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Editorial

In the current scenario of Global warming and Climate change new pathogens are emerging causing various livestock and poultry diseases including zoonotic diseases. The environment also has direct impact on livestock production systems. Recent natural calamities like earthquake, flood, landslides also predispose certain livestock and poultry deseases. This Nepalese Veterianry Journal has been playing a key role in documentation of research, clinical studies, socio economic studies, policies to be undertaken and is ready in hand information regarding recent activities of Veterinary and Animal sciences in Nepal and relevant useful informations from other coutries.

The present day demand is on the papers with molecular studies (on DNA and RNA). There are very few papers with molecular touch. The authors hope that there will be more papers on molecular studies of Nepalese pathogens which will ultimately help in disease control strategy and vaccine production according to national demand. There are very limited information regarding effect of climate change on animal health and livestock production system. It requires more studies for generation of information.

Nepal Veterinary Association is playing a key role in dissemination of technical knowledge by organiging trainings, conferences, technical seminars, involving in various activities in animal health and regular publication of Nepalese Veterinary Journal. With the concept of one health policy lot of issues regarding zoonotic diseases eg. birdflu, rabies, brucellosis and leptospirosis etc. have been addressed in this Journal. The studies carried out so far is really not enough unless causative agent are isolated and identified. So, future studies should concerntrate on these aspects

Lot of Veterinarians of this country has exposure in foreign countries and has carried out outstanding research on animal health. They can give inputs in carrying out similar type of studies to enhance the working facilities, capabilities inturn improving quality scientific papers. The editorial board expects necessary efforts from new generation Veterinarians for quality research and publication on livestock and poultry diseases including zoonotic diseases.

The editorial board members are highly thankful and appreciated for their efforts in correction and reviewing scientific papers and thank all the authors for their contribution. Thanks are due to all personnels involved direcly or indirectly for publication of this Journal. Last but not least, Thanks goes to Ms. Pramina Shrestha, who has really done very hard secretorial job.

Dr. U. M. Singh, Ph. D. (Vet. Path.)

Editor-in-Chief

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Sero-prevalence of Brucellosis during Premonsoon and Post Monsoon Seasons in Different Farm Animal Species of Nepal

U. M. Singh, P. Shrestha, M. Prajapati, M. P. Acharya, S. P. Shrestha., D. R. Khanal and B. R. Joshi

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ABSTRACT

Brucellosis in human causes Bang's disease or Malta fever or Undulant fever in man which is a disease commonly seen in shepherds of migratory sheep and goat flocks. In animals it is manifested as abortion, mostly during third trimester of pregnancy and infertility. The transmission of this disease is via aborted placenta and aborted materials and contaminated fomites. A study was conducted to find the prevalence of Brucellosis in different livestock species by ID Screen Brucellosis serum Indirect Multispecies ELISA kit (ID Vet, France). Serum samples were collected from cattle, buffaloes, sheep, goat, pigs, yaks and chyangras during premonsoon and post monsoosn seasons. Premonsoon samples comprised of a total of 1280 serum samples of different ecozones mountain (523), hills (387) and terai (370). During premonsoon, the total samples tested from goat, sheep, cattle, buffalo, pigs, yak and chyangra were 393, 110, 355, 227, 91, 70 and 34 respectively. A total of 38 samples (2.92%) were found positive by ELISA. Ecozonewise 17(3.25%) samples from mountain, 5(1.29%) from hills and 16(4.32%) samples from terai were positive. Similarly, postmonsoon samples comprised of a total of 1256 serum and 25(1.99%) samples were found positive by ELISA. Samples of different ecozones montain (481), hills (362) and terai (413) were tested. During postmonsoon, the total samples tested from goat, sheep, cattle, buffalo, pigs, yak and chyangra were 381, 102, 362, 281, 60, 35 and 35 respectively. Ecozonewise 18(3.75%) samples from mountain, 4(1.10%) from hills and 3(0.73%) samples from terai were positive. Species wise highest prevalence was observed in sheep (9.09%) followed by goat (4.07%), Pig (2.0%), cattle (1.97%) and buffalo (1.32%) during premonsoon season. Similarly highest prevalence was observed in yaks (17.14%) followed by sheep (3.92%), goat (2.89%), buffalo (0.71%) and cattle (0.55%) during post monsoon season. Season wise there was no significant difference in two seasons.

INTRODUCTION

Brucellosis is a highly contagious infectious zoonotic disease. This disease affects different species of animals mainly cattle, buffaloes, sheep, goats and pigs. The disease is manifested by occurrence of reproductive disorder abortion, retained placenta and infertility. It is also called as Bang's disease, Malta fever or Undulant fever in man Transmission: Oral, nasal, conjunctiva and skin. The disease is transmitted through vaginal discharges, fetuses, placenta, urine, manure, raw milk. Major route of transmission is by ingestion. Mode of transmission to man is by contact, inhalation and accidental inoculation.

Brucella organisms are Gramm negative coccobacilli, bacteria and there are six species: *Brucella abortus* – affects, cattle, sheep, goat, pig horse and humans, *Brucella melitensis*- affects goats, sheep, cattle and humans, *Brucella ovis*- affects Sheep and *Brucella suis*- which affects pigs and humans. *Brucella melitensis* is most virulent species for humans followed by *Brucella suis, Brucella abortus* (Hirsh *et al.,* 2004).

Clinical signs in cattle are manifested are late abortion 5-9 months, retained placenta, placentitis, permanent sterility, still birth, infertility, metritis, orchitis epididymitis and hygroma of carpal joint-arthritis. In goat, abortion occurs in 4th month. In human the signs are acute irregular (undulant) fever, cold weakness, headache, joint pain, sweating. It is asymptomatic in non pregnant animals. The main objective of this study was to find the prevalence of brucellosis in different domestic livestock species from three ecozones.

MATERIALS AND METHODS

Samplings were carried out twice, during the premonsoon and post monsoon seasons. Species for sample collection were Cattle, Buffaloes, Goat, Sheep, Pigs and Yak. Premonsoon samples composed of a total of 1280 samples which included Mountain (523), Hills (387) and Terai (370) samples .During premonsoon, the total samples collected from goat, sheep, cattle, buffalo, pigs, yak and chyangra were 393, 110, 355, 227, 91, 70 and 34 respectively. Similarly Post monsoon samples composed of a total of 1256 samples also included Mountain (481), Hills (362) and Terai (413) samples. During postmonsoon, the total samples collected from goat, sheep, cattle, buffalo, pigs, yak and chyangra were 381, 102, 362, 281, 60, 35 and 35 respectively. ID Screen Brucellosis serum Indirect Multispecies ELISA kit ID Vet, France was used for conducting ELISA. Mountain samples were collected from Jumla, Mustang,

Rsuwa and Murtidhunga, Dhankuta. Hill samples were collected from Baitadi, Surkhet, Lamjung and Dhankuta. Similarly terai samples were from Kanchanpur, Bardia, Bara and Sunsari.

