of western development region were analysed for three years to understand the pattern of disease in these districts. The outbreak records were collated from the disease epidemiology record of the districts. Some of the outbreaks in the nearby villages were investigated in detail by visiting the outbreak area, collecting the pathological samples from the sick or dead animals and collecting detailed information on disease outbreak pattern and its distribution pattern using survey questionnaire.

Isolation, identification and characterisation of causative organism

Sample collection

A total of 7 reported HS outbreaks were investigated and samples (long bones of dead animals and blood and nasal swabs from sick animals) were collected from 19 buffaloes and two cows. Samples were transported to Veterinary Investigation laboratory of ARS, Lumle under cool condition. Samples collected from different areas were as follows (Table 1).

Table 1 Samples collected from animals in different areas

Districts	Village	Animals species		Type of sample
		Cows	Buffaloes	
Kashi	Kaski Kot	-	1	Nasal swab, long bone
	Lumle	-	2	Long bone
	Bhichok	-	7	4 Nasal swabs, 3 long bones
	Pumdi Bhumdi	-	2	Blood, long bone
	Dhampus	-	1	Long bone
	Dansing	1	2	Long bone 4 blood
Parbat	Deopur	1	4	Blood, long bone
	Total	2	19	

Isolation and identification of *P. multocida*

Aseptically collected bone marrow and blood samples were plated on blood agar plates and incubated at 37° C for 24 hrs. Blood smears were stained by Giemsa stain and examined for bipolar microorganisms. After 24 hrs of incubation, the blood agar plates were examined for typical *P. multocida* colonies. The confirmation of *P. multocida* was done by biochemical tests using API kits.

Capsular typing of *P. multocida*

Indirect haemagglutination (IHA) test procedure described by Carter (1955) with some modifications described by Wijewardana (1992) was followed for capsular typing of the isolated *P. multocida*. Hyperimmune rabbit antiserum used in the test were obtained from FAO Regional laboratory (Asian region) Peradeniya, Sri Lanka. The procedure consists of following steps:

Preparation of antigen for IHA test

P. multocida, reference strains and recovered isolates of HS from cows and buffaloes were plated on blood agar plates and incubated at 37° C for 18-24 hours. The growth on each plate was examined by rapid slide agglutination with type B antiserum. The growth was then harvested using 2ml of 0.3% formal saline per plate. The bacterial cell suspension was centrifuged at 8000 rpm at 4° C for 15 minutes. The supernatant was removed and sediment was re suspended with normal saline (NS) to obtain opacity of 25 times that of Wellcome opacity tube No 6, which contains approximately 10^8 microorganisms per ml. to obtain this concentration, one part of the cell suspension was mixed with 24 parts of the saline solution. One part of this standardised suspension was further diluted to 1:3 times by addition of normal saline. Then, diluted suspension was heated at 56° C for 30 minutes in a water bath with frequent shaking. Heat-treated suspension was centrifuged at 8000 rpm for 15 minutes at 4° C. The clear supernatant was separated and stored at -20° C and used as capsular antigen.

Preparation of sheep erythrocytes (RBC)

Sheep blood was collected aseptically in EDTA vacutainer tube by jugular vein puncture. The blood was centrifuged at 2000 rpm for 5 minutes. Supernatant was removed and sediment (packed cells) was washed 3 times with NS by centrifugation and supernatant was removed after centrifugation. The washed RBCs were sensitised by mixing one volume of the packed RBCs with 15 volume of the antigen and kept for 30 minutes at 37° C in a water bath with frequent shaking. The cells were washed 3 times with NS by centrifugation at 2000 rpm for 5 minutes. Then suspension of sensitised RBCs was prepared in NS to obtain 1% concentration. All antisera were inactivated at 56° C for 30 minutes. These sensitised RBCs were absorbed with all known types of antiserum (A, B, C, D, E and F), for which 3 volume of each antiserum was mixed with 1 volume of washed packed sheep RBC and kept for 2 hrs at 37° C with periodic shaking. Then absorbed serum was centrifuged at 65 rpm for 2 minutes. Absorption was carried in the micro centrifuge.

Indirect Haemagglutination test procedure

The test was performed in 96 well round bottom microtiter plates. 160 µl NS was dispensed in each well in the first column and 100 µl of NS to other wells. Inactivated and absorbed serum (40 µl) was added in the first column wells. The serum was mixed and 100 µl of the mixed serum was transferred to penultimate wells. Last well was left as control without serum. Then 100 µl of 1% sensitised RBCs suspension was added to all wells. A negative control row was made with unsensitised RBCs. The plate was shaken and left for 2 hrs at room temperature.

Any type of agglutination of RBCs was considered as positive and no agglutination or a small button of deposited RBCs was regarded as negative. All serotypes were further reconfirmed at FAO Regional Reference Laboratory, Sri Lanka.

Somatic typing of P. multocida

The somatic typing method was adopted from Heddlestone *et al.*, (1972). The reference antiserum were obtained from FAO Regional Reference Laboratory, Sri Lanka. The test procedure consists of following steps.

Preparation of Heddlestone's antigen (H)

The 24 hr growth from a heavily inoculated dextrose Starch Agar (DSA) plate was harvested in 1.0 ml of solution containing 0.02 M phosphate buffer, 8.5% NaCl, 0.3% formalin (pH 7.0). This cell suspension was standardised to 25 times that of Wellcome opacity tube no 6 (1 drop of suspension with 24 drops of normal saline). The suspension was heated in a boiling water bath for 1 hr and centrifuged to sediment the cells. The supernatant was labelled as "H" antigen and stored at -20° C.

Test Procedure

The test was performed on the glass slides in which molten agar was poured and wells of three patterns of 7 wells (6 peripheral and 1 central) were cut on each slide. The diameter of a well was 4 mm, while the distance between the wells was 6 mm. The 16 antisera were placed in outer wells and the antigen was placed in the central well. Each antigen was tested against 16 antiserum. The slides were incubated for 18-24 hrs at 37° C in a humid chamber and examined against a dark background. A precipitation line appearing between the antigen well and the corresponding antiserum was considered as specific homologous reaction.

Determination of vaccination response in buffalo calves

The vaccination response of buffalo calves was studied in 13 unvaccinated buffalo calves aged between 6-9 months. Ten buffalo calves were vaccinated sub-cutaneously with 5 ml alum precipitated HS vaccine (Batch No. 2. 2057, Biological Products Division, Tripureswor, Kathmandu) and 3 buffalo calves were left as unvaccinated controls. Serum samples were collected from all animals on day 0 before vaccination and at day 7, day 14, day 30 and then at monthly intervals for up to 6 months post vaccination. At 6 months, all previously vaccinated calves were again revaccinated with a booster dose of vaccine and serum samples were collected from the vaccinated and unvaccinated animals to monitor the anamnestic response post vaccination at 7, 14 and 30 days post booster vaccination. In the serum samples, antibody titre of *P. multocida* was monitored by Indirect Haemagglutination Test (IHAT) using the vaccine strain of *P. multocida* organisms according to the method described by Wijewardana (1992).

Mouse Protection test

Serum samples collected from 10 vaccinated and 3 control animals were tested for the presence of mouse protection antibodies. Serum samples were grouped as serum with IHA titre, no IHA titre and serum from unvaccinated animals. Groups of 4 mice were injected sub cutaneously with 0.5 ml of each serum. Each mouse was challenged 24 hours later with intraperitoneal injection of 0.1 ml suspension of a 10⁻⁶ dilution of an 18-hrs nutrient broth culture of *P. multocida*. This procedure was adopted for serum samples collected at different intervals from the vaccinated and unvaccinated animals. Survival of any mice in the group at 48-hrs post challenge was recorded and was regarded as having the presence of protective antibodies in the serum.

RESULTS

HS outbreaks in the hill districts

The number of HS outbreaks reported in 11 hill districts during 1998-2001 is presented as Table 2. The number of outbreaks reported from these districts were based on the field reports from the field staff and unconfirmed by laboratory investigations.

Hence, the reported outbreaks would represent their trend rather than their actual number occurring in the districts.

Table 2 The number of HS outbreaks reported from the Western hill districts between 1998-2001.

Year	Districts										
	Arghakhanchi	Baglung	Gorkha	Kaski	Lamjung	Myagdi	Palpa	Parbat	Syangja	Tanahun	Gulmi
1998-99	11	1	4	17	2	2	2	-	5	10	-
1999-00	2	1	-	28	-	2	-	2	8	18	11
2000-01	2	1	-	28	-	2	2	10	13	3	-

Seasonal distribution of the outbreaks

Seasonal distribution of the outbreaks was analysed for the year 1998-2000 but not for the year 2000-2001. The seasonal distribution of the outbreaks is presented in Table 3. From the table, it is evident that higher proportions of the outbreaks occur during the summer season (March-May) followed by rainy and autumn season with least number of outbreaks recorded during the winter season (December- February).

Table 3 Seasonal distribution of HS outbreaks in different districts

Districts	Total number outbreaks	Seasonal distribution of the outbreaks			
		Summer (Mar-May)	Rainy (June-Aug)	Autumn (Sept-Nov)	Winter (Dec-Feb)
Arghakhanchi	13	9	2	1	1
Baglung	2	-	2	-	-
Gorkha	4	3	-	-	1
Kaski	45	8	20	17	-
Lamjung	2	-	-	1	1
Myagdi	4	2	-	2	-
Palpa	2	-	-	2	-
Parbat	2	-	2	-	-
Syangja	13	12	-	-	1
Tanahun	28	14	2	4	8
Gulmi	11	8	-	3	-
Total	126	56 (44.4)	28 (22.2)	30 (23.8)	12 (9.5)

(Note: The figures in the parenthesis indicate percentage of the total outbreaks)

Sequence of outbreak in some areas

On analysing the sequence of outbreak in some of the reported areas, it was found that in some of the villages, the outbreaks are reported more frequently. This repeated outbreak sequence in a particular area is presented in Table 4.

Table 4 Sequence of HS outbreaks in some of the villages

Districts	Reported area	Sequence of reported outbreaks			
		Ist outbreak	IInd outbreak	IIIrd outbreak	IVth outbreak
Baglung	Adhikari Chaur	July, 1997	August, 1998		
Baglung	Kalika, Mulpani	July, 1996	August, 1996	Feb., 1997	March, 1997
Kaski	Bharat Pokhari	Nov., 1996	August, 1997		
Kaski	Lumle	June, 1996	July, 1997		
Gorkha	Taranagar	July, 1996	Sept., 1996	Nov., 1996	June, 1997
Tanahun	Dulegauda	July, 1996	April, 1997	May, 1997	
Tanahun	Bhanu	May, 1997	June, 1997		
Tanahun	Purkot	Dec., 1997	May, 1998		
Arghakhanchi	Thada	July, 1996	Feb., 1997	March, 1997	
Lamjung	Chiti	Janu., 1997	May, 1997		
Myagdi	Ghatan	May, 1997	June, 1997		
Myagdi	Arthunle	May, 1997	June, 1997		
Myagdi	Ratnechaur	Feb., 1997	March, 1997		

In these repeated outbreaks, it is difficult in some cases, to ascertain whether the outbreaks were the new outbreaks or the continuation of the same outbreak in a nearby area. It was particularly difficult when the outbreak was reported in a nearby village during the following month of the previous outbreak. Hence, Some of the reported outbreaks might be the continuation of the same outbreak but was reported as two outbreaks. However, in some of the other cases, the gap between the two outbreaks was long and distinct to be recorded as a new outbreak.

Animal mortality in the outbreaks

The mortality pattern of affected animals in the outbreaks is presented as Table 5.

Table 5 Mortality pattern of affected animals in the HS outbreaks during 1998-2001.

Districts	No of outbreaks	Total affected	Total died
Arghakanchi	15	59	2 (3.4%)
Baglung	3	6	6 (100%)
Gorkha	4	26	8 (30.7%)
Kaski	73	118	63 (53.4%)
Lamjung	2	496	5 (1.0%)
Myagdi	6	7	3 (42.8%)
Palpa	4	533	69 (12.9%)
Parbat	12	132	26 (19.7%)

Districts	No of outbreaks	Total affected	Total died
Syangia	26	218	30 (13.8%)
Tanahun	31	184	149 (80.9)
Gulmi	11	31	0 (0.0%)
Total	187	1810	361 (19.9%)

(Source: Central Epidemiology Unit, Tripureshwor, Kathmandu)

Note: Figures in parenthesis represent percent mortality.

From the above table, the mean mortality in these outbreaks was found to be about 20% of the affected cases, however, the mortality in individual outbreaks ranged from 0% to 100%, which again shows the poor recording of morbidity and mortality in disease outbreaks.

Serotypes of isolated organisms from the outbreaks

The serotypes of *P. multocida* isolated from different areas are presented in Table 6. A total of 18 samples were subjected for bacteriological examination. *P. multocida* was isolated from 12 samples. Out of 4 blood samples, *P. multocida* was isolated from two samples only. Similarly organisms were isolated from two nasal swabs from the 6 swabs subjected to bacterial culture.

Table 6 Isolated serotypes from HS outbreaks

Districts	Area	Animal Species	Number of animals	Type of samples	Number of samples	Total isolates	Identified serotypes
Kaski	Kaski Kot	Buffalo	1	Nasal swab	1	1	B:2
	Lumle	„	2	Nasal swab	1	1	B:2
				Bone marrow	1	1	B:2
	Bhichok	„	7	Nasal swab	4	0	No growth
				Bone marrow	3	3	B:2
	Dhampus	„	1	Blood	1	1	B:2
	Dansing	Cow	1	Bone marrow	1	1	B:2
	Pumdi	Buffalo	2	Blood	1	1	B:2
	Bhumdi			Bone marrow	1	1	B:2
Parbat	Deopur	„	4	Blood	2	0	No growth
				Bone marrow	2	2	B:2
Total			18		18	12	

P. multocida was isolated from all bone marrow samples. Blood smears stained with Giemsa stain showed bipolar microorganisms with short coccobacilli.

IHA titre in vaccinated and unvaccinated calves

Serum samples collected from 13 buffalo calves were tested for the detection of IHA titre up to 6 months post vaccination. IHA test results are presented in Table 7.