RESULTS AND DISCUSSION

Overall prevalence of brucellosis in Nepal was found to be 2.48%. There was no significant difference in the prevalence in all the species of animals by statistical analysis.

Prevalence of Brucellosis during premonsoon

During pre monsoon , the total sample of goat, sheep, cattle, buffalo ,pigs, yak and changra are 393, 110, 355, 227, 91, 70 and 34 respectively were tested. Overall prevalence was 2.97% and highest prevalence was observed in sheep (9.09%) followed by goat (4.07%), pig (2.97%), cattle (1.97%), buffalo (1.32%). all the samples from yak during premonsoon were found to be negative (Fig. 1).

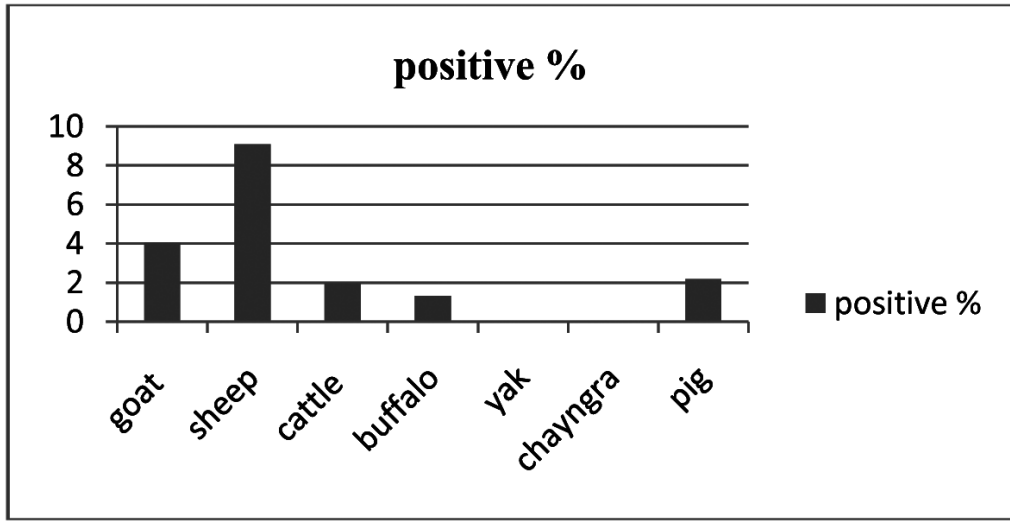

Fig. 1: Species wise prevalence of brucellosis in Nepalese livestock

Prevalence during post monsoon

During postmonsoon, the total sample of goat, sheep, cattle, buffalo ,pigs, yak and chyangra are 381, 102, 362, 281, 60, 35 and 35 respectively were tested. Overall prevalence was 1.99% and highest prevalence was observed in yaks (17.14%) followed by sheep (3.92%), goat (2.89%), buffalo (0.71%) and cattle (0.55%) (Fig. 2).

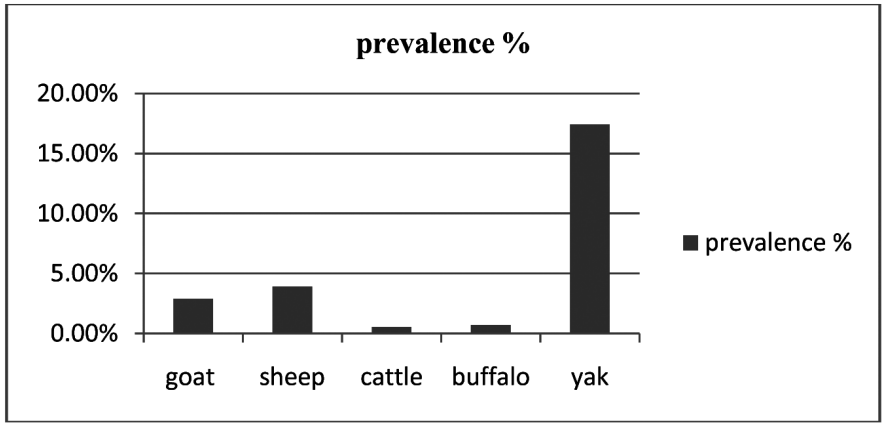

Fig. 2: Species wise prevalence during postmonsoon seasons

Ecozonewise Prevalence

During premonsoon, prevalence of disease in mountain was 3.25%, in hills 1.29% and in terai 4.32%. Similarly during post monsoon it was observed as 3.75% in mountain, 1.10% in hills and 0.73%in terai (Fig. 3).

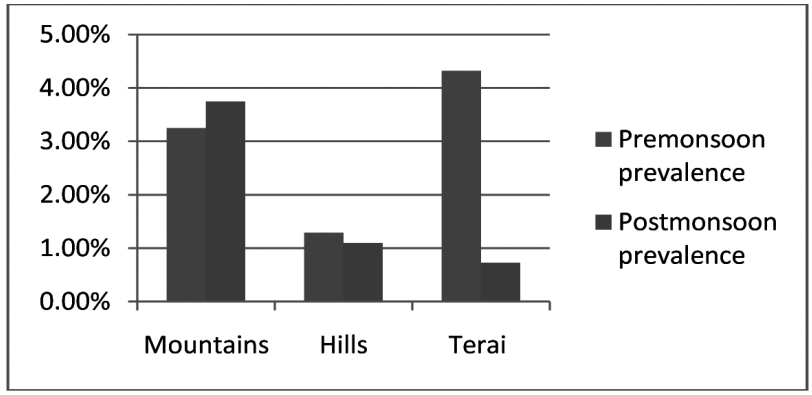

Fig. 3: Prevalence according to ecozone during pre and post monsoon seasons.

In premonsoon and post monsoon samples sheep were found to have higher positivity. In yak the positivity during post monsoon season could be due to influence of migration and infection picked up during that period. Several studies conducted on brucellosis by various authors have detected 1.28% to 5.5% prevalence in cattle, 1.93% in buffaloes which is similar to the findings of this study (Jha *et al.*, 1993, Pradhan 1996, Joshi and Joshi, 2001).

Among the infertility problems 46.1 % of total aborted cases in cattle were found to be positive for brucellosis and buffaloes were found to be negative for brucellosis (Joshi and Joshi, 2001).