Table 7 Percentage of buffalo calves showing IHA titre*

Groups	Number of animals	Percentage of buffalo calves showing IHA titre								
		0 day	7 day	14 day	1 month	2 month	3 month	4 month	5 month	6 month
Vaccinated	10	0	40	40	40	30	20	10	10	10
Un-vaccinated	3	0	0	0	0	0	0	0	0	0

* The dilution of serum samples was tested at 1 in 10 onwards.

None of the buffalo calves showed IHA titre on zero day (before vaccination). IHA titres were detected in 40% of the vaccinated animals only. In 20% of the serum samples, the titre remained up to 3 months post vaccination at 1 in 40 dilution. The titres were found up to 1 in 80 dilution on 7th and 14th day post vaccination. Then the titre dropped to 1 in 20 and 1 in 10 dilutions in most of the animals. No IHA titre was detected in 60% of the vaccinated buffalo calves throughout the monitoring period and in unvaccinated animals.

Passive mouse protection test

The result of passive mouse protection tests as the percent of mice survived at 48 hours post challenge is presented in Table 8.

Table 8 Percent of mice survived at 48 hours post challenge

Type of serum sample	Percent of mice protected (4 mice challenged)									
	First vaccination							Booster vaccination		
	0 day	7 days	14 days	30 days	60 days	90 days	above 90 days	7 day	14 day	45 day
Serum with no IHA titre	0	25	50	50	50	75	0	100	75	100
Serum with high IHA titre	0	25	25	25	25	75	0	50	75	75
Un vaccinated	0	0	0	0	0	0	0	0	0	0

The result showed that there was no difference in the protection ability of the serum samples with IHA titres or without IHA titre in the vaccinated animals while, no protection was provided by unvaccinated serum samples.

DISCUSSION

Haemorrhagic Septicemia is a serious disease of farm animals in Nepal with considerable economic loss. Outbreaks of this disease in all hill districts of the western development region and in all seasons of the year indicates its wide spread and scattered occurrence. It has been reported that Septicemic Pasturellosis (HS) occurs in outbreaks during the period of environmental stress caused by wet and chilly weather

and exhaustion from heavy work, which increases the susceptibility of the animals for the disease with infection caused by ingestion of contaminated foodstuffs originating from clinically normal carriers which constitute about 45% of the population in the disease endemic areas (Blood and Radostits, 1989). The contact between the susceptible animals and the carriers or infected animals need to be close and immediate as the organisms do not survive on the pasture for more than 24 hours (Bain, 1963). In the hills of Nepal, the outbreaks reported in all seasons indicate that the immune status of animals might have been compromised in all seasons due to various factors. These factors include poor feeding during winter and early summer months, work stress in working animals during autumn and summer months and exposure to the extreme weather during winter months. It was reported that carrier state in the animals remains in latent and active form and the organisms could be isolated in the tonsils for long periods after infection (De Alwis *et al.*, 1990). The organisms reappear intermittently in the nasopharynx of the healthy carriers, which becomes a source of infection to the incontact susceptible animals.

In the hills of Nepal, although, the seasonal distribution of outbreaks was not distinct, yet occurrence of 44 percent of the reported outbreaks during summer season (March-May) indicate that animals might have been most heavily stressed during this season. It is however, also possible that, the higher number of outbreaks during the summer months was perhaps related to the increased contact between the animals during the fallow grazing season after the harvest of summer crops. The nutritionally stressed animals exposed to the active carriers thus could pick up the infection leading to disease outbreak during this season. The outbreaks during the rainy season might be the extension of the summer outbreaks in the same or in the adjoining areas. Similarly, the increased contact between the animals during autumn and winter grazing might lead for the outbreaks during these seasons. This situation will not however, be applicable for animals managed under continuous grazing management, where other stress causing factors will govern the outbreak of disease.

One of the characteristic features of these outbreaks was the lower morbidity and mortality than that reported in the texts (Blood and Radostits, 1989). Similarly, for enzootic areas of Sri Lanka, mortality in buffaloes was recorded to be 29% (De Alwis, 1981). The lower morbidity and mortality recorded in these outbreaks might be due to the smaller herd size reared by individual farmers and the reduced frequency of contact between the animals. Increase in the stall feeding management and the reduction in the area for grazing pastures might also have contributed for reducing the chances of contact between the animals.

The occurrence of outbreaks in all seasons also suggests that the carriers can transmit the disease to the susceptible population throughout the year. Any specific precursors for the development of disease in carrier animals or its transmission could not be identified. However, the increased frequencies of outbreaks recorded during the

summer season suggest that the causative factors might be the increased contact between the susceptible animals during the season.

All reported outbreaks were not investigated in detail, and isolation of the organisms was attempted in some outbreaks only, however, isolation of the organisms was not successful in all sampled cases. The difficulty of isolating *P. multocida*, from clinical cases and nasal swabs has also been reported by Wijewardhana, (1992) which was also observed in the present investigation. Despite this, isolation of *P. multocida* from 66% of the sampled cases confirms the presence of HS in the hills of Nepal. This finding also clears the doubt that HS might not be present in the hills as has been suspected by some workers. It was also found that all the isolates recovered in these outbreaks belong to one serotype B:2 (6:B), which has also been recorded in other parts of south Asia (De Alwis, 1984). All these isolates were found to be pathogenic to mice. The study confirms that *P. multocida* serotype B:2 causes HS in buffaloes and cattle in the western hills of Nepal.

The number of outbreaks reported in each district was quite variable, with some districts showing very high number of outbreaks and others very low. This situation perhaps reflects the state of disease reporting system in the districts rather than the pattern of outbreaks. It is also possible that all outbreaks were not reported in some districts while in others the continuation of one outbreak might have been reported twice as separate outbreaks. This inconsistency in outbreak reporting clearly reflects the need to standardise the reporting system and training for the district veterinary officials on disease reporting and outbreak investigation.

The responses of buffalo calves to the locally produced alum precipitated vaccine showed that there was no relation of the level of IHA titre with the protective immunity of the animals, as serum with high IHA titre or without detectable IHA titre have similar level of protective immune response. None of the serum samples had mouse protective antibodies before the vaccination or after 90 days of vaccination. Similarly, none of the unvaccinated animals have any protective antibodies. Although, IHA titre was undetectable in many animals, yet mouse protective antibodies were present in these serum samples for up to 3 months. It is thus apparent that the protective antibodies and the IHA antibodies are different in animals. It was thus obvious that determination of IHA titre might not be the useful tool for evaluating the protective response of the vaccines. Although, high IHA titre has been reported as a good indicator of natural exposure and solid immunity, IHA titre to vaccination has been reported to be poor (De Alwis *et al.*, 1987 cited by De Alwis *et al.*, 1990), which was also recorded in the present study. It was also evident that the protective immunity lasted only for three months in vaccinated animals. Survival of any mice in the test group indicate an immune serum (OIE, 1916), thus it is evident that in the vaccinated animals, protection was offered from day 7-post vaccination till 90 days. This finding is in agreement with that of De Alwis *et al.*, (1981) from Sri Lanka, who reported that the alum-precipitated vaccine gave protection for 3-4 months and the oil adjuvant

CONCLUSIONS

- The following are the conclusions drawn from this study:
- Hemorrhagic septicemia is a serious disease of economic significance in the hills of Nepal.
- The outbreaks of the disease occur throughout the year with higher number of outbreaks during the summer seasons.
- Mortality of the affected animals is around 20% of the affected cases.
- The causative organism of this disease is *Pasturella multocida* of serotype B:2.
- The available alum precipitate vaccine can protect the buffaloes for 3 months only.

RECOMMENDATIONS

The following recommendations could be made from the findings of this study:

Animals should be vaccinated against HS during early summer and then during the early autumn months.

Alum precipitated vaccine should be repeated at three monthly intervals in the grazing animals, hence vaccination with oil adjuvant vaccine should be used for preventive vaccination programmes.

More research should be focussed on the development of other approaches as well as for the development of more potent novel vaccines for better management of this disease.

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STRATEGIC DRENCHING AGAINST FASCIOLOSIS IN CATTLE IN THE WESTERN HILLS OF NEPAL

H. K. Shrestha and H. D. Joshi¹

ABSTRACT

A study was carried out to evaluate the effectiveness of strategic drenching against fasciolosis in cattle in the western hills of Nepal. Thirty Fasciola infected cows in each of the four villages (Pakuwa in Parbat and Kundule in Baglung district in the mid hills, and Bhendabari and Sisuwa in Kaski district in the low hills) were randomly allocated in two groups. Animals in the treatment group were drenched with 2.5% Albendazole suspension at 12 mg per kg body weight (110-130 ml per cow) once in November and second time in February and the animals in the other group were left untreated. Faecal samples were collected at monthly intervals, and were examined by standard sedimentation method for the presence of Fasciola eggs. The strategic drenching reduced the overall infection in treated animals. In the mid hills, the strategic drenching was very effective in controlling the infection for the whole year. However, in the low hills, it was not as effective in controlling the infection as was in the mid hills. The likely reason for this difference could be the difference in animal management system influencing the severity and frequency of the infection at the two altitudes. The effect of strategic drenching on the body condition of cattle was significant only in the low hills for February drenching. Farmers' response to the strategic drenching, in general, was good because of the improvement in body condition and milk production in the animals. Study on economic benefits by strategic drenching of cattle in different agro-ecological zones is the further area of research

INTRODUCTION

Fasciolosis is one of the major disease of cattle causing serious loss in production and productivity in these animals. Fasciolosis, locally known as 'Mate' or 'Namle', alone causes an estimated annual loss of Rs 146 million in these animals (Lohani and Rasali, 1995). Eckert and Eichenberger (1970) have reported that the *Fasciola* infected cows produced 10 to 40 % less milk than uninfected cows, and 7.5 to 24 % weight loss in infected calves. The disease prevalence in different parts of the country has been reported to be more than 50 % in cattle and buffaloes (Lowcock, 1982; Joshi, 1986). Previous studies has identified the disease prevalence to be mainly affected by the

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availability of Khetland, paddy cultivation and permanent water sources rather than altitude (Joshi, 1988). Rice straw, which is the major feed for livestock during winter months has been reported as the major source of *Fasciola* infection for stall fed animals (Joshi, 1987; Mahato, 1993). Green grasses from near permanent water sources or water lodging areas in monsoon are another potential source of *Fasciola* infection. Therefore, in the Nepalese hills, the major risk period for *Fasciola* infection is during post monsoon and winter months (September to February).

The general practice to control fasciolosis in Nepal is to drench animals twice a year with a fasciocide. Farmers drench their animals when they are clinically ill, and by that time the production has already been adversely affected. Obviously, this could not be the cost-effective way of controlling the disease. Proper timing of drenching based on the local epidemiology of the disease is necessary. A strategic control plan by drenching cattle in November (to control infections from grasses around the sources of water) and February (to control infections from feeding fresh rice straw) was formulated as technological intervention. This experiment, therefore, was undertaken to verify the effectiveness of the above drenching schedule against *Fasciola* infection in cattle in the western hills of Nepal.

MATERIALS AND METHODS

The study was conducted from October 1994 to December 1995 in four villages of Lumle Agricultural Research Centre (LARC) research command area (RCA). They are- Pakuwa (Parbat district) and Kundule (Baglung district) in the mid hills, and Bhendabari (Dhital, Kaski district) and Sisuwa (Kaski district) in the low hills. These sites were selected as representative of mid and low hills, and based on the cattle population in the area.

Faecal samples of more than sixty lactating or pregnant cows from each of the four villages were collected and examined for the presence of *Fasciola* eggs by the standard sedimentation technique (MAFF, 1986). Cattle with *Fasciola* eggs in faeces were regarded as infected. Among the *Fasciola* infected cattle, pair matching was done based on parity. From these, fifteen pairs were randomly selected, and were again randomly allocated into two groups, namely, treatment and control groups, each having 15 animals.

Albendazole suspension (2.5 % w/v) which was reported to be effective against fasciolosis, and without any side effects, and commonly available in Nepalese market, was selected as the anthelmintic drug for drenching. The anthelmintic was administered at the recommended dose rate of 12 mg/kg body weight (i.e., 110- 130 ml per adult animal) once during second half of November 1994 (first drenching) and the other during second half of February 1995 (second drenching) to the animals included in the treatment group. For the animals in control group, a placebo (diluted mineral mixture suspension) was administered during the same time, and were kept under farmers management.

Faecal samples were collected at monthly intervals from cattle in both groups, and transported to the Veterinary Investigation Laboratory, LARC. Standard sedimentation technique (MAFF 1986) was used to examine the faecal samples. Animals with *Fasciola* eggs in faeces were considered positive for *Fasciola* infection.

Body condition scoring (1-9 scale) of the experimental animals were done as per the guidelines of Nicholson and Butterworth (1985) four times just before November 1994 drenching, mid-December 1994, before February 1995 drenching and mid-March 1995.

A questionnaire survey was conducted at the end of the trial period to record the feeds provided, and their sources for each month of the period of experiment.

The detailed observation on each cow, results of faecal examination and fortnightly milk production of each cow were recorded. Farmers' feedback and responses for the strategic drenching were recorded in a questionnaire survey sheet.

Data Analysis

The prevalence of fasciolosis was determined as percentage of cattle infected out of the total animals sampled for faecal examinations at each site. Similarly, cattle under farmer's management group, which were treated for fasciolosis by the farmers, were excluded from the analysis. Repeated measure analysis (Genstat 5 Release 3.1) was carried out to examine the effectiveness of the strategic drenching on fasciolosis and interaction with factors such as time, altitude, and location and management systems. For analysis of body condition scores, differences between the condition scores of each animal before and after drenching in November and February separately were calculated. The differences in body condition scores in two different treatment groups were then compared by using Kolmogorov-smirnov two-sample test (Genstat 5 Release 3.1) for significance.

RESULTS

The summary of the results of repeated measures analysis is shown in Table 1. On repeated measures analysis, the strategic drenching was found highly effective in reducing the *Fasciola* infection among the treated cattle when compared with that among untreated ones ($p < 0.005$). Among the various factors examined, only altitude ($P < 0.005$) and time (months) ($P < 0.05$), and interaction between time and altitude ($P < 0.05$) influenced the effectiveness of strategic drenching significantly. Other factors like management system and location were not significant ($P > 0.10$). However, the interaction between the strategic drenching and the time was significant at 90% level of confidence only ($p < 0.10$).