In this study the prevalence of brucellosis in buffalo were 1.32% during premonsoon and 0.71% in post monsoon similar to 2.86% prevalence in slaughtered buffaloes (Dhakal *et al.,* 2005).

In sheep, prevalence during premonsoon was higher (9.09%) compare to post monsoon season (3.92%) however there was no significant difference in the statistical analysis. By Rose Bengal Plate Agglutination (RBPT) test and serum agglutination test 2.7% of 730 serum samples from sheep were found to be positive for brucellosis which was lower than the findings of this study (Jha *et al.*, 1992).

In goat, 36 (3.7%) out of 813 serum samples of goats of Koshi hills were found to be positive by RBPT and Serum agglutination test. In this study there were prevalence of 4.07% during premonsoon and 2.89% during post monsoon season, which were found to be similar. However, higher prevalence (17.14%) in goats has been recorded by Shrestha *et al.,* (2008) and 5, 36% in slaughtetred goats Dhakal *et al.,* (2005). Chyangras were found to be negative.

In pigs during premonsoon season were found to be 2.97% which was lower than the findings (7.18%) of Shrestha *et al.,* (2008). and in yak the samples were detected positive (17.14%) only during post monsoon season

Indiscriminate breeding practices, absence of animal quarantine facilities, poor disease control management, lack of knowledge about the disease among farmers

CONCLUSION

Brucellosis was detected in all the livestock species of all ecozones. This study had detected the antibodies to the Brucella infection. Detailed study has to be carried out for culture and isolation of organism from aborted samples. Molecular identification of organisms is to be carried out.

ACKNOWLEDGEMENT

The authors are thankful to all the site veterinary officers and technicians and field data recorders from different ecozones for collection of serum samples. Similarly thanks goes to Directors of Regional Agricultural Research Stations for necessary support of the programme. Thanks to the World Bank for over all funding of the programme through the Zoonosis Control Programme- NARC component. The authors are thankful to the laboratory technicians and supporting staffs of Animal Health Research Division, NARC Khumaltar for conducting laboratory analysis of the samples.

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Nepalese Vet. J. 32:7-12

Occurrence of Hypodermosis in Yaks of Mustang

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ABSTRACT

Hypodermosis, or warble fly, is a subcutaneous myiasis caused by the larvae of Hypoderma spp. Hypodermosis is a major parasitic disease due to the pernicious effects of larvae migration. In this disease the migrating fly larvae damage hides, trigger serious allergic reactions, including anaphylactic shock, and reduce milk and meat production. On 6th to 12th February 2013, during a field visit to Kobang VDC of Mustang, out of 221 yaks, 37 were found infested with Warble fly larvae. Since 2008, this problem has been noticed and found increasing in every successive year. The infested yaks were given Ivermectin (Kepromec), Pheniramine maleate (Avil) and inj. B.complex. The farmers were suggested to give Ivermectin in early December for prevention of that year. On follow up, cases were reduced drastically on those Yaks in which Ivermectin was given. In those yaks in which Ivermectin was given in December, 2013 did not show any hypoderma infestation.

Key words: Hypodermosis, Myiasis, larvae and yak

INTRODUCTION

Mustang district is one of the remote districts of high altitude northern Himalayan region of western Nepal. Kowang is one of the VDC of Mustang district which lies at the height of 2300 meter having temperate climate. Yak keeping was found very popular in that area as they are main source of meat and milk of the local people. Every year in the month of April, the *Thakalis* (native people) organize the festival of '*Bow and Arrow Shooting*' for seven days where fresh blood of Yaks are sold for drinking purpose. People believe that by drinking raw blood will cure various diseases and enhance sexual vigour. A glass (about 150ml) of blood cost about 1 US Dollar. Many diseases affect Yak reducing milk and meat production among which parasitic diseases are very common as the animals are rarely dewormed and vaccinated. Hypodermosis is one of the external parasitic disease which is also found in cattle, wild animals, sheep, deer and horses (Li, *et al.,* 2014). A study suggested that Hypodermosis in livestock is caused by *Hypoderma bovis* (warble fly), *H.lineatum, H. sinense*, *H. qinghaiensis*, and *H. diana* (Yin, *et al.,* 2003). It can also infest human beings (Lagace-Wiens *et al.,* 2008; Puente *et al.*, 2010).

H.bovis, *H.lineatum* and *H.sinense* are the common parasites affecting yaks and dairy cattles. The causative arthropod parasite is not yet reported in Yaks of Nepal. The migration and the development of the Hypoderma larvae in the cattle take about 10-11 months leading to the condition of Hypodermosis (Catts & Mullen, 2002; Karatepe *et al.*, 2013). A visible swelling in the skin of the dorsum may indicate the presence of Hypoderma larvae; however, a small number of subcutaneous Hypoderma larvae may not result in any symptoms.

Life cycle of Hypoderma

Adult hypoderma, also known as heel flies are about 15 mm long, hairy and bee like in appearance. Adults are not directly harmful to animals, neither they bite nor sting. Only their egg laying activity is the cause of nuisance to animals. Due to absence of mouthparts, hypoderma don't feed but they survive with the nutritional reserves stored before birth. They lays eggs in late spring or in early summer in about 5 to 8 eggs in rows of single hair and can deposit up to 800 eggs on a host during her life time, which is usually less than a week. It takes 3 to 7 days for eggs to be hatched which is called as first stage larva. First stage larva then migrates to the base of the hair shaft and penetrates the skin. It then migrates through the fascial planes between muscles, along connective tissue, or along nerve pathways secreting proteolytic enzymes that fascilitate their movement. Depending on the hypoderma species, larva migrates to two different regions during fall and winter. *H.lineatum* larva migrates to the submucosal connective tissue of the oesophageal wall, where they accumulate for 2-4 months. *H.bovis* larva migrate to the region of the spinal canal, where they are found in the epidural fat between the dura mater and the periosteum for a similar period (Kahn, 2005).

These larvae arrive in the subdermal tissue of the back of the host where they make breathing holes through the skin by early winter. Cysts or warbles formed around the larvae undergo 2 molts (second and third stage). The warble stage lasts in 4–8 weeks. The third-stage larvae finally emerge through the breathing holes, drop to the ground, and pupate. From theses Pupae, flies emerge in 1–3 months, depending on weather conditions. Adult flies, which do not feed, live <1 week. This whole stage of life cycle completes in 1 year (Kahn, 2005).

For the 2 species, seasonal events are similar except that those for *H lineatum* **occur** ~6–8 week earlier than those of *H bovis*. These events vary from year to year but correlate with local and regional climatic conditions (Kahn, 2005).