Table 1 Repeated measure Analysis of Variance of *Fasciola* infection in cattle in the western hills of Nepal.

Source of variation	Degrees of freedom (df)	Adjusted d.f.	S.S.	M.S.	F-value	F-probability
<u>Subject Stratum</u>						
Altitude	1	-	40854.8	40854.8	20.37	<0.005
Location	3	-	7024.7	2341.6	1.17	>0.10
Management System	1	-	321.6	321.6	0.16	>0.10
Treatment groups	1	-	50711.2	50711.2	25.29	<0.005
Management System x Treatment group	1	-	518.4	518.4	0.26	>0.10
Residual	6	-	12031.8	2005.3	3.61	
<u>Subject x Time Stratum</u>						
Time	12	3	86836.2	7236.4	13.02	<0.001
Time x Altitude	12	3	22414.3	1867.9	3.36	<0.05
Time x Location	36	9	17599.8	488.9	0.88	>0.10
Time x Management System	12	3	4284.4	357.0	0.64	>0.10
Time x Treatment group	12	3	38048.6	3170.7	5.71	<0.10
Time x Management System x Treatment group	12	3	2318.5	193.2	0.35	>0.10
Residual	72	18	40005.4	555.6		
Total	181		322969.8			

Note: Degrees of freedom in the subject x time stratum were multiplied by 0.2484 before examining F-values.

The prevalence of *Fasciolosis* in cattle in different treatment groups in the mid and low hills are illustrated in Figures 1 and 2, respectively. Among the untreated cattle in the mid hills, the prevalence of *Fasciola* infection naturally decreased to <8% after the month of May, except in August, when the prevalence was 33%. The prevalence increased later to 42% in November. However, in the low hills, the prevalence in untreated animals remained at very high level (>50%) throughout the year, and increased upto 89% in November.

In the mid hills, the strategic drenching significantly reduced the prevalence of *Fasciola* infection in treated cattle for the whole of the year (Figure 1). The infection dropped to 7% and less after the first drenching, and remained at low level for rest of the year (except in the month of April when 40% infection was observed). In contrast, in the animals under farmer's management, the infection remained higher (>58%) until April, and thereafter reduced to very low level for the rest of the months. The infection picked up again (to about 40%) in November in both treatment and control groups.

In the low hills, under farmer's management, the *Fasciola* infection remained at very high level (>80%) until April, and at moderate level (44-80) afterwards. In treated animals, the infection was brought down to less than 5% after second (February) drenching. However, the infection increased to >30% after June. The infection increased to >80% in both the groups in November. Differences in the prevalence of *Fasciolosis* between the treated and untreated animals was significant ($P < 0.05$) till August, after which it became non-significant. However, the prevalence of infection among the treated animals in the low hills was generally higher than that recorded among treated animals in the mid hills. Increase in the prevalence from June, and a further increase in September in animals drenched in February (Figure 2) indicates that the source of *Fasciola* infection still existed in the low hills even after February.

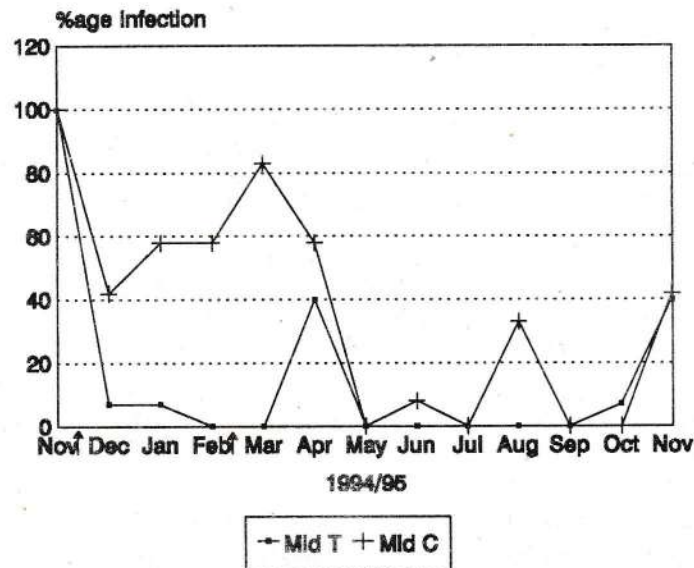


Figure 1 Prevalence of *Fasciola* infection in cattle in the two groups in the mid hills.

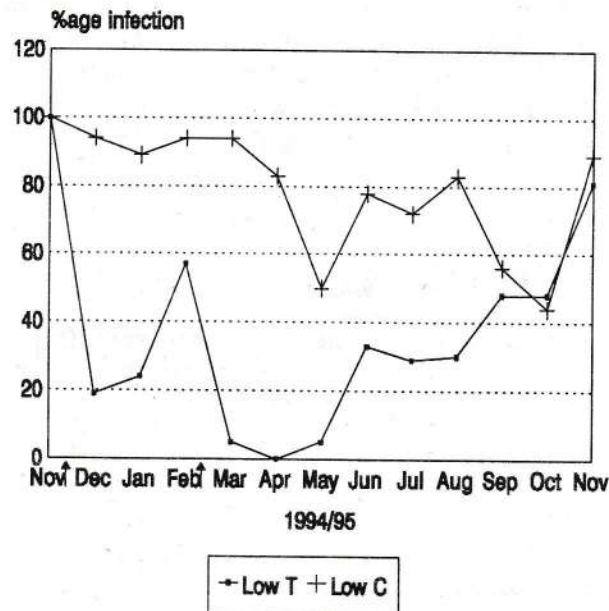


Figure 2 Prevalence of *Fasciola* infection in cattle in the two groups in the low hills.

Management of Experimental Animals

Feeding management in the mid hills

In Pakuwa and Kundule, most of the cattle were stallfed. They were fed rice straw from September/October to April/May only. During this period, they were also provided with tree fodder occasionally. Grasses from dry land (forest/bariland) were fed from June to October. In addition, maize/wheat stover were also fed at different times. Only few cattle were allowed to graze dry land/forest throughout the year intermittently.

Feeding management in the low hills

In Sisuwa and Bhendabari, most of the cattle were managed under semi-stallfed system. They were fed with rice straw almost throughout the year depending on its availability in the households. Tree fodders were not available. Most of the animals were allowed to graze in open communal grazing lands from June to November and in fallow rice field from November to June after the rice harvest.

Effect of Strategic Drenching on Body Condition

In the low hills, the difference in body condition scores after and before February drenching were significantly different ($P=0.04$) indicating effect of drenching on body condition during that time. However, there were no significant difference in body condition for November drenching. Similarly, the differences were not significant for both drenching in the mid hills. Overall, the differences between condition scores of cattle after and before drenching in November and February (separately) were not significant for the two groups ($P=0.38$ and $P=0.20$, respectively) (Table 2).

Table 2 The effect of strategic drenching on body condition.

The effect of drenching	Kolmogorov Smirnov Two Sample Test		Probability (P)
	Maximum Differences	Chi-square (2df)	
Overall			
November (1994) drenching	0.1222	1.941	0.38
February (1995) drenching	0.2222	3.232	0.20
Mid hills			
November (1994) drenching	0.1167	0.3630	0.83
February (1995) drenching	0.08333	0.1852	0.91
Low hills			
November (1994) drenching	0.2222	1.915	0.38
February (1995) drenching	0.4127	6.603	0.04

Farmers Feedback on Strategic Drenching

Farmers responded favourably to the drenching programme. There were no side effects of the anthelmintic, it was easy to administer (animals accepted it easily when given mixed with concentrate), and subjectively, some improvement in milk production and body condition were observed by the farmers. All farmers reported increased appetite in their animals. Though all farmers showed willingness to follow the strategic drenching, only few actually adopted in the following year (data not presented).

DISCUSSION

The effectiveness of the drenching schedule varied between the altitudes and management system of the animals. In the mid hills, the infection was seen from November to March/April and after that it remained at low level before picking up the infection from the grasses in the post-monsoon period. Thus, strategic drenching was effective in reducing the *Fasciola* infection in cattle significantly from November to March/April, and kept the prevalence of *Fasciola* infection low throughout the year. The drenching was effective in the mid hills, and the animals were maintained almost free from infection for the whole year. However, in the low hills, this strategic treatment was not as effective as the infection was seen throughout the year. This might be due to feeding of rice straw, and/or allowing the animals to graze throughout the year. Therefore, though infection was brought down for up to August following drenching, animals might have been picking infection continuously from the rice straw and from the grazing fields resulting the lower efficacy of treatment. The strategic drenching, though controlled the possible infection during post monsoonal and winter months, was not adequate in controlling infection during other period. The pattern of prevalence of *Fasciolosis* in the low hills suggests that there is need of one or two additional treatments, perhaps between June and August for the year round control.

Fasciola metacercariae contaminated grasses and rice straws are the major sources of *Fasciola* infection in stall fed animals in Nepal (Joshi, 1987). This restricts the infection of the animals to a limited period (post-monsoon and winter months, considering that rice straw is fed during the winter months) in most of the areas. On analysing the feeding management of experimental animals at two altitudes, a distinct difference was observed. In the mid hills, animals were mostly stall fed, and rice straw was fed to animals from September/ October to April/ May and grasses from wetland (Simkhet) and near permanent water sources were generally not available. Therefore, in the mid hills, the major source of *Fasciola* infection seems to be mainly the rice straw which was fed from September/ October to April/May. In contrast, in the low hills, cattle were fed rice straw for a longer duration and animals were allowed to graze in the open field (near riverside) and fallow rice field throughout the year. Therefore, unlike in the mid hills, the potential for *Fasciola* infection to cattle in the low hills was almost throughout the year. This might be the reason for the differences observed in the effectiveness of strategic drenching against *Fasciolosis* in cattle in the mid and the low hills.

The effect of strategic drenching on body condition of cattle was found significant only for the February drenching but not for November drenching in the low hills. In the mid hills, no effect on body condition was observed for both treatments. The reason for this might be due to nutritional factors. In the low hills in February, animals in untreated group had been affected by both nutritional stress (low plane of nutrition) and by heavy *Fasciola* infection. Removal of this infection in treated animals would have contributed for the better body condition of treated animals. In contrast, in the mid hills, the nutritional status of the animals were better than that of low hills (availability of tree

fodder), and the parasite burden might be comparatively low even in untreated cattle. Better nutrition could have been able to overcome the stress of parasitism, and hence the lack of differences between the groups.

In general, farmer's response to the strategic drenching was good. No side effects were reported, and the anthelmintic was easy to administer. Farmers generally reported improvement in body condition, better appetite, and in some cases increase in milk production. Though all the farmers were willing to adopt the practice of strategic drenching to control *Fasciolasis* in their cattle, only few understood its importance and potential benefits. There is need for more extension education to the farmers on the importance of strategic drenching. It is also necessary to have quantitative analysis of economic benefits of the strategic drenching to cattle. Considering a similar susceptibility of buffaloes to this disease (Joshi, 1988), and similar agroclimatic conditions of the area, similar drenching schedules with slight modifications to adjust their feeding management could probably be adopted to control the diseases in buffaloes.

CONCLUSION

The strategic drenching was effective in controlling the *Fasciola* infection in cattle for throughout the year in the mid hills, however, it was not equally effective in controlling the disease in the low hills. The pattern of prevalence of infection in the low hills suggest that there is a need of one or two more treatments after February to keep the infection at low level throughout the year, and this needs further verification. It should be taken into consideration that the risk period may vary within altitude, animal management and feed resources. There is need of extension education to the farmers on the importance and potential benefits of the preventive treatment. Analysis of economic benefits of the strategic treatment in different agro-ecological zones is the further area of research.

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SERO-PREVALENCE OF *PESTI DES PETITS RUMINANTS* (PPR) IN SHEEP AND GOATS IN NEPAL

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ABSTRACT

A total of 437 serum samples from sheep and goats were examined by Competitive ELISA, among them 170 samples (38.65%) were found positive for Pesti des petits ruminants (PPR). The highest sero prevalence was found in Rupendehi district of Western region. The antibody for PPR was detected from hill areas like Mustang and Darchula, as well.

INTRODUCTION

Pesti des petits ruminants (PPR) is an acute contagious viral disease caused by Morbillivirus. It affects small ruminants especially goats, sheep and occasionally wild animals. Among these, goats are highly susceptible (OIE Manual, 1996). PPR disease exists in Africa, Middle East and Southern Asia. The first outbreak of PPR in Nepal was reported from Dhanusha, Bara, Parsa, Rautahat and Sarlahi districts of central region of Nepal (Jha *et al.*, 1995). The first outbreak detected along the southern border may be due to the import of infected goats from the neighboring country, India. This shows that PPR might have already entered in Nepal and thus became endemic in small ruminant population of the country. It causes about 50 % mortality which can reach up to 100 % in severe cases (OIE manual, 1996) and so it is the disease of small ruminants having great economic importance.

The disease is not of public health importance but is transmitted between animal to animal. It is characterized by fever, oculonasal discharge, stomatitis, pneumonia, and diarrhea. The incubation period is 4-5 days. Mortality and morbidity rates are higher in young animals than in adults that occur up to 50% and 100% respectively. This disease leads to great economic loss, which was estimated to be US \$ 1.5 million in 2001 (Mahato & Gongol, 2002). This study was undertaken with the objective to evaluate the status of PPR in different districts of the country.

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MATERIALS AND METHODS

A total of 437 serum samples from the unvaccinated animals (goat 388 and sheep 49) from the suspected outbreak areas were collected from four different regions i.e. Central (206), Western (103), Mid-Western (93) and Far- Western (35) between July 2001-2002. The serum samples collected from different districts are shown in Table 1. The serum samples were transported to the Central Veterinary Laboratory (CVL) in cool boxes maintaining the cold chain and stored at -20°C in a cold room. The samples were thawed at room temperature before conducting the test. Competitive ELISA (cELISA) method (OIE Manual, 1996) was used to detect antibody of PPR in serum samples and for this, ELISA kits were supplied by BDSL, UK. Briefly the methodology was as follows:

PPR antigen was first coated in plates (Nunc). The plates were then incubated for 1 hour. First two rows were kept for control. The coated plates were washed 4 times by PBS (pH 7.2). After that blocking buffer was added in each well. Except in control rows, test serum samples were added to rest of the wells. Then monoclonal antibodies (Mab) were added in all wells except in first two wells of conjugate controls. The plate was again incubated for one hour and then washed by PBS for 4 times. Horse Radish Peroxidase (HRP), anti rabbit conjugate was added to all wells and incubated for 1 hour. After incubation the plates were washed 4 times and Ortho- phenylenediamine (OPD) substrate with hydrogen peroxide (H_2O_2) was added in all wells. After 10 minutes, stopping solution i.e. Sulphuric acid (H_2SO_4) was added to stop the reaction. The reading was taken at 492 nm wavelength.

RESULTS AND DISCUSSION

Out of 437 samples, 170 samples (38.90%) were found positive for antibody of PPR (Table 1). Among the four development regions, the highest prevalence rate was found in the western region (53.39%) followed by the central region (36.41%) and the mid-western region (32.26%). The lowest prevalence rate of PPR was found in the Far Western region (11.76%). The prevalence rates in sheep and goats were 30.61% and 39.95%, respectively (Table 2).

Out of 16 districts, PPR antibody was detected in serum samples collected from 15 districts (Table 3), Kailali being the exception without any positive sample. The highest prevalence of PPR was found in Rupendehi (85.71%), whereas, it was 9.68% in Rasuwa district (Table 3).

Table 1 Region wise sero-prevalence of PPR

S.N	Regions	Animal Species	No. of Tested Samples	No. of Positive Samples	Percentage
1	Central	Goat	206	78	36.41
2	Western	Goat	103	55	53.39
3	Mid- Western	Sheep	31	9	29.03
		Goat	62	20	32.26
4	Far- Western	Sheep	18	6	33.33
		Goat	17	2	11.76
Total			437	170	38.90

Table 2 Sero-prevalence of PPR in Sheep and Goats

S.N	Animal Species	No of Tested Samples	No of Positive Samples	Percent (%)
1	Goat	388	155	39.95
2	Sheep	49	15	30.61

Table 3 District wise Sero-prevalence of PPR

S.N	Districts	Animal Species	No of Samples tested	No of Positive Samples	Percentage positive
1	Sarlahi	Goat	19	7	36.84
2	Kathmandu	Goat	55	36	65.45
3	Nuwakot	Goat	70	16	22.86
4	Rasuwa	Goat	31	3	9.68
5	Makwanpur	Goat	11	8	72.73
6	Parsa	Goat	20	8	40
7	Snyanga	Goat	16	2	12.5
8	Kaski	Goat	8	3	37.5
9	Mustang	Goat	42	26	61.90
10	Gulmi	Goat	30	18	60
11	Rupendhi	Goat	7	6	85.71
12	Dang	Goat	20	4	20
13	Surkhet	Goat	42	16	38.09
		Sheep	31	9	29.03
14	Kailali	Goat	9	0	0
15	Dadeldhuda	Goat	8	2	25
16	Darchula	Sheep	18	6	33.33
Total			437	170	

DISCUSSIONS

Out of 437 serum samples of sheep and goats, 170 samples (38.90%) were found positive for PPR antibodies. This is lower than the serological report of Jha *et. al.* (2000). This study was carried out in 16 districts of four development regions of the country. The positive cases from Western and Mid-western regions might be due to the movement of animals across the border. A large numbers of goats were imported from India for meat, and bucks for breeding purpose that entered in Central as well as in Far-western regions which could be the single and most important factor responsible for PPR outbreak. Similarly, the highest prevalence rate found in Rupendehi (85.71%) district might be due to its close contact with Indian border. However, the positive prevalence in Mustang (61.90%), Rasuwa (9.68%), Darchula (33.33%), and Dadeldhura (25%) might be due to the importation of bucks from infected areas for breeding purposes (Ghimire and Mahato, 2000) or due to the introduction of new goats from the infected areas. In Kailali district, all samples were found negative for PPR the reason for which is unclear but it might simply be the sampling of animals from uninfected regions of the district.

The member of samples collected from sheep were less than those of goats. Though unequal sample size, when compare had higher prevalence of PPR than sheep. This proves that goats are more susceptible to PPR compared to sheep (OIE, 1996). On other hand sheep were less in number and there was low outbreak of PPR in sheep population under natural condition, which might suffer badly under stress conditions (Mahato and Gongal, 2002).

As PPR is important for the small ruminant population of the whole nation, the control strategy has to be developed for mass vaccination in the outbreak areas.

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LEGISLATION FOR OVERALL EFFECTIVENESS OF VETERINARY SERVICES IN NEPAL

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ABSTRACT

In Nepal, number of Acts have been promulgated after the restoration of democracy. Conventional Livestock Administrative Acts are not compliant with the changed socioeconomic and international development perspective. In the present context, these Acts need essential amendment. Recently promulgated Animal Health and Livestock Services Act 2054 and Animal Slaughterhouse and Meat Inspection Act 2054, pave ways for the sustainable livestock development through the control of Animal Disease, quality of livestock products and extending standard veterinary services. Scientifically formulated regulations will complement these Acts. Responsible authority at the Department for the enforcement, follow up and update the Acts and regulation, is important.

INTRODUCTION

Laws, the ratified document of right and duties between the people and government, are reciprocal with justice. The main objective of the law is the safe conduct of the fundamental law of the country. Precedence of the constitution is followed during the formulation of all acts. The constitution of the kingdom of Nepal 2047, article 131 incorporates the dismissal provision of irrelevant Acts. Violation of the legitimate rules is an offense and punishable.

Historical Background

Hindu Legal system is the origin of modern Nepalese Legal system. In the ancient time, law and religion were synonyms, Religious negligence was an infringement. On the basis of the religious code of conduct, Kirati, Gopal, Lichhabi, Malla and many other kings enacted laws, regulations and by-laws in the name of Sanad, Sawal, Sthithi, Royal order, Rukka, Mulukisawal, During that time, the rules of law were least practiced in the absence of constitution. Rules drawn up by the king Ram Shah was testimonial in Nepalese history. A book "Human Legislation System" drafted by Jaystithi Malla (B.S. 1437) was the first codified law.

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Primeminister J.B Rana impressed by the British Parliament and Napoleon code, formulated a Kausal to draft Muliki Ain (Law of the land). His Majesty the king Surendra Bir Bikram Shah Dev declared Muluki Ain, the first law in the modern legal history, in 1910 B.S.. Main features of this Act were judiciary supremacy and equal penalty for alike offense. Since then, this act amended many times but the amendment made in 2020 B.S., hold an extreme view for social change.

Origin of Livestock Acts

As mentioned in religious scriptures, animals and birds were worshipped as god. From ancient period to now, slaying of a cow is a serious crime (panchkhat) by the law. Two percent Sale tax on the livestock had mentioned in Manusmriti. Such tax was called Bhog in Kirati era and were imposed on male buffalo and pigs.

Prevailing livestock governing Acts

- Muliki Ain Part 3 Mahal 6 contains about Animal ownership, loosing and getting animals, Part 4 Mahal 5 article 3, 10 and 12. Letting the fire in the cowshed, Mahal 7, Four footed Animals, and Mahal 16 mentions the Bestiality.
- District Development Committee, Village Development Committee, Act 2047
- Infectious Disease Control Act 2020.

Modern Legal Concept

Offense in the past may not be an offense today. The pace of change is accelerating steadily in science and technology. Futuristic science and technology, its use and abuse, legal education and public awareness, judicial professionalism and activism, system of writ, have modernized the legal system. Social norms, public aspiration, moral value, business code of conduct, concern of environment, urbanization, liberalized economy and free trade, have increased government responsibility. Internal peace and security, obligations of a *welfare state* have animated the government to practice the rules of law, for the national development.

Need and Urgency of Veterinary Legislation

In changing world, government will adapt to the New World order of things in livestock production. Regulatory frameworks become globalized for many livestock products, veterinary medicines, and infectious disease control and food operation. Among many regulatory factors of the need and urgency of veterinary legislation, is its non-existence. Conventional Veterinary Acts in practice are not congenial with the objective of sustainable livestock development. Traditional Acts do not provide the legal support to implement priority programs effectively to fulfill public interest and welfare. Veterinary requirement will become more precise in the coming century. Suitable Acts are essential to encounter such prospective changes. All external and internal factors indicate the need and urgency of appropriate veterinary acts.

Main Objectives of Veterinary Acts:

- To systematize and develop livestock activities legally.
- To regulate animal production and livestock trade (marketing, export and import of livestock, its products and other inputs).
- To protect the welfare of consumers by providing wholesome meat and meat products and prohibit adulteration.
- To set up quality assurance of livestock products.
- To organize Animal Slaughterhouse and execute Meat Inspection / Meat Treatment System.
- Guarantee the professional right and duties of the veterinarians by legal recognition of veterinary services.
- Suggest government in veterinary technical matters through an organized technical expertise e.g. Veterinary Council.
- Prepare the ethical code of conduct for practicing veterinarians for their professional and moral development.

These Acts will promote

- Modernize livestock development which is envisaged in long term Agriculture prospective plan
- Shaping up implementation strategies of current policies, directives and strategies for the sustainable livestock development.
- Legal backstopping especially to Animal Disease control and Quality control of animal products.
- International recognition and cooperation of the national veterinary service.
- Promote professional ethical standard and assure quality of veterinary services.
- Promote private practices and privatization process.
- Control the epidemic, emerging animal diseases and zoonoses in war footing basis.

Acts are designed to suit the animal production system, socioeconomic situation, and environmental needs of the country and international requirement. These Acts fulfill the national or international statutory requirement and set a vision of more efficient, equitable and employment intensive livestock sector, harmonizing the private and government veterinary services. Act also entails the government to fulfill or enforce public policies. The main objective of these Acts is for the development of livestock not its control.

Silent Features of These Acts

- Specific in the respective subject: Preferred objectives of the acts will be accomplished after the formulation of related regulations. Contents of the Acts are compliant with the socioeconomic and cultural practices of the farming communities.

- **Enforcement** proposed is practical. Step by step enforcement has been proposed.
- Interpretation is uncomplicated: Language, style and words used are simple.
- There is no ambiguity, ambiguous and obscure words.
- **Brief but complete:** these acts content the subject matter as per the national requirement.
- **Delegated legislation:** Subject matter specialists will draft scientific framework of regulation and by-laws under delegated authority of the acts.
- **Administrative justice and law:** Ministry of Agriculture and Department of Livestock Services is empowered to prove the instances or argument and final decision under these acts
- **Quasi judicial office:** Considering the perishable natures of livestock products and for effective legal enforcement, Animal Quarantine Officer and District Livestock Service Officer have been vested a *quase -- judicial* power. Veterinarians will be the judges of facts.
- **Finance bill:** Legitimate power of revenue collection for the service provided is reserved.
- **Government bill:** Only the minister can present these bills in the parliament.
- **Authority cannot be transferred:** authority entrusted by Act can not be transferred to other person.
- **System of appeal:** If the subject is not under the local or territorial jurisdiction or wrong procedure or interpretation, plight of appeal to review the soundness of decision of an inferior authority had instituted **Amendment:** Infections Disease Control Act 2020 had amended by the omission of the word pays, panchhi, wa..
- **Shall be as the prevailing law:** Subjects or legal procedures not specified in these Acts shall be concluded as article 4 of the Muluki Ain.
- **His Majesty's government shall be plaintiff:** inquiry, investigation and register of cases shall be administered by His Majesty government
- **Power to amend Annex:** By the notices in the *Nepal Gazette* His Majesty government retain power to modify the annex.
- **Penalty and punishment:** Penalty and punishment proposed are perfect considering the austerity of the offense.

Acts and the Animal Disease Control

- Establishment of Slaughterhouse/ Meat Inspection System will be a fruitful laboratory for disease investigation and disease control.
- Quarantine System (interior and international) will direct quality import and export of animal and animal products.
- Establishment of Veterinary Council will spread out standard veterinary practices Sanitary and hygienic standard will be useful for disease control.
- Animal Disease Control will enhance livestock production and productivity.

Lacking in these Acts:

- Subject matter related to the vetero-legal cases has not been covered in the Act. Materials pertinent to meat microbiology and industry are not mentioned.
- Central Bureau of Meat Inspection or Meat Board, as the apex body for enforcement of act is not yet commissioned.
- Control and supply of Veterinary medicine is not managed

Suggestion

- A qualified legal advisor must be appointed in the Department of Livestock Services.
- Implementation strategies of the Acts should be designed instantly.
- Contingency plan taking FMD as a model should be prepared.
- Manpower, especially for meat inspection, should be trained.
- Slaughterhouse and Meat inspection Act enforcement strategies should be prepared though intensive discussion with consumers, Butchers and political leaders such as Mayors, Village Development Committee chairman.
- Ad-hoc Veterinary Council should be formed immediately.
- Economical model of slaughterhouse convenient to private parties should be composed in consultation with FAO and other international organizations.
- Drafting new Acts as per need and timely revision should be a continuous process.

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CONSERVING ANIMAL GENETIC RESOURCES IN NEPAL: ROLE AND OPPORTUNITY FOR THE VETERINARIAN

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ABSTRACT

Veterinarians can play a vital role in identifying indigenous animals with unique morphological and production characteristics. This is made possible when livestock and poultry are examined routinely for prophylactic measures and treatment of diseases. Active participation in acquiring knowledge and skills in the newly emerging technologies of molecular biology and reproduction will allow the veterinary profession to be in the forefront. The close association between the veterinarian and the animal species provides a unique opportunity for the profession to demonstrate leadership, dedication and a strong commitment towards conservation.

INTRODUCTION

Human societies have relied for centuries on animal agriculture to provide food, fibre, power, fertilizer and employment. This is more apparent in developing countries where human activities are interwoven with the raising of livestock and poultry. Nepal has a strong animal agriculture with 14 percent of the national gross domestic product derived from livestock. More than 90 percent of the farmers in rural areas supplement their income by the sale of products from domestic animals. Furthermore, domestic animals are idolized and sacrificed in nearly every religious ritual. Thus, the significant economical and cultural importance of these animals to the Nepalese society deserves special attention in the planning of policies and national strategies.