Clinical findings and pathogenesis

Pathological effect of warble fly is caused mainly in the larval stage when the larvae infest in the animal bodies. During penetration by newly hatched larvae, it causes extreme pain to the animals and causes hypodermal rash. Warbles are found at the back from tail head to shoulders, and from top-line to about one third the distances down the sides. Usually the cysts are firm and raised considerably above the normal contour to the skin. Mostly young animals get infested than adult animals. Death of migrating larvae near oesophageal tissue (H. *bovis*) leads to severe reactions that are fatal to animals. It causes inflammation of the oesophageal wall, dysphagia, drooling and bloat. Death of migrating larvae in the spinal cord (H. *lineatum*) leads to stiffness, ataxia, muscular weakness and paralysis of hindlimbs (Kahn, 2005)

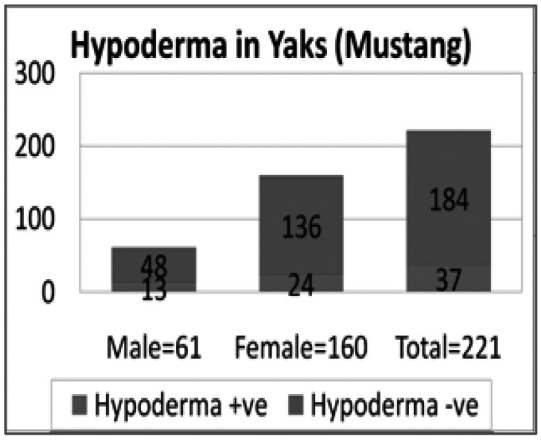
The larvae's migration and growth inside the body, deprives nourishes from yak and effects on yak's health. Appetite, milk yield, meat performance and hide quality are reduced. The yak becomes thin and their reproductive capacity decreases. The quality of fur will change as a result of the larvae's drilling; disease-resistant force drops as a result of infection. In addition, the adults cause great annoyance to the cattle such as panic, restlessness and running crazily when warble flies are laying eggs in summer. Moreover, the warble flies sometimes attack yaks and cause injuries and abortion (Hongzhi & Aihua, 2004).

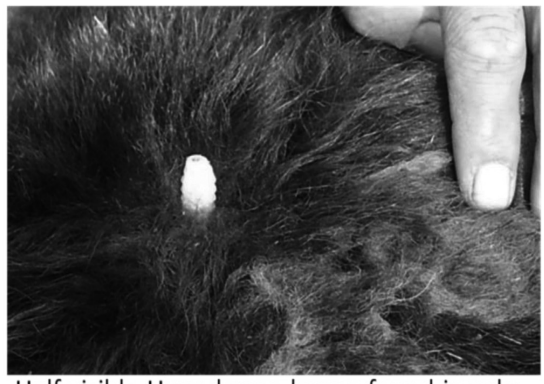
CASE STUDY

On 6th to 12th February 2013, during a field visit to Kowang VDC of Mustang district, most of the yaks were found infested with warble fly. There were a total of 221 yaks in which 160 were female and 61 were male. Out of 221 yaks, 37 were found infested with Hypoderma. The problem was noticed there in 2008. Since then the problem has been found increasing every successive year (Through personal communication with local people)

More than 10 Hypoderma was found infesting a single animal causing irritation, eruption of skin layer, rough hair coat resulting loss of production like meat, milk, hides etc. Infestation of Hypoderma causes great economic loss to farmers by reducing growth of calves, deterioration of general body condition and reduction in disease resistance power (Boulard, 2002; Hassan, *et al.,* 2010). A study done in

Qinghai province of China suggested 20% reduction in milk production though they consumed extra 500 kilograms of pasture each year (Yin, *et al.,* 2003).





Half visible Hypoderma larvae found in yaks of Mustang

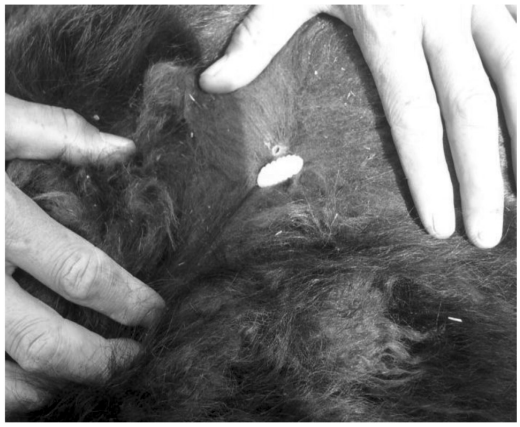
TREATMENT

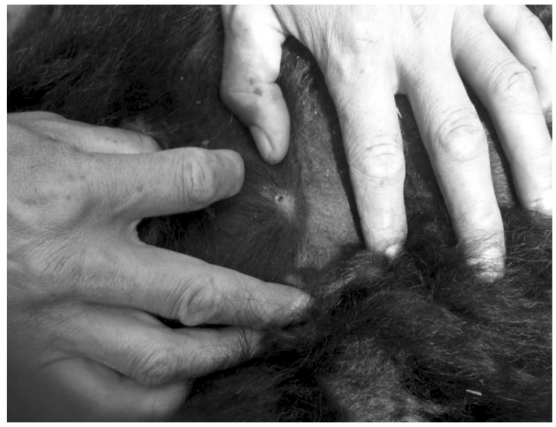
In the affected animals, larvae were taken out by hand expulsion. Drugs used were:

- Ivermectin- 7 to 10 ml s/c (75mg /50kg body weight
- Avil 10-15ml I/m
- Vit B Complex – 10ml I/m.

Farmers were advised to give Ivermectin in early December for prevention in that year.

During April 2014, during follow up, cases were reduced drastically on those Yaks in which Ivermectin was given. In those yaks in which Ivermectin was given in December, 2013 did not show any hypoderma infestation.





DIAGNOSIS, CONTROL AND PREVENTION

Warble fly infestation can be diagnosed by clinical signs and symptoms and by palpation of 2nd and 3rd stage larvae on back of infected animals during the spring and summer months (Yin, *et al.*, 2003). Disease can also be diagnosed by serological methods (Karatepe, *et al.*, 2013). Disease can be prevented by using an effective dose of Ivermectin (Li, *et al.,* 2014; Wangdi, 2011). On small groups of tractable animals, extraction by instrument or hand expulsion (by squeezing) of the individual grubs is effective. Rarely, when this procedure is performed carelessly, the grub is crushed in its cyst and an anaphylactic reaction may result (Kahn, 2005).