Major commercial companies, which own breeding populations of cattle, pig chicken and turkey, have benefited financially from the genetic response to artificial selection. However, selection has greatly reduced the genetic variability associated with morphological and production characteristics. This has aroused serious concerns over the decreasing rate of genetic response. Unfortunately, the mainstream agriculture producers and the general public are not aware of the importance of genetic resources and the narrowing genetic base of domestic animal species. Many individuals involved in animal agriculture will recall the indigenous animal populations, once common in the hills and mountains, which are now absent from the landscape. Because of these facts, the conservation of domestic animal genetic resources has thus become a preoccupation in many countries around the world including Nepal. To address animal genetic resource conservation issues, biotechnology programs have been developed in North America

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(Shrestha, 1990; Gerrits and Shrestha, 1995). Furthermore, the Food and Agriculture Organization of the United Nations has published a list of domestic animals, of which the existing diversity is increasingly under threat (Scherf, 1991). The detailed description of domestic animal genetic resources in Nepal has been reviewed (Shrestha, 1998).

It is common occurrence for veterinarians during the routine practice of disease prevention and treatment to come in contact with animals having unique morphological and production characteristics. All indigenous animals need to be identified, characterized, protected and preserved through the dedication, professionalism and support of individuals participating in conservation activities. Veterinarians can play a key role in conservation issues as they are, and will always be an integral part of the process.

Importation of Exotic Breeds

The supply of food to meet the growing requirements of the human population deserves special attention. In an effort to improve productivity, exotic breeds with potential for production have been imported into the country for nearly a century. These animals were, and are used extensively to upgrade indigenous livestock and poultry populations across the country. Later, crossbred populations that produce significantly more milk, meat and eggs were developed. Concurrently animals as a source of power and manure were replaced by increased mechanization and the use of chemical fertilizers. An increase in productivity has certainly been achieved, but at high input costs. Consequently, there has been an unprecedented demand for technical services and skills to address increasing animal health, nutrition and management needs. Thus, the introduction of exotic breeds has increased the need for veterinary and agricultural professional services across the country and also created employment.

Decline of Indigenous Animals

The steady conversion to crossbred populations has had serious consequences on the structure and management of animal enterprises. There has been a drastic decline in the number of indigenous animals with unique morphological and production characteristics. Thus, in both livestock and poultry, potential genetic variation for hardiness and disease resistance, which may possibly be important and valuable in the future, have been overlooked. The rapid increase in crossbreeding activity must therefore be considered as a threat that could ultimately lead to the extinction of many indigenous animals. The technology to artificially recreate the array of genotypes represented in currently existing populations is not available today and may not exist for a number of decades. Veterinarians can play important role by documenting any increases in the number of subclinical and clinical cases of disease encountered in these crossbred animals. Similarly, the susceptibility of indigenous animals to diseases as a result of the changing environment should be noted.

Emerging Technologies

Qualitative and quantitative variations in genes on specific chromosomes or gene combinations detected at the DNA or gene product level have been known to be associated with morphological and production characteristics. Measuring genetic distance among animal populations based on highly polymorphic microsatellite markers or mitochondrial DNA D-loop sequences or fingerprinting, will help to locate populations that are distant on the evolutionary scale, thereby increasing the possibility of locating a large number of segregating genes in the diversified populations. DNA from divergent genetic populations with distinct features is being increasingly used to develop maps for livestock and poultry species. Although knowledge in this area is rapidly expanding, the average mammalian genome is thought to consist of 50,000 to 1,00,000 genes.

Unfortunately, only a few of these genes have been found so far. A number of markers that have already been identified could be used as tools to rapidly screen breeding populations for specific characteristics. This technology may also be used in combination with quantitative methodology to improve the accuracy of predicting breeding values based on genetic merit. There is a need to maintain breeding populations with specific attributes or with a large amount of variability. These populations will act as a reservoir of specific nucleotide sequences in the DNA related to morphological and production characteristics in the animals. In the future, veterinarians who acquire the knowledge and technical skills to identify and to utilize major genes from exotic animals will play a key role in improving animal populations.

Newly developed reproductive technology used in creating transgenic animals may improve the resistance of breeding populations to specific diseases. Specific genes from cryopreserved DNA of indigenous animals could be incorporated into the genome of the transgenic animals. A number of leading veterinarians in developed countries have used this approach to increase the amount of genetic variability by utilizing the technology of gene transfer among species relatively close from an evolutionary point-of-view. The potential benefit through the savings in handling, vaccination and the use of anti parasite and antibacterial drugs could result in significant savings to the producer and provide a safer food supply to the consumer.

There are wide variations in genetic resources, both among and within animal populations. This great potential might be exploited to meet the changing consumer preferences and environmental conditions, simultaneously contributing to the efficiency of production. A major problem in the conservation of domestic animals is the lack of funding. Additional budgetary allocations are needed to increase total research effort in animal agriculture including the conservation and the use of domestic animal genetic resources. The inability to obtain additional funds may seriously compromise the competitiveness of animal agriculture.

CONCLUSION

Domestic animals are of great commercial importance and represent an important sector of agriculture. However, the technology, human resources and mechanisms to effect the long term identification and conservation of unique, valuable domestic animals does not exist in the country. The identification and utilization of domestic animals with important and divergent genetic resources will play an important role in the future of domestic animal improvement.

Veterinarians can play an important role in the conservation of Nepal's animal genetic resources by documenting diseases encountered, identifying unique animals and demonstrating leadership by actively participating in the newly emerging technologies of cryopreservation and biotechnology.

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A REVIEW OF FISH DISEASE PROBLEMS IN NEPAL

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ABSTRACT

Several reports and literatures were studied to evaluate the loss in fish production caused by infectious and non-infectious diseases of fish in Nepal. The parasitic diseases (both internal as well as external parasites) were the most prevalent. Diseases caused by protozoa, helminthes and crustacea were economically important. Fries and fingerlings were mostly affected by protozoan and helminth diseases while crustacean diseases mainly affected brood fish. Among the bacterial, fungal and viral diseases, the losses caused by EUS (epizootic Ulcerative Syndrome) was significant. The disease problem has increased with the intensification of fish production system in the country.

INTRODUCTION

In Nepal about 185 indigenous fish species have been identified and five species of exotic fish are successfully introduced (Anon, 1997). Commercial fish farming started in Nepal since 1947 with the introduction of Indian Major carps. Common carp was introduced from India in 1956 and from Israel in 1960. Grass carp was also imported from India in 1967, Silver Carp from Japan in 1972 and Rainbow trout from Japan in 1988 (Anon, 1998).

Commercially produced fish in Nepal are Rohu (*Labeo rohita*), Naini (*Cirrhinus mrigala*), Bhakur (*Catla catla*), Common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), Bighead carp (*Aristichthys nobilis*), Grass carp (*Ctenopoma ruyangodon idella*) and rainbow trout (*Onchorynchos mykiss*) and other indigenous fish such as Asla (*Schizothorax spp*), Sahar (*Tor spp*), Katla (*Accrossochilus spp*) are captured from rivers. Tilapia (*Tilapia nilotica*) and Puntius (*Puntius gonionotus*) are under study.

Among different systems of fish culture, the extensive and semi-intensive systems are in general practice. The intensive system, which requires high level of technology, is being adopted in some lake systems. In Phewa Lake and Kulekhani hydroelectric reservoir, fish are cultured in cages; while, pen and enclosure system is practiced in Begnas and Rupa lakes. Fish culture (Pisciculture) is also integrated with livestock and horticulture systems. Fish is also captured from river and other water bodies.

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Disease problems in fish were low in the past. With the increase in fish culture practices, disease incidences and intensities have also increased. It is estimated that annual loss in production due to fish diseases range from 25 to 30 percent, which is equivalent to about 4 to 5 million rupees every year. It is also estimated that parasitic diseases alone cause 8 to 12 percent loss in production (Anon., 1996).

The first incidence of parasitic disease, Gill fluke (*Dactylogyrus spp*) was observed in 1965 in common carp fry in Godawary Fish Farm. It caused mortality in the nursery ponds, especially where fry population was heavy. The first case of Cestode infection (*Cariophyllaeus spp*) was detected from the intestine of common carp yearlings in Godawary Fish Farm in 1965. White spot disease (Ichthyophthiriasis) caused severe mortality of fry, fingerlings as well as adult fishes in 1967. Trichodina was also first detected in the hatchling of Chinese carp (Grass carp and Silver carp) from Godawari Fish Farm in 1972.

First account of fish disease EUS (Epizootic Ulcerative Syndrome) came in March 1972 from Queensland, Australia. It first appeared in Nepal in 1989/90 and caused 15-20% loss in production equivalent to 30 million rupees.

METHODOLOGY

On the basis of observations and reports from different government fish farms, the occurrence of fish diseases has been reviewed. The commonly occurring fish diseases have been presented in Table 1.

Table 1 Fish parasites recorded in various fish farms in Nepal.

Farms	Affected fish species	Protozoan and Parasitic diseases	Recorded period
Fisheries development centre(FDC), Bhairahawa	Common carp, Silver carp, Rohu, Naini	<i>Trichodina</i> sps, <i>Monogenea</i> , Tapeworm, <i>Argulus</i> sps	1998
FDC Bhandara	Grass carp	<i>Trichodina</i> sps,	1998
FDC Hetauda	Bighead carp, Common carp	<i>Trichodina</i> sps, <i>Monogenea</i>	1998
FDC Janakpur	Common carp, Bighead carp, Naini	<i>Trichodina</i> sps, <i>Monogenea</i>	1998
FDC Fattepur	Grass carp, Common carp	<i>Trichodina</i> sps, <i>Monogenea</i>	1998
FDC Lahan	Grass carp	<i>Trichodina</i> sps, <i>Monogenea</i>	1998
Central Fish hatchery, Balaju	Common carp, Grass carp	<i>Trichodina</i> sps, <i>Monogenea</i> , Tapeworm	

Thakur

The parasites were found in mucus and gills of fish causing a moderate to light infection. *Trichodina* sps affected mostly fry below 3 cm, while *Monogenea* affected mostly the fingerlings below 60 gms. Tapeworms were mostly recorded in fingerlings of common carps.

In addition following diseases have also been reported in Nepal.

A. Protozoan Diseases:

1. Costiasis or Blue Slime Disease.
2. Ichthyophthiriasis or White Spot Disease.

B. Crustacean Disease:

1. Lernaeasis

C. Bacterial Diseases:

1. Tail Rot or Fin Rot Disease
2. Dropsy

D. Fungal Diseases:

1. Saprolegniasis
2. Branchiomycosis or Gill Rot Disease
3. Epizootic Ulcerative syndrome (EUS)

E. Viral Disease:

1. Spring Viremia of Carp (SVC)

F. Environmental Diseases:

1. Gas Bubble Disease or Gas Embolism in Hatchlings.
2. Asphyxiation

Three species of Protozoa, two species of monogenetic trematodes, nematode and tape worms have been recorded in the carps.

Trichodina spp is distributed all over Nepal. This protozoon mainly affected small sized fish. Monogenetic trematodes parasitized fry and fingerlings. The incidences of Nematode and Cestode infection are less. Parasitic infection is more common in fish seed than the table and brood fish in which Argulosis is observed.

EUS, seen in both indigenous and cultured species, affected large sized fish causing severe economic losses.

DISCUSSION

Fishes in Nepal suffer from different types of diseases. Mainly parasitic diseases are reported which cause heavy mortality in hatchlings, fries and fingerlings. Larger fish and brood fish are susceptible to protozoan and helminth diseases but are vulnerable to crustacean diseases. Infection with *Argulus* and *Lernea* may not be fatal but cause ulcerated lesion-permitting attack by bacteria, fungi and viruses.

The incidences of bacterial, fungal and viral diseases are low. Reports on these diseases are rare. Confirmation of viral disease in Nepal is difficult due to lack of laboratory facilities. EUS, now known to be caused by a fungus, causes heavy loss to marketable and brood fish.

Serious attention is required from all concerned authorities to control disease problems in fish. The future of the fish industry and recreational fisheries depends upon the development of effective methods to maintain healthy fish population.

RECOMMENDATION

With the expansion and development of pisciculture in Nepal, there is demand skilled manpower to tackle disease problems in fish. Technicians involved in this field must be allowed to remain in the same field. Good working environment must be developed. Fish is cultured mainly in the Terai of Nepal but the only laboratory which looks after diseases in fish is located at Balaju in Kathmandu. Laboratories in different places of Terai must be established and other facilities especially opportunity for higher education must be developed.

ACKNOWLEDGEMENT

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REPORT ON OUTBREAK OF CAPRINE BABESIOSIS IN CHAUMALA VILLAGE OF KAILALI DISTRICT, FAR WEST NEPAL

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INTRODUCTION

Goat rearing is an important agricultural enterprise in the far western region. Some districts of far-west regions are recently emerging as the potential goat rearing areas of the country. The poverty alleviation goat-rearing program of HMG has given stimulus for its expansion. The combined goat population of the four districts is figured to be around 400,000 which constitutes about 6% of the country's total goat population. Goats are mainly raised for meat contributing about 19.4% of the total meat production (CBS. 2001/2002).

Among the various diseases affecting goats, babesiosis had not been reported previously, though it has been reported in cattle population from different parts of the country including from far west region. *Babesia* are intra-erythrocytic protozoan parasite of domestic animals and are the cause of haemoglobinuria and anaemia. They are transmitted by ticks of various genera in which both transovarian and transtadial transmission can occur. *Babesia motasi* and *B. ovis* are of clinical importance in goats, former being of particular importance for the severity of the disease caused by it. These haemoprotozoan parasites are transmitted by ticks belonging mainly to *Haemaphysalis* and *Rhipicephalus* genera. The present paper reports the outbreak of babesiosis in the goat flocks of Rajipur, Chaumala of Kailali district, which is known as a potential pocket area of goat keeping.

Climatic Condition and Goat Management in the area

The maximum temperature of the district during the summer months (April to June) ranges from 35-43° C. The weather remains hot and humid during the monsoon (June to August) with temperature fluctuation between 33 and 38° C. However, the winter season marked fall in the ambient temperature i.e. between 15 and 25° C. The goats are generally housed inside the shed at night and taken to the community grazing land or to forest for grazing during the day. Goats are basically dependent on grazing for their feeding with little supplementation of concentrates and salts only occasionally at home. There is a dense forest area near the village close to foothills. There is always a possibility of infestation of ticks, which might have infested the wild animals earlier.