CONCLUSION

Hypodermosis is one of the main parasitic disease problems in Yaks of Mustang causing great economical loss in terms of milk, meat and hide. However, in this context, there is not any data concerning the incidence and degree of Hypodermosis in Yaks. The epidemiology of disease is still poorly understood. Surveillance of Hypodermosis in Yaks of other Himalayan regions of the country namely Dolpa, Solukhumbu, Jiri etc. should also be regarded in the future to assess the prevalence and production loss. More studies should be conducted on the epidemiology of disease for effective control measures to enhance the production and economical impact of the disease be assessed.

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Nepalese Vet. J. 32:13-19

Prevalence of *Toxoplasma gondii* in Different Species of Farm Animals in Nepal

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ABSTRACT

Toxoplasmosis, caused by Toxoplasma gondii, is one of the most common parasitic infections seen in human beings and other animals in most parts of the world. In Nepal, limited infoemation is available regarding this disease in farm animals. This study was undertaken to determine seroprevalence of this disease in different farm animals. A total 2692 serum samples from cattle, sheep, goat and pig were obtained randomly from different 34 districts of five different regions of Nepal and tested for Toxoplasmosis with a commercial indirect ELISA kit (ID Vet Innovative Diagnostic, France). The test results revealed that the overall seroprevalence of Toxoplasmosis was 14.64% (394/2692). Sheep had a higher overall prevalence (25.3%) compared to the 19.20% of goats, 12.14% of cattle and 19.15% of pig ($p<0.05$). Animals sampled from the Western and Far-Western regions had prevalence of 19.57% and 20.65%, respectively, which were higher than the prevalence rate found in the animals of Eastern (15.44%), Central (12.77%) and Mid-Western Region (13.57%)($p<0.05$)

Key words: Toxoplasmosis; cattle; sheep; goat; pig; ELISA

INTRODUCTION

Toxoplasmosis, caused by *Toxoplasma gondii,* is one of the most common parasitic infections found in human beings and other animals in most parts of the world. The disease has been reported in many countries with different climate. The definitive hosts are domestic cats and various species of wild felids and the intermediate hosts are mammals and birds (Nematollahi and Moghddam, 2008). Intermediate hosts of the parasite become infected by ingestion of sporulated oocysts, cyst-contaminated meats, especially from pig and sheep and also after contact with free tachyzoites or congenitally by placentary via (Pepin *et al*., 1997).

Among livestock, sheep and goats are more widely infected with *T. gondii*. It causes abortions and neonatal mortality in goats and sheep (Dubey and Beattie, 1988). But

the infection in cattle does not usually cause clinical symptoms as they have a high natural resistance to the parasite (Dubey and Thulliez, 1994). Apart from causing significant economic loss in small ruminant breeders; it causes toxoplasmosis in humans through consumption of raw meat of infected sheep and cattle (Sevgili *et al.*, 2005).

In Nepal, there is limited data available on the prevalence of toxoplasmosis in human beings but no data available in animals so far. It is well known that meat from persistently infected animals is one of the most important potential sources of human toxoplasmosis (Lundén and Uggla, 1992). So it is necessary to investigate the prevalence of *T. gondii* infection among farm animals. The main purpose of this study was to access the seroprevalence, *T. gondii* among goats, sheep, cattle and pigs in different districts of Nepal.

MATERIALS AND METHODS

This was a cross sectional study carried out from July 2013 to March 2014. A total 2692 serum samples were randomly collected from cattle (1812), goat (703), sheep (83) and pig (94) from thirty four districts of different five regions of Nepal. 518 serum samples from Fourteen districts of eastern region, 1456 serum samples from ten district of Central region, 184 samples from four districts of Western region, 258 samples from three districts of mid-western region and 276 serum samples from three districts of far - western region.

Serum samples were collected aseptically in a sterile vaccutainer following centrifugation of blood samples obtained from jugular vein of cattle, sheep and goat and ear vein of pig. All serum samples were stored at -20^0C until tested. Antibodies to *T. gondii* were tested by indirect enzyme linked imunosorbent assay (iELISA) by using commercial Kit (ID.vet Innovative Diagnostic, France).

RESULTS

In this study, out of 2692 serum samples of different species of animals, 394 samples were found to be seropositive for IgG antibodies for *T. gondii*. The test results revealed that the overall seroprevalence of Toxoplasmosis was 14.64%. Sheep had a higher overall prevalence (25.3%) compared to the 19.20% of goats, 19.15% of pig and 12.14% of cattle which was statistically significant (Table 1). However when compared in between different groups, the difference in prevalence was found to be statistically significant in cattle and goat ($p<0.00001$), cattle and sheep ($p=0.0004333$) and cattle and pig ($p=0.0450$). The prevalence was not found to be statistically significant between goat and sheep, goat and pig, sheep and pig.

Table 1: Seroprevalence of *T. gondii* in farm animals

S.No.	Species	Positive Number	Negative Number	% Positive	p Value
1	Cattle	220	1592	12.14%	p<0.00001
2	Goat	135	568	19.20%	
3	Sheep	21	62	25%	
4	Pig	18	76	19.15%	
	Total	**394**	**2298**	**14.64%**	

Animals sampled from the Far-Western and Western regions had prevalence of 20.65%, and19.57% respectively, which were higher than other development regions. Details of these are given in (Table 2). However, when compared in between different groups, the difference in prevalence was found to be statistically significant in central and western regions (p=0.011), central and far western regions (p=0.00055) and mid western and far western regions (p=0.030). The prevalence was not found to be statistically significant between eastern and central regions, Eastern and western regions, eastern and mid western regions, eastern and far western regions, central and mid western regions, western and mid western regions, and western and far western regions.

Table 2: Seroprevalence of *T. gondii* of different develop regions in goats, sheep, cows and pigs

S.No.	Region	Positive Number	Negative Number	Positive %	p Value
1	Eastern Region	80	438	15.44%	p<0.002864
2	Central Region	186	1270	12.77%	
3	Western Region	36	148	19.57%	
4	Mid Western Region	35	223	13.57%	
5	Far Western Region	57	219	20.65%	
	Total	**394**	**2298**	**14.64%**	

DISCUSSION

Toxoplasmosis in farm animals has been widely studied due to its importance to public health, since the dissemination of the parasite for man can occur either through the direct contact with domestic animals or by consuming products of animal origin. Thus, evaluation of serological tests becomes important in order to use sensitive and

specific tests in serological surveys, since the majority of the infected animals are usually asymptomatic

Although *Toxoplasma gondii* is found in most parts of the world, there are relatively few reports on toxoplasmosis in Nepal. The prevalence of *Toxoplasma gondii* infections in Nepal has been reported in pregnant women (Rai *et al.*, 1998) and in some high and low risk groups (Rai *et al.,* 1994) but no reports on toxoplasmosis in farm animals exist. The present study revealed toxoplasma antibodies in cattle, sheep, goat and pigs. The data is based on a single blood sample from each host from among an apparently healthy animal population. In the present study, the overall prevalence of toxoplasmosis in farm animals is 14.64% which is higher than that reported 9.2% from Guangxi, China (Lv *et al.,* 1994) and lower than 32.9% from Pakistan (Shah *et al.,* 2013) and 19% from Rahim Yar Khan (Punjab), Pakistan (Ramzan *et al.,* 2009).