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Epidemic Background

The disease outbreak was observed in the Rajipur village of Chaumala Village Development Committee, which is near the Eastwest highway and approximately 5 Km. away from the foothills. The outbreak was seen in a flock of over 250 goats in the month of July-August 2003. Altogether 28 (11.2%) adult goats in the flock were reported to have died due to the disease within a period of one month and over 20 animals were clinically ill. Earlier during mid June, there were reports of tick infestation in goat population at that area. Most of the sick animals were crossbred of Ajmeri (Rajasthan Breed). Three years back, there was an outbreak of Goat pox in the same lot. The sick animals as complained by the farmers appeared depressed and dull with high fever that did not respond to antibiotic therapy namely Oxytetracycline and Procaine Penicillin. The animals gradually lost condition, gave up feeding and passed red coloured urine towards the terminal stage and finally died following a course of 5-6 days illness. Few animals were reported to have fallen sick suddenly with high fever that immediately went prostrate and died within few hours.

Clinical Findings

Clinical examination of sick animals revealed high temperature of 105-106.4° F, anaemia with pale and icteric conjunctiva and pale mucus membrane of the mouth suggestive of hemoprotozoan infection.

Post mortem and laboratory findings

Post mortem examination of the dead goats showed grossly enlarged and friable liver with pale yellowish discoloration. Gall bladder was found to be distended with granular bile content.

Blood smear prepared from sick animals stained with Giemsa revealed heavy infection of erythrocytes with pyriform blood protozoan parasites. The parasites were diagnosed as Babesia species (*B. motasi* and *B. ovis*) on the basis of their shape and size. The haematological examination of the blood from sick and healthy animals showed severe anaemia (Table 1).

Table 1: Haematological findings in diseased and normal goats:

Haematological picture of diseased and normal goats									
	Total RBC/ cu.mm	Total WBC/ cu.mm	Hb.	PCV	Lymph ocyte	Neutro phil	Eosinop hil	Mono cyte	Basop
Diseased	1.6x10 ⁶	3x10 ³		10%	38%	36%	26%	Nil	Nil
Healthy	13x10 ⁶	9x10 ³	12g/dl	28%	62%	30%	5%		0-2%

A severe anaemia with erythrocyte count as low as $2 \times 10^6/\mu\text{l}$ and Hb level down to $3\text{g}/\mu\text{l}$ was present in most clinical cases. These findings are in agreement with several earlier reports from abroad (Blood *et al*, 1995).

Treatment

Treatment to the entire sick population of animals could not be extended because most of the farmers had already sold the whole flock of goats with sick animals. However, some sick animals were treated with Berenil @ 3 mg/Kg body weight, IM resulting a dramatic recovery of the treated animals. As a measure to control the tick population in animals Ivermetin injection was recommended for use in all animals in the VDC just before the onset of monsoon. Although, blood protozoans are regarded as the problem of large ruminants, this outbreak in goats had suggested that babesiosis could become a health problem in small ruminants too resulting a loss in their production and productivity.

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PREVALENCE OF CANINE TRANSMISSIBLE VENERAL TUMOR AND EFFICACY OF ITS SURGICAL TREATMENT

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ABSTRACT

A study was conducted on 2,870 canine cases brought at Central Veterinary Hospital, Tripureshwor, Kathmandu, from June to November 2001. A total of 28 tumor cases were observed and twelve cases were identified as Canine Transmissible Veneral Tumor after clinical and histopathological examinations. The disease was found to be more common in females than in males. The study showed the average age of male and females to be 4.5 years and 7.2 years respectively which ranged from 2.5 to 6 years in males and 3 to 13 years in females. Though the disease was more common in small breeds, trend on the definitive breed susceptibility was not evident. Reoccurrence of only 22.2 percent of cases following surgical treatment showed effectiveness of surgical treatment against this problem. Hematological picture was found to be within the normal range in all examined cases.

INTRODUCTION

Canine Transmissible Veneral Tumor (CTVT) is a unique neoplasm, appearing in cauliflower like, pedunculated, nodular, papillary, or multi-lobulated forms. The disease is transmitted by transplantation of tumor cells to the external genitalia during mating, to ruptured skin, or to mucous membranes of the dog. It is found in both sexes. The major sites are the genitalia, including vagina in bitch and glans penis and prepuce in male. Metastasis is very rare, and if occurs, is mainly found in regional lymph nodes. The size ranges from a small nodule to a large mass up to 10 centimeters. It is firm and friable. The surface of the tumor when exposed outside may bleed and ulcerate.

The disease was studied in a considerable detail at the turn of the nineteenth century by Sticker and was erroneously called contagious lymphoma. Transmissible Veneral Tumor (TVT) has also been called condyloma, veneral granuloma, infectious sarcoma, contagious lymphosarcoma, sticker tumor and histiocytoma.

The disease is reported from many regions of the world and is usually endemic where dogs are free-roaming, particularly in cities and rural areas. Virus has been suggested to be the causative agent but it is still unknown. Chromosomal studies show that most veneral tumor cells contain 59 chromosomes but dog's somatic cells contain 78 chromosomes. The specific feature of this tumor is that it may regress spontaneously or

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may metastasize to other parts of the body like regional lymph nodes, liver, spleen, intestine, lung, eye and brain. The immunological response of the host may determine the tumor behavior. In normal adult dogs the tumor is irregular and is benign in type in contrast to the immunosuppressed old dogs and neonatal pups where rapid growth and metastasis of the tumor is a common feature. There is no evidence that the tumor is transmissible to man.

There is no therapeutic treatment for the problem except for surgical intervention. The present paper reports on the incidence of CTVT in dogs brought for treatment at Central Veterinary Hospital (CVH), Tripureshwor between June to November 2001, and the responses of surgical intervention for this disease.

MATERIALS AND METHODS

A total of 2,870 canine cases brought at CVH from June to November 2001 were the materials for this study. During the study, following methodologies were applied.

Tentative diagnosis was based on case history, symptoms and clinical examination and histopathology was carried out for confirmative diagnosis. History of mating was the primary consideration. Tumors were diagnosed on the basis of symptoms like vulvar/prepuccial bleeding or discharge, difficulty in micturition and the gross appearance of tumor on clinical examination.

Hematological examination was carried out for Total Erythrocyte Count, Differential Leukocyte Count, Total Leukocyte Count, Erythrocyte Sedimentation Rate, Packed Cell Volume and Hemoglobin before surgical intervention.

Treatment

Treatment was done by surgical excision under general anaesthesia. Xylaxine and Ketamine hydrochloride were given as anaesthesia at the dose rate of 1-2 mg and 10-15 mg per kg body weight respectively. Tumor was excised from healthy tissue beneath the base of the tumor along with stump. Later, cauterization with Copper Sulphate crystals was done. Extensive tumor mass of vagina was removed by episiotomy. Males were castrated at the same time where as females were spayed by ovariohysterectomy after one month of the treatment. Follow up was done at the end of November. The patients were examined for the re-occurrence of the tumor.

RESULTS

Out of the total canine cases, 28 dogs were found to have different types of tumor. A total of twelve tumors were identified as CTVT. Canine Transmissible Veneral Tumor shared 0.41 per cent of total cases and 42.37 percent of total tumors of dog. This figure shows low prevalence rate of CTVT in dogs. However, CTVT contributed high prevalence among the dog's tumor. Three males and nine females belonging to six breeds were found to be affected by this disease (Table 1).

Table 1 Prevalence of CTVT in dogs at CVH (June-November 2001)

Sex	Breed						Total
	Pom cross	Terrior cross	Labrador cross	Local	Apso cross	Bhote	
Male	1	0	0	1	0	1	3
Female	2	3	2	1	1	0	9
Total*	3 (25)	3 (25)	2 (16.66)	2 (16.66)	1 (8.33)	1 (8.33)	12 (100)

Nine of the 12 tumor cases were treated by surgical excision. The three remaining cases were not intervened surgically because two animals were very old and the third case was tried with chemotherapy, (vincristine sulphate at the rate of 0.05 mg per kg body weight) but showed severe side effects like vomiting, diarrhoea and nausea. During the follow up, reoccurrence of the problem was found in two cases (22.22 percent) of the surgically treated dogs. One bitch (11 per cent) passed away among the nine treated patients probably due to extensive bleeding and post-operative complications. It was found that the efficacy of surgical treatment was 66.67 per cent. Table 2 shows the efficacy of surgical treatment in CTVT as recorded in November 2001.

Table 2 Efficacy of surgical treatment in CTVT as recorded in 2001.

Sex	Follow up result			Total
	Cured	Reoccured	Death	
Male	2	1	0	3
Female	4	1	1	6
Total	6(66.67)	2 (22.22)	1 (11.11)	9

DISCUSSION

Out of 2,870 registered canine cases, CTVT was confirmed pathologically in 0.41 percent of the cases. Among dog's tumor CTVT alone shared 42.63 per cent. From the study, CTVT was found to be more prevalent in females (75 per cent). Jubb and Kennedy (1970) also reported CTVT to be more common in females. The age of infected male and female dogs ranged from 2.5 to 6 years and 3 to 13 years with an average of 4.5 years and 7.2 years respectively in the present study. Das and Sahay (1989) reported the average age of male and female dogs to be 5.5 and 7.2 years respectively.

It was difficult to identify breed effect in this disease during this study, because most of the dogs were crossbreeds. Although the involvement of small breeds was seen to be more in this study, it cannot be stated clearly due to the small sample size. In other studies, Das and Sahay (1989) have reported the predominance of large breed dogs in this disease.

Hematological study of tumors showed blood parameters within a normal range. According to report of Das and Sahay (1989), the average haematological parameters do not deviate except on the occurrence of polycythemia in CTVT. Similarly, histopathological features of the tumors were suggestive of TVT in all examined tissues.

During follow up, reoccurrence was found in two dogs (22.22 percent). One bitch died because of extensive bleeding and probably due to infection. Effectiveness of surgical treatment combined with cauterization with copper sulphate crystals was found to be 66.67 percent indicating the effectiveness of surgical therapy for this problem.

All TVT cases brought at CVH revealed the history of mating with stray dogs/bitches. Dogs and bitches with TVT were reported to be active and their general health conditions were found normal except twelve and thirteen years old two females who had attained senile ages. Clinical signs observed were bleeding from genitalia, blood mixed urine and straining at the time of micturition in all cases, protruding mass from the vagina in few females, bulging appearance at the site of glans in the affected males.

Two males were castrated at the time of removal of the tumor. Four females were spayed by ovariectomy after a couple of months of removal of the tumor. In one male, multiple growths were noticed on the surface of penis. An extensive tumor inside vagina in one bitch was removed through an episiotomy.

CONCLUSION

CTVT was found to be quite common canine tumor problem in cases reported at Central Veterinary Hospital. This high prevalence rate was probably due to the chances of pet dogs to mate with stray dogs, because CTVT is common in free-roaming dogs. The disease was more prevalent in females. The disease was found to be common in all age groups in females but more in young males. Reoccurrence rate of 22.22 percent suggested that the surgical excision combined with cauterization was comparatively best and cost-effective treatment in this disease.

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PREVALENCE OF INTERNAL PARASITES IN YAK AND OTHER ANIMALS OF LANGTANG VALLEY

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ABSTRACT

A study conducted to determine the prevalence of internal parasites in the animals reared in the high altitude Langtang valley of Nepal showed that all species of farm animals are infected with gastrointestinal parasites. While yaks and chauries at Chandanbari were found to be infected with Fasciola and Trichostrongylus, no infection of Fasciola was recorded at Jingbu and in cattle and buffaloes of Dhunche. In horses at Kyanjin Gompa, Strongyles spp, Parascaris equorum and Oxyuris equi were the most common parasites. This study provided some indication on the prevalence of internal parasites of animals reared in the high altitude valleys of Himalayas, although the small sample size and short duration were the limitations of this study.

INTRODUCTION

The Langtang Valley is a high altitude mountainous region in the Himalayas of northeastern Nepal. The Himalayan people of Nepal are predominantly an agricultural community. Yak and Chauries (yak x local hill cow hybrid, which have a higher milk yield than female yak) are the predominant livestock of the region, providing milk, a source of fuel, and serve as pack animals. Yak and Chauries survive particularly well in the region due to the high altitude (up to 5000m). Domestic Yak and Chauries are free ranging and migratory animals (Joshi, 1982). Farmers, their families, and the herds migrate to higher elevations, following the path of the melting snow line to fresh grazing areas. Other livestock in the region include Tibetan horses, cows, water buffalo, goats and sheep.

The prevalence of parasites has been studied to a limited extent in Yaks of India and Nepal (RangaRao and Sharma, 1994). However, these studies have not focused specifically on internal parasites in Yaks of the Langtang Valley. High prevalence of internal parasites has been described in studies done on Yak population in Cheese production areas in Nepal (Joshi *et al*, 1996). Information regarding the prevalence of parasites in livestock of the Langtang Valley may be useful in improving the overall health of livestock in the region. The purpose of this study was to determine the prevalence of internal parasites of free-ranging domestic Yak, Chauries, water buffaloes and horses in the Langtang Valley.

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MATERIALS AND METHODS

Yak and Chauries were studied at two different sites, Chandanbari (elevation 3350m) and Jangbu (elevation 5000m). Samples at Chandanbari were collected from a total of seven Chauries belonging to two different farmers. Samples at Jangbu came from a total of 21 Yak and Chauries belonging to nine different farmers. At both locations, animals belonging to different farmers grazed on the same pasture, but were brought in twice daily to their respective owners for milking. Fecal samples were collected during milking times. Local custom prohibited obtaining feces per rectum; animals were closely monitored and feces collected from the ground in a clean latex glove or zip-lock bag immediately after the animal defecated.

Samples were collected from eight horses at Kyanjin Gompa (elevation 3850m), using the same method as above. Horses were also free ranging on pasture. Samples were also collected from seven water buffaloes by the same method in the town of Dhunche (elevation 1950m). Water buffaloes were housed in permanent corals and were used for milking.