Among the farm animals the highest prevalence of toxoplasmosis was in sheep (25%) which is similar to 25 % India (Mirdha *et al.*, 1999) and 24.5% Iran (Hashemi. *et al.,* 1996). In the present study, seroprevalence for *T. gondii* in sheep was low as compared to 35% Mazandaran province, Iran (Sharif *et al.,*_2007), 46.2% Brazil (Costa *et al.,* 2012), 44.1% West Indies (Chikweto *et al.,* 2011) and 36% Pakistan (Shah, 2013).In contrast, it is higher than 17.65% from Bangladesh (Samad *et al.,* 1993)., and 4.4% from Northeastern China (Yang *et al.,* 2013).

This might possibly be due to the difference in climatic conditions. In this study, the prevalence of toxoplasmosis in goats is 19.20% which is similar to 19.25% from Khoozestan Province, south-west Iran (Hoghooghi-Rad *et al.,* 1993)_and 19.6% from India (Mirdha *et al.,* 1999). Prevalence of toxoplasmosis in sera of goats in present study is low compared to 53.84% from Pakistan (Shah M., *et al.,* 2013), 35.5% from Malaysia (Chandrawathani *et al.,* 2008). But it is higher than 12.09% from Bangladesh (Samad *et al.,* 1993).

Likewise, the seroprevalence of toxoplasmosis in cattle is 12.14% which is lower than 16.10% of cattle from Bangladesh (Samad *et al.,* 1993), 25% of cattle from South West Pakistan and 52% from India (Mirdha *et al.,* 1999). But seropositivity for T. gondii in cows is extremely high compared to 1% in Brazil (Costa *et al.,* 2012), 0% in Khoozestan Province, south-west Iran (Hoghooghi-Rad and Afraa, 1993) and 5.7% in China (Lv, *et al.,* 1994).

In our study, the seroprevalence of toxoplasmosis in pig is 19.15% which is higher than that reported to 4.2% from Latvia (Deksne and Kirjušina, *et al.,* 2013), 9.2% and Serbia (Klun *et al.,* 2011)

Many factors such as management and hygienic standards in livestock breeding, density of cat population and environmental conditions are effective on acquisition of *T. gondii* oocysts (Tenter, *et al.,* 2000). Humidity and warm temperature favor the oocyst survival. Toxoplasmosis is more common in those areas where the environment is warm and humid as compared to dry and cold areas (Dubey, 1988). This may be attributed to the fact that ingestion of oocysts from cat faeces is the primary mode of infection of ruminants. This preliminary survey shows that *Toxoplasma gondii* infections among farm animals are quite common in Nepal.

CONCLUSION

The study gives preliminary information about the disease in different species of farm animals in Nepal which may represent a potential source for human infection with *T. gondii.* Hence, further epizootiological and parasitological investigations on toxoplasmosis in farm animals and human at the country level are required to determine the magnitude of the infection.

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Nepalese Vet. J. 32:20-26

Prevalence of Fasciolosis in River Basin Area of Saptari District

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ABSTRACT

A study on prevalence of fasciola in cattle and buffalo of Dauribharauwa V.D.C in Koshi river basin area of Saptari district was conducted from January 2012 to May 2012. A total number of 125 faecal samples were collected and were examined for the presence of Fasciola by sedimentation method as described by Hansen and Perry (1993). The overall prevalence of Fasciola was 72.8%. The prevalence of Fasciola according to species was found to be 74.66% and 70% in cattle and buffalo respectively. The prevalence of Fasciola according to sex was found to be 81.81% in female whereas 69.04 % in male cattle. But it was found to be 56.25% in male whereas 76.47% in female buffalo. The prevalence of Fasciola according to age was found to be 40%, 76%, and 78.33% in group less than two year, two to five years, and above five years, respectively.

INTRODUCTION

Fasciolosis is the most important among such diseases which reduce the productivity of livestock in Nepal. More than 50% prevalence of the disease has been reported in cattle and buffaloes in different parts of the country (Joshi, 1988). Under Nepalese condition, the major risk period of the disease is between September to February.

Fasciolosis is a highly prevalent disease in the animal population in most of the districts of Nepal where the climate and farming practices favour the prevalence of the disease. Although a proper survey in different parts of the country has not been conducted to determine the prevalence of this disease, the number of positive faecal samples reported from the veterinary hospitals in the different parts of the country indicates its widespread occurrence and high prevalence rate within the country. The prevalence may be far higher than those reported by the district veterinary hospitals because of the incomplete coverage of the veterinary services and the diagnostic facilities in the District. In 1973, Singh, Basnyat, Eichenberger and Bommili (1973;

cited by Mahato 1993) reported an infection rate of 50-90 percent in animals in areas below 1800 m and estimated an annual economic loss of Rs. 200 million due to this disease alone.

The disease is distributed in all parts of the country in all farm animals. The disease is a problem from the buffaloes of terai to the yaks of Himalayas (Joshi 1983). The disease is well recognized by Nepalese farmers and called by different local names like *namle, lew*, *galphulo, mate* in different regions of the country.

The extent of the problem, as recognized by farmers, is reflected by the drug sale (60%) that is incurred for the treatment of fasciolosis.

MATERIALS AND METHODS

Site selection

The study was carried out in Duribharuwa VDC of Sapatri district from February to July. This is the rural river basin area, where livestock are raised in the densely human populated area. Various studies have not been carried out on prevalence of fasciola in different animal of species. The area of Saptari is 1363 Km2. Total Population 570,282 (2011) and having population density 420Km2.

Collection of sample

For fresh sample collection, dung were collected in the morning from the rectum of the animal. In the cases where it was impossible to collect rectal samples; fresh faecal samples were collected from central part of faecal pit taking care not to contaminate any foreign materials with dung. The collected samples were kept in the separate plastic sleeves and marked individually for their identification. The sleeve was tightened close to the dung so as to exclude air from the container. The laboratory work was performed at the District Livestock Services Office (DLSO) Saptari.