All samples were analyzed by two methods: Modified McMaster technique for determining prevalence and eggs per gram (EPG) of protozoa and nematode eggs; and sedimentation technique for determining prevalence of trematode eggs. None of the animals studied had ever been treated with deworming medication previously. All samples were collected during the rainy season (monsoon).

RESULTS AND DISCUSSION

Examination of seven fecal samples from chauries in the Chandanbari site showed the prevalence of *Eimeria* spp., *Trichostrongylus* spp., *Strongyloides* spp., *Fasciola hepatica*, and *Toxocara vitulorum* (Table 1). Examination of 21 fecal samples from yak and chauries in the Jangbu site showed the prevalence of *Trichostrongylus* spp., *Strongyloides* spp., and *Toxocara vitulorum*.

Table 1 Prevalence of internal parasites in yak and chauries (fecal examination)

Site	<i>EIMERIA</i> SPP. (%)	<i>TRICHOSTRONG</i> <i>YLUS</i> SPP. (%)	<i>STRONGYLOID</i> <i>ES</i> SPP. (%)	<i>TOXOCARA</i> <i>VITULORUM</i> (%)	<i>FASCIOLA</i>
Chandanbari (3350 m)	71.4	14.3	85.7	57.1	42.9
Jangbu (5000 m)	0	33	23.8	4.7	0

Fecal examination of 12 cows and water buffaloes samples in Dunche showed the prevalence of *Trichostrongylus* spp., *Eimeria* spp., and *Paramphistomum* (Table 2).

Table 2 Prevalence of internal parasites in cows and water buffaloes on fecal examination

Site	<i>EIMERIA SPP.</i> (%)	<i>TRICHOSTRONGYLUS SPP.</i> (%)	<i>PARAMPHISTOMUM</i> (%)
Dhunchhe	50	67	16.6

Examination of eight fecal samples from horses in Kyanjin Gumpa showed the prevalence of Strongyles, *Eimeria* spp., *Parascaris equorum*, and *Oxyuris equi* (Table 3).

Table 3 Prevalence of internal parasites in horses on fecal examination

Site	<i>Eimeria spp.</i> (%)	<i>Strongyles spp.</i> (%)	<i>Parascaris equorum</i> (%)	<i>Oxyuris equi</i> (%)
Kyanjin Gumpa	25	100	37.5	50

Like other studies of yak and chauries (RangaRao and Sharma, 1994), this study showed that *Fasciola* spp was the predominant trematode. The lower prevalence of *Fasciola* at higher elevation may be due to the inability of *Lymnaea* snails to live at such high elevations. Further studies are needed to determine this difference. Prevalence and average EPG of nematodes are also consistent with other studies (RangaRao and Sharma, 1994).

Further studies are needed in order to have a broader understanding of the internal parasites of livestock in the Langtang Valley. This study was limited by time and sample size. Future studies could include larger sample size; test the effects of anthelmintic medications on milk production and overall health, and sample collection during the dry season, which may affect the prevalence of *Fasciola* in bovids.

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EVALUATION OF ARTIFICIAL INSEMINATION PROGRAMME IN RUPANDEHI AND KASKI DISTRICTS OF WEST NEPAL

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ABSTRACT

The result of artificial insemination in cattle and buffaloes for conception rate, number of male calves born and their survival trend were studied in Kaski and Rupandehi districts of Nepal. Among the 318 cattle and 224 artificially inseminated buffaloes monitored in Kaski, 190 cattle and 124 buffaloes were found pregnant with the conception rate of 59.7% and 55.4% respectively. Similarly, among the 1080 inseminated cattle and 123 buffaloes monitored in Rupandehi 586 cattle and 50 buffaloes were found pregnant resulting the conception rate of 54.3 and 40.6 percent respectively. The number of inseminations/conception for cattle and buffaloes were 1.67 and 1.80 respectively in Kaski, and 1.84 and 2.46 in Rupandehi. One hundred eighty-five male and 194 female calves were born from 406 cattle in Rupandehi resulting 48.8:51.2 male female calf ratio, with 20 cases of abortion. Similarly, 55 male and 57 female calves were born from 117 cattle including 5 abortion cases resulting the male female calf ratio of 49:51 in Kaski district. In buffaloes, the male and female calf ratio were 48:52 and 50:50 in these districts respectively. About 95% female calves were found well grown at the time recording while most of the male calves were either already dead or very poorly maintained.

INTRODUCTION

Artificial insemination (AI) service is included in the regular programme of almost all District Livestock Service Offices (DLSOs) of Terai region and in some Hill districts mainly Kathmandu, Lalitpur and Kaski. A total of 41032 animals were artificially inseminated in Nepal during the year 1999/2000 (Annon, 1999/2000a), among which, 11768 inseminations were carried out in the Western region (Annon., 1999/2000b). However, the information about the conception rate of these inseminations, the number of male and female calves born and their present status are usually lacking. Hence the present study was carried out to evaluate the outcome of AI carried out in Kaski during 1999/2000 and in Rupandehi district during 2000/2001. The objective was to evaluate the effectiveness of AI services in the districts and to identify the reasons for the success or failure of this service.

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MATERIALS AND METHODS

Cattle and buffaloes inseminated during July to October, 1999 in Kaski and July to August, 2000 in Rupandehi were monitored in October and July/August months of the succeeding year respectively. The formats with owners' names, address, date of insemination, species and breed of animals, semen straw number (bull number) were provided to the technicians. They visited the farmer/farms to record the number of conceived animals, the date of parturition, number of abortions, and male and female calf born and present status of these calves. The technicians other than inseminators were selected to record the information in order to avoid the biasness if any. Similarly, the insemination monitoring results recorded by Semen Bank Bhairahwa in FY 1999/2000 are also included in this study to obtain more accurate information.

RESULTS AND DISCUSSION

Conception Rate

Out of 318 inseminated cows of different breeds monitored, 190 were found pregnant in Kaski district resulting in 59.7% conception rate in cows (Table 2). One hundred twenty-four buffaloes from 224 recorded were found pregnant resulting in 55.4% conception rate. Among the 1080 cows and 123 buffaloes monitored in Rupandehi district 586 cows and 50 buffaloes were found to be pregnant resulting 54.3% and 40.6% conception rate respectively (Table 1). The rate of conception was variable from one AI center to another indicating its dependency on the skill of inseminators. This could be the reason of higher conception rate in Kaski district. However, the conception rate recorded in both districts is still higher than the average conception rate of 51.23 in Western region (Annon., 1999/2000b) and 36.2% in Eastern region (Annon., 1999/2000c).

Table 1 Conception rate of artificially inseminated cattle and buffaloes in Rupandehi district.

S N	A.I. Center	Monitoring Office	No. of recorded animals			No. of Pregnant animals			Conception Rate		
			Cow	Buff	Total	Cow	Buff	Total	Cow	Buff	Avg.
1	DLSO Bhairahwa	DLSO	155	19	174	81	9	90	52.26	37.37	55.52
		Seman Bank	98	18	116	58	13	71	59.18	72.22	
		Total	253	37	290	139	22	161	54.94	59.46	
2	LSC Jogikuti	DLSO	300	23	323	174	12	186	58	52.20	57.14
		Seman Bank	87	10	97	51	3	54	58.82	30	
		Total	387	33	420	225	15	240	58.14	45.45	
3	LSC Kerwani	DLSO	168	16	184	103	5	108	61.31	31.25	54.98
		Seman Bank	81	6	87	37	4	41	45.68	66.67	
		Total	249	22	271	140	9	149	56.22	40.91	
4	LSC Butwal	DLSO	96	11	107	42	2	44	43.75	18.18	45.86
		Seman Bank	41	9	50	26	2	28	63.41	22.22	
		Total	137	20	157	68	4	72	49.64	20	
5	LSSC Manpakadi	DLSO	30	7	37	6	0	6	20	0	21.54
		Seman Bank	24	4	28	8	0	8	33.33	0	
		Total	54	11	65	14	0	14	25.93	0	
District Total			1080	123	1203	586	50	636	54.26	40.65	52.87

Table 2 Conception rate of artificially inseminated cattle and buffaloes in Kaski district.

S N	A.I. Center	Monitoring Office	No. of recorded animals			No. of Pregnant animals			Conception Rate		
			Cow	Buff	Total	Cow	Buff	Total	Cow	Buff	Avg.
1	DLSO Pokhara	DLSO	156	73	229	88	40	128	56.41	54.79	56.37
		Seman Bank	124	16	140	73	7	80	58.87	43.75	
		Total	280	89	369	161	47	208	57.50	52.81	
2	Livestock Service Center	DLSO	38	135	173	29	77	106	76.31	57.03	61.27
		Seman Bank	-	-	-	-	-	-	-	-	
		Total	38	135	173	29	77	106	76.31	57.03	
District Total			318	224	542	190	124	314	59.75	55.36	57.93

Number of Services per Conception

586 cows and 50 buffaloes were found pregnant after a total of 1080 and 123 inseminations, respectively for these animals in Rupandehi (Table 1 & 3). Thus 1.84 and 2.46 inseminations were required to be pregnant for one cow and one buffalo respectively. Similarly, the number of service per conception was again within the range of recommended goal for well managed herds, as 1.60-1.75 service per conception has been considered one of the measurements of breeding success.

Table 3 Number of Insemination per Conception

S N	District	No. of recorded animals			No. of Pregnant animals			No. of Insemination/ Conception		
		Cow	Buff	Total	Cow	Buff	Total	Cow	Buff	Average
1	Rupandehi	1080	123	1203	586	50	636	1.84	2.46	1.89
2	Kaski	318	224	542	190	124	314	1.67	1.80	1.72

Male Female Calf Ratio, Calf Survival trend and Incidences of Abortion

Based on the calving records of 406 cows and 28 buffaloes, in Rupandehi the number of male and female calves born from cows were 185 and 194, and from buffaloes were 13 and 14 respectively (Table 4). Similarly, from the total recorded calving of 112 cows in Kaski, the number of male and female calves born were 55 and 57 respectively and from 116 buffaloes the number of male and female calves were 58 each.

The tendency of the farmers to grow female calves of cattle is found appreciable in both districts but most of the male calves were reported dead within a month or two due to starvation in Kaski. In Rupandehi, the recorders found about 68% male calves were alive by the age of two months. Milk market problem in this district compelled the farmers to provide milk even for male calf. It is also the general tendency of buffalo keeping

farmers of road sides and market areas of Kaski to remove the male calf immediately after birth. 'Male calf raising is not profitable' was the farmers' argument.

Out of 117 Pregnant cows and 117 buffaloes, incidences of abortion recorded in 3-7 months of gestation was 5 and 1 (4.27%, 0.85%) in Kaski. Similarly, out of 406 pregnant cows and 28 buffaloes, 20 cows and 1 buffalo aborted (4.9% and 3.6%) in Rupandehi district.

Table 4 Incidences of Abortion, Male Female Calves Ratio and the Calves' Survival trend

S. N	Output Description	Outputs			
		Rupandehi		Kaski	
		Cow	Buffalo	Cow	Buffalo
1	No. of Pregnant animals monitored	406	28	117	117
2	Incidences of Abortion	20	1	5	1
3	No. of Male calves born	185	13	55	58
4	No. of Female calves born	194	14	57	58
5	Male Female calves Ratio	48.81:51.19	48.15:51.85	49:51	50:50
6	No. of Male Live calves at 2 month age	126	13	11	Most of the male calves already removed
7	No. of Female Live calves at 2 month age	187	14	54	
8	% of Male Live calves at 2 month age	68.12	100	20	
9	% of Female Live calves at 2 month age	96.39	100	94.74	

CONCLUSION

Although artificial insemination is commonly practiced in many parts of the world, the use of this breeding technique is very limited in Nepal. Scattered breedable animals in subsistence level of farming system and low level of commercial dairy farming particularly in hills is one of the constraints to include many animals in this practice. Scarce resources such as transport facilities, limited number of technicians are greatly limiting the use of AI. Conception Rate is another key factor for livestock farmers to select AI or natural breeding. It is generally observed that inexperienced technicians are posted in newly established AI centers, resulting very low conception rate, which discourages the farmers for AI. Ignorance of most of the farmers about the time for insemination, lack of skill of the technicians to detect 'estrus', hence untimely insemination, identification and subsequent treatment for repeat breeder animals are

some of the reasons for poor conception rate in the artificially inseminated animals. Refresher practical training must be arranged to improve the skills of the inseminators.

Several times it is discussed that 62.5% exotic blood level in cattle is more appropriate for most of our farming conditions. However, neither the semen supplier units distribute the semen in this manner nor the AI centers in the field attempt to follow this pattern. In fact, progeny record keeping is becoming extremely difficult due to indiscriminate insemination. Hence there is urgent need to develop and implement strategic breeding at least in some selected dairy pockets of the country.

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STUDY ON PIG MORTALITY IN EASTERN NEPAL

S.R. Aryal¹

ABSTRACT

A study on pig mortality in Eastern Nepal was conducted during July 1999 – June 2000. Farmers were interviewed in different season of the year to collect information on mortality. The study showed an average of 10.23 percent mortality in pigs in this region. Pre-weaners had the highest mortality (20.45%) followed by post-weaners (3.49%) and adults (1.86%). Pre-weaning (32.86%) and post-weaning (5.44%) mortality were found higher in rainy season whereas, adult mortality (2.60%) was found higher in summer. Among the ailments cause of death physical injuries/crushing by the sow accounted for highest mortality in pre-weaners (52.94%) and pyrexia accounted for highest mortality in post-weaners (66%) and adults (75%) respectively.

INTRODUCTION

Pigs are important domestic animal species in Eastern Nepal. Pig farming is emerging as a potential source of commercial meat production as well as a source of income for the poor people. Out of 4.3 million pigs in Nepal, 45 percent are raised in the Eastern hills (CBS, 1993). Mortality has been found to be a major source of economic loss in pig rearing. Although pig mortality is the cause of important economic losses in the enterprise, it is often neglected by pig raisers and farmers. This study was undertaken to analyze the extent and significance of pig mortality from different age groups to find out the major causes of death and to recommend possible remedial measures.

MATERIALS AND METHODS

Pig breeding farmers of eastern Nepal having 1-5 breeding sows and fattening pigs were taken for the study. The management practices, hygienic conditions, housing facilities and type of feed were almost the same among the farmers. The pattern of mortality was studied according to age, season and probable ailments/cause of the death. The groups were divided as pre-weaners (0-2 months), post weaners (2-12 months) and adults

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(above 12 months). Data on mortality was collected three times a year (July - October, November - February and March - June) to cover three season of a year (rainy, winter and summer).