Examination of sample

The samples collected in the morning were examined in the same day. During the study, direct technique and standard sedimentation technique were employed. Animals with fasciola eggs in dung were considered positive for fasciolosis. Gross examination of the faecal sample was performed for consistency, color, and presence of segment of parasite/mucous/blood. Microscopical examination was performed for the detection of eggs of fasciola helminth parasites as described by Soulsby (1978). A small quantity of dung were placed with a tooth pick on a slide, mixed with a drop of distilled water, spread out and examined directly.

RESULTS AND DISCUSSION

A total of 125 faecal samples were collected from Duribaruwa VDC of Saptari district in which Cattle 75 and Buffalo 50.

Gross examination of faecal sample

Consistency

The consistency of the faecal sample observed during the time of its collection was found to be of three different types: semi-solid, firm and slurry. The percentage of semi-solid type was found to be maximum (54.4%), followed by slurry type (40.8%) and firm (4.80%). The data is presented in Fig. 1.

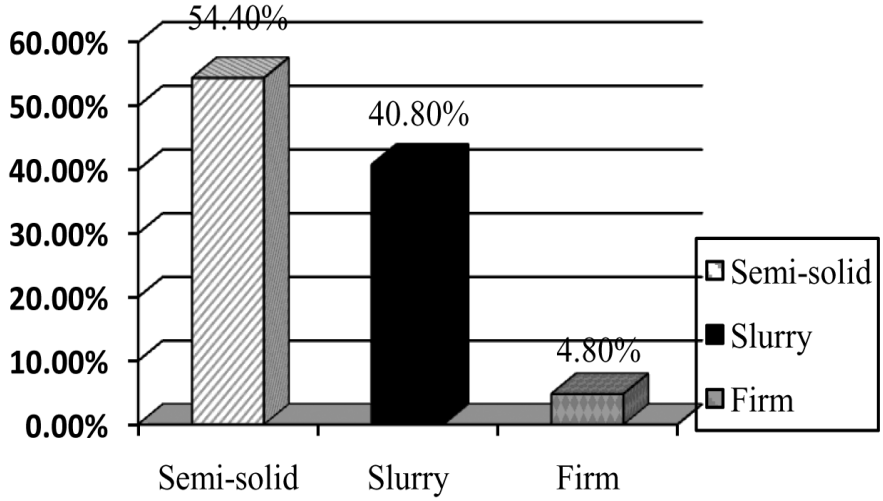

Fig. 1: Consistency of collected faecal samples

The difference in consistency of the faecal materials may be due to the type of feed to the animals, habits, health condition and the presence of fasciolosis in the animal. The animal producing slurry type of dung may be infected with fasciola causes diarrhea in infected animal.

Dung colour

The colour of the dung material of cattle collected for the examination of fasciolosis was also noted at the time of its collection. Most of the samples were greenish black (85.60%), followed by yellowish brown (8%), yellowish green (4%) and greenish grey (2.40%). The data is presented in Fig. 2.

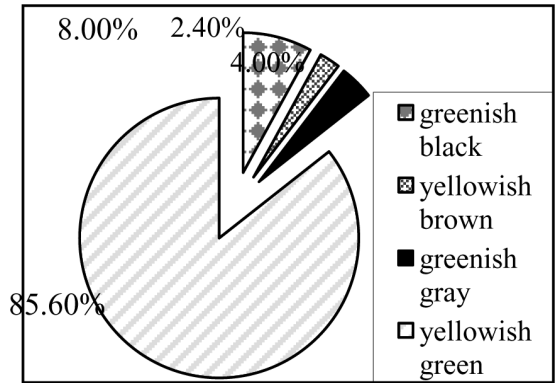

Fig. 2: Colour of the collected faecal samples

The colour of dung is mainly due to the types of the feed to the animal, for example greenish color of dung is indicative of feeding green grasses to the animal,

Faecal content

While examining the faecal materials grossly, segment of parasites, adult parasites were absent and the sample wasn't stained with the mucus or blood. There was presence of plenty of undigested fibres and pollen grains in all the samples. The presence of undigested fibres in plenty amount may be justified by the feeding habit of the cattle. Cattle were fed plenty of roughages, i.e. straw which does not digest completely and undigested fibers can be seen in the dung.

Microscopic examination of faecal sample

Overall prevalence of Fasciola

The faecal samples were examined by sedimentation method. Of 125 sample, 91 (72.8%) were found to be positive and rest 34 (27.2%) were found negative. The data is presented in Fig. 3.

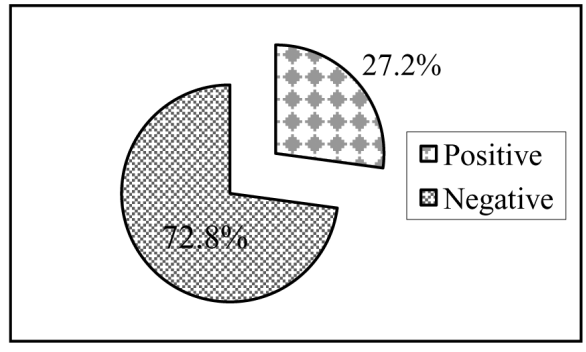

Fig. 3: Prevalence of Fasciola in the study area

The findings of the current study is comparable to Jaiswal (2006) in which70.70% were positive for overall parasitic during study on fascioliasis in ruminants at Dhanusa district and slightly more than Buddhathoki (2008) in which 67. 77 % Fasciola is the positive in the dang district as this study area (river basine) is similar to Jaiswal and different to Buddhathoki.

Prevalence According to species

A total samples 125 (75 cattle and 50 Buffaloes) dung samples were tested for presence of Fasciola. Out of 75 samples of cattle 56 (74.66%) samples were found positive where as 35 (70%) samples were found positive in buffaloes faecal samples (Fig. 4).

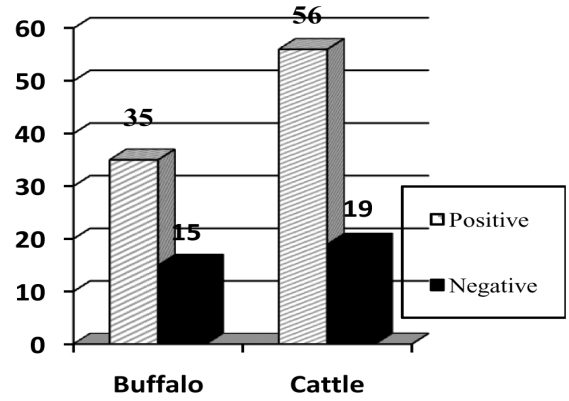

Fig. 4: Prevalence of Fasciola according to species.