A total of 78 farmers of Udyapur, Sunsari, Dhankuta and Jhapa districts were interviewed for mortality in their pigs during three different seasons of the year. The information on ailments/cause of death was collected on the basis of the history and symptoms reported by the farmers. The ailments/cause were grouped and categorised as starvation, pneumonia, diarrhoea, physical injuries/crushing, distokia and pyrexia.

RESULTS AND DISCUSSION

A total of 10.23 percent mortality was found in pigs in this study (Table 1). The pre-weaning, post-weaning and adult mortality was found to be 20.45 percent, 3.49 percent and 1.86 percent respectively (Table 1). The highest incidence of mortality was recorded in pre-weaners than in post-weaners and adults which might be due to higher susceptibility of piglets to environmental stress and diseases.

Table 1 Age-wise mortality rate of pigs.

Age (months)	Mortality rate
Pre-weaners (0.2)	153/748 (20.45)
Post-weaners (0-12)	21/602 (3.49)
Adults (above 12)	8/429 (1.86)
Mean pig mortality	182/1779 (10.23)

Aryal, *et al.* (1992) earlier had reported 10 percent pre-weaning mortality in Pakhribas pigs when they were weaned at 60 days of age and Rai and Kshatri (1994) had reported 16 percent mortality in Pakhribas pig nucleus breeding units in the Koshi hills of Nepal. Gatenby and Chemjong (1992 as cited by Aryal *et al.*, 1996) reported 21.5 and 14.6 percent pre-weaning mortality in local and Pakhribas cross-bred respectively in the hills of eastern Nepal. The pre-weaning mortality rate of pig in present study was found to be higher than in Pakhribas farm and lower than in farmers' conditions than the previous study. However, the farmers in Eastern Nepal, generally slaughter the pigs when they are sick rather than to treat the sick animals. In such circumstances, the mortality rate could still be higher as there is a chance of death of sick animals.

According to seasonal variation, highest pre-weaning mortality was found in rainy season (32.86%) followed by summer (16%) and winter (14.89%) (Table.2). Likewise in post-weaning group highest mortality was recorded in rainy season (5.44%) followed by summer (3.14%) and winter (2.5%) (Table.2). On the other hand, highest adult mortality was found in summer (2.6%) followed by rainy (1.89%) and winter (0.97%). The exact reason of higher mortality in rainy season in pre-weaners and post-weaners is not known, however, it may be due to relatively higher humidity accompanied with high temperature creating favorable condition for the growth and multiplication of harmful organisms that may possibly led to higher incidence of diseases.

Table 2 Season-wise mortality rate of pre-weaners, post-weaners and adults

Seasons	Pre-weaners	Post-weaners	Adults
Rainy (Ashad-Ashwin)	70/213 (32.86)	8/147 (5.44)	4/211 (1.89)
Winter (Kartik-Magh)	35/235 (14.89)	5/200 (2.5)	1/103 (0.97)
Summer (Falgun-Jestha)	48/300 (16)	8/255 (3.14)	3/115 (2.60)
Total	153/748 (20.45)	21/602 (3.49)	8/429 (1.86)

Among the ailments or cause of death, more than 50 percent pre-weaning mortality (52.94%) was due to physical injuries/crushing by the sows followed by diarrhoea (21.57%), pneumonia (15.03%) and starvation (10.46%) (Table.3). This result suggests that management factor is responsible for the majority of pre-weaning mortality. In a study, in the United States of America, Tubbs *et al.* (1993) found that 43.2% of mortality was due to trauma followed by starvation (20%), unknown causes (13%) and scours (10.85%). Likewise Rautiainen (1994) in Finland found that, 80% pre-weaning mortality was due to non-infectious causes such as trauma, hypoxia, starvation and the results of the present study are similar to the other workers. These results agree well with the present study.

Among the ailments/cause of death in post-weaners and adults, 66% and 75% mortality were due to pyrexia respectively (Table.3). However the exact cause of pyrexia was not known.

Table 3 Mortality of pig based on different ailment/causes

Types	Total number died	Ailments/cause					
		Starvation	Physical injuries/crushing	Pneumonia	Diarrhoea	Distokia	Pyrexia
Pre-weaner	153/748 (20.45)	16 (10.46)	81 (52.94)	23 (15.03)	33 (231.57)	-	-
Post-weaner	21/602 (3.49)	-	-	3 (14.29)	4 (19.05)	-	14(66)
Adults	8/429 (1.86)	-	-	-	-	2(25)	6(75)

Note: Numerator denotes the number of death, denominator denotes the total number of piglets born, & post-weaners and adults in the farm; figures in the brackets indicates percentage

Result of the present study showed that lack of awareness among farmers and lack of proper management of the pigs were the major causes of pig mortality in eastern Nepal. Better management practices such as use of farrowings pen, care and protection of newly born piglets from the sow, hygiene of the farrowing pen and the surrounding environment may reduce the mortality in pre-weaners. Likewise better hygiene of the surroundings and regular vaccination against important swine diseases may help to reduce the losses in post-weaners and adults. Thus extension services and training to create awareness in the farmers are recommended to reduce pig mortality in the region.

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SEASONAL OCCURANCE OF HAEMORRHAGIC SEPTICAEMIA IN MID-WESTERN REGION OF NEPAL

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ABSTRACT

Haemorrhagic Septicaemia (HS) is one of the most important endemic diseases in the Mid-Western region of the country. Monthly occurrence of HS outbreaks reported in the Mid-West region for four years were analyzed. It was found that the disease had two-peaks in its occurrence every year. The possible strategy to control these outbreaks could be the vaccination of animals during the month of April and September.

INTRODUCTION

The mid-western region comprises of 15 districts of Rapti, Bheri and Karnali zones of Nepal and is located in between western and far-western region. This region extends right from Terai to inner Terai, Hills and up to Mountains. The most remote known districts like Humla, Mugu, Kalikot Dolpa and Jumla of Karnali zones also lie in this region. So, most of the districts of this region are remote and not easily accessible. The main domesticated livestock include cattle, buffaloes, goats, sheep, yak, and horses. The domesticated livestock and poultry rearing is mostly in traditional subsistence system which is an integral part of agricultural system in this region. Hemorrhage septicemia (H.S.) is one of the most commonly occurring disease in this region. The present paper analyses the pattern of occurrence of this disease in this region, which would assist to develop the strategy for control of this disease in this region.

MATERIALS AND METHODS

The monthly epidemiological reports produced by 15 District Livestock Service Offices (DLSO's) of the mid-western region between 1994 to 1998 were analysed to see the occurrence of H.S. in the mid-western region. Total number of cases of H.S. in a month reported from 15 districts of this regions was taken as an indicator of its occurrence. Number of cases reported every month was compiled for the period of 4 years (Table 1).

RESULTS AND DISCUSSION

Though the epidemiological reporting system in Nepal has several shortcomings, like reporting based on symptomatic diagnosis, irregular reporting, but it is the only information system that is directly reported from the farms and fields. As the most

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common shortcoming in these reports are the number of outbreaks, number of deaths and population at risk, hence to rule out these mistakes only the number of cases was taken as an indicator of the occurrence of this disease. The number of cases of H.S. reported from the districts for four years is presented in Table 1.

Table 1 Outbreaks of H.S. in Mid-Western Region during 1994-1998.

Months	1994-95	1995-96	1996-97	1997-98	Total of 4 yrs.
July	137	167	85	45	434
August	252	56	59	212	579
September	641	50	70	0	761
October	175	1281	97	95	1648
November	4	965	5	30	1004
December	13	10	0	42	65
January	17	11	0	22	50
February	16	134	3	26	179
March	11	43	65	70	189
April	33	3	65	4	105
May	926	56	19	192	1193
June	312	143	168	0	623

From the above table, it is evident that, although the disease occurs throughout the year, its occurrence was high during the month of October, November and May with two peaks, one during the October- November months and other during May. The disease is in low profile during the winter months (December to March) but begins to increase with the summer months. This pattern gives an indication that the Haemorrhagic Septicaemia (H.S) in the Mid-Western region of Nepal follow the trend with two main season of its outbreak i.e. one before the monsoon and another in monsoon or soon after monsoon. Hence, to control these outbreaks, vaccination in the month of April and September can be proposed assuming that oil adjuvant vaccine give protection for 5 months. This strategy will be helpful to reduce the loss due to H.S. at regional well as at the national level.

INCLUSION BODY HEPATITIS-HYDROPERICARDIUM SYNDROME IN BROILERS IN NEPAL: A CASE REPORT

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ABSTRACT

Nepalese poultry industry has experienced a new emerging disease of economic importance in the year 2003. On the basis of postmortem and histopathological examinations of the affected birds, the disease was tentatively diagnosed to be Hydropericardium Syndrome. Subsequently, the provisional diagnosis was confirmed as Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS) at IVRI. The disease was confined at four locations in Kathmandu, two locations in Bhaktapur and one location in Kabhre Palanchok districts. The disease has been recorded only in broiler birds.

INTRODUCTION

Hydropericardium Syndrome is an emerging disease of economic importance causing heavy mortality. The mortality rate ranges from 10-80%. Some farms do experience 100% mortality. Generally, it is a disease of broiler birds but it also affects layers and parent stocks. It is commonly known as Angara disease in Pakistan. In India, farmers generally call this disease as Leechi heart disease. It is so called because the heart of the affected bird typically resembles a peeled Indian Leechi fruit. Synonymously, it is also known as Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS) and Hydropericardium-Hepatitis Syndrome (HP-HS).

The disease is caused by some unknown factors in combination with an Adenovirus. Among the 12 serotypes of fowl Adenovirus (FAV), serotype 4 has been found to involve in causing IBH-HPS in South American countries, viz. Peru, Ecuador, Russia, Pakistan, Japan and India. Immunosuppressive effect from Infectious Bursal Disease (IBD), or other diseases and factors enhance pathogenicity of Adenovirus infections. The disease was first reported in Angara Goth, Karachi, Pakistan in 1987. Use of the term Infectious Hydropericardium was suggested instead of HPS because of infectious nature of the FAV serotype 4 used to reproduce hydropericardium in young chicken.

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CASE HISTORY

An outbreak of a disease in commercial broiler farms was reported from Kathmandu valley during June 2003. Increased feed intake, drowsiness, and leg stretching were some of the symptoms. The characteristic posture, with their chest and beak resting on the ground and closed eyelids were noticed. The birds were found dead in the same posture after four to five hours.

Post mortem examinations of the affected birds showed following abnormalities:

- Liver swollen, fragile and pale yellow in color. Yellowish tiny necrotic foci are present on the surface of liver.
- Accumulation of straw colored fluid and in some cases jelly like substance in the pericardial cavity. Appearance of tiny reddish patches on the myocardium suggesting petichial haemorrhage.
- Spleen was found to be quite double in size than that of normal. Pinpoint, stellate shaped reddish patches on the surface of spleen indicative of haemorrhage.
- Lungs dark in color and cyanosed suggesting early congestion.
- Kidney swollen and pale in color. Sub capsular tiny red spots were found to be distributed extensively over the surface of the kidney suggesting severe haemorrhage. The uppermost lobe of the left kidney was seriously affected in all cases.
- Bursa of fabricius and thymus were atrophied. Thymus was congested in most of the cases.
- The leg bones of the majority of broiler birds were weak, easily broken on slight finger pressure and it was rubbery in one of the bird.

Different organs collected during postmortem were preserved in 10% neutral buffered formalin. These samples were processed, impregnated, made in block, cut the section and stained as per routine histological procedure in histopathology unit, Central Veterinary Laboratory, Kathmandu.

- Histopathological examination of liver showed severe hemorrhage, hepatocytes with pyknotic nuclei, fatty degeneration and focal necrosis of the hepatocytes. vacuolation of the endothelial cells of the tunica intima of blood vessels. Presence of basophilic intranuclear inclusion bodies in some of the hepatocytes. The nuclei of such hepatocytes were highly basophilic and greatly enlarged. Infiltration of mononuclear cells especially around the blood vessels.

- Heart revealed hemorrhage but not extensive in nature. Edema of the individual myofibrils with degenerative changes. Infiltration of mononuclear cells between the separated myofibrillar bundles.
- Kidney with extensive hemorrhage and edematous nephrons. The renal corpuscles showed basophilic staining characteristics.
- The brain was normal.
- The section of spleen shows severe hemorrhage. Lymphocytosis in the medullary area. Lymphocytes with pyknotic nuclei in the regions of artery in the red pulp.

The samples collected from field (29 serum, 2 pericardial fluids, one set of sample including Heart, liver, spleen, kidney and Bursa of fabricius in 10 % neutral buffered formalin and another set of samples including liver, heart, kidney and Bursa of fabricius in ice pack) were sent to IVRI, India where the disease was confirmed to be Inclusion Body Hepatitis-Hydropericardium Syndrome (IBH-HPS).

DISCUSSION

The clinical pattern of the disease and its postmortem details were the same as that reported in other countries. However, average mortality rate was less. Morbidity was not apparent in our context, which is contradictory. Increased feed intake, leg stretching and easily breakable leg bones seem to be new clinical symptoms. The histopathological findings in the affected liver, heart, kidney and spleen were similar as published in different international journals and standard textbooks. However, the findings may vary on examination of a large number of histopathological samples collected from different farms.

Nepal had never experienced IBH-HPS in the past or if it were, probably no authentic report is available. The disease has got a quick recognition due to serious financial loss to the poultry farmers and its quick spread from farm to farm. It has appeared as a new challenge to Nepalese poultry industry especially among broiler birds in Kathmandu, Bhaktpur and Kabhrepalanchok districts.

RECOMMENDATION

- An epidemiological survey of the disease needs to be carried out to know the current status of the disease.
- A Control strategy seems to be formulated and adopted so that the disease does not spread in a new area or locality.
- More often, the disease exists in concurrence with Chicken Infectious Anaemia Virus (CIAV). Therefore, a detailed investigation of haematological and biochemical

pictures of blood from affected birds may be carried out. And samples collected from the field cases needs to be tested against CIAV also.

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