Prevalence According to sex

The prevalence of Fasciola was found to be 81.81 % (27/33) in female where as 69.04 % (29/42) in bullocks. But it was found to be 56.25% (9/16) in buffalo bulls where as 76.47% (26/34) in buffalo cow (Fig. 5).

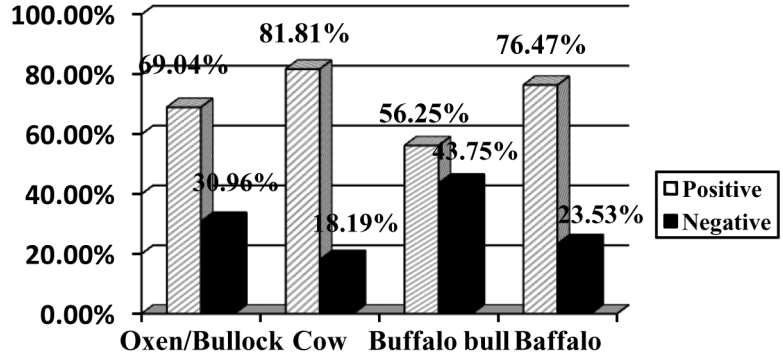

Fig. 5: Prevalence of Fasciola with respect to sex

In both species (cattle and buffalo) females have higher prevalence compared to the males. The finding is comparable with the similar findings of Mahato (1993). The higher prevalence in female may be due to the fact that most of the male are sold when they attain marketing age and a higher proportion of these animals are generally young which are less susceptible to infection. Second reason may be due to female animals are mostly stall fed during pregnancy. These animals provided with mainly rice straw which may have been infected with metacercaria and have greater chance of getting infection.

Prevalence According to age

For finding the prevalence of Fasciola according to age, the animals were divided into three groups (0-2 years, 2-5 years, ≥ 5 years).

It was found that 6(40%) out of 15 at 0-2 yrs of age both cattle and buffalo are positive while 38(76%) out of 50 animals were positive between 2-5 yrs of age. Similarly 47(78.33%) out of 60 cattle and buffalo were positive in more than 5 yrs of age. data is presented in Table 1.

Table 1: Prevalence of fasciola with respect to age

	0-2 years	**2-5 years**	**≥ 5 years**	**Total**
Cattle Positive	4	23	29	56
Cattle Negative	5	6	8	19
Buffalo Positive	2	15	18	35
Buffalo Negative	4	6	5	15
Total	15	50	60	125

The findings indicate that the younger animal gets less infection than adult one. Liver fluke infection is more common in adult than young animal, for liver fluke infection, as the age of animal increase, the chance of infection is more.

The prevalence of Fasciola increased with increasing age. This could be due to longer exposure of older animals to infection and to carrying residual infection from previous years.

CONCLUSIONS

A study on prevalence of Fasciola in cattle and buffalo of Dauribharauwa V.D.C established in Koshi river basin area of Saptari district was conducted from January 2012 to May 2012. A total number of 125 faecal samples were collected through random sampling technique. The Dung samples were examined for the presence of fasciola by sedimentation method as describe by Hansen and Perry (1993). The

overall prevalence of fasciola was (72.8%). The prevalence of fasciola according to species was found to be 74.66% and 70% in cattle and buffalo respectively. The prevalence of fasciola according to sex was found to be 81.81% in female where as 69.04 % in male cattle. But it was found to be 56.25% in male where as 76.47% in female buffalo. The prevalence of fasciola according to age was found to be 40%, 76%, and 78.33% in group less than two year, two to five year, and above five year respectively

The faecal samples were examined for the presence of fasciolosis by sedimentation method. Out of 125 samples 72.80% (91/125) were found to be positive. In the Koshi river basin area of Duribharuwa VDC of Saptari district .The prevalence of fasciolosis was found to be 74.66 % and 70% in cattle and buffalo respectively. The prevalence of Fasciola was found to be 81.81% in cow where as 69.04 % in male cattle. But it was found to be 76.4% in female buffalo and 56.25% in male buffalo.

For finding the prevalence of Fasciola according to age, the animals were divided into three groups. It was found that 6, 38, and 47 animals were positive out of 15, 50 and 60 animals resulting 40%, 76% and 78.33% infection in group Less than two year, two to five year, and above five year respectively.

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Nepalese Vet. J. 32:27-43

Assessment of Microbial Qquality of Milk Produced by Small Dairies in Kathmandu

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ABSTRACT

Milk is one of the most complete foods available in nature. The milk market of Kathmandu city is not only limited to processed milk, the contribution of product from small dairies also being quite significant. Although milk and milk products supply all required nutrients, they can also serve as the vehicle for food and water borne diseases as well as zoonotic diseases. Considering the importance of microbial quality of the milk and its role into the public health, a study was conducted to evaluate the microbiological quality of milk produced from selected small dairies in Kathmandu in the year 2009. Altogether 20 dairies were selected from four different locations and the milk samples were tested for pH, titratable acidity, total plate count, coliform count and yeast and mold count. Results indicated that acidity of milk from the eastern part of Kathmandu was comparatively higher and so was the pH value. The total plate count of all dairies was in the range of 1.272×10^6 *cfu/ml to* 4.272×10^6 *cfu/ml. Similarly, coliform count was found in the range of* 0.9×10^2 *cfu/ml to* 4.27×10^2 *cfu/ ml and the yeast and mold count was in the range of* 3.450×10^4 *cfu/ ml and* 8.000×10^4 *cfu/ml. The study revealed that the microbial quality of milk sold by small dairies in Katmandu valley is poor. Hence, there is need of serious efforts from all the concerned farmers, dairy processors and government authorities to maintain the quality of fluid milk.*

Key words: Microbial quality, chemical composition, raw milk.

INTRODUCTION

The milk, when secreted in a healthy udder is almost sterile. The contamination of milk with spoilage and disease producing microorganism may occur at any stage of production, collection, processing, marketing and utilization (Pelczynska and Ezyaztof, 1995). Microbiological quality of the milk needs to be rigorously controlled with regard to both number and type of micro flora present (Adesiyun *et al*., 1995).

Pasteurized milk must be clean, fresh and have long keeping quality as well as chemically and microbiologically safe. According to Nepal Food Law, the coliform count in Pasteurized milk must be nil per ml of milk. So, all dairy industries must produce milk and milk products of high microbial quality.

Pasteurized milk is one of the better-processed milk products, which has nutrition composition and taste similar to fresh milk. Pasteurized milk needs a strict quality control to guarantee the composition of nutrition and food safety (Varnam and Sutherland, 1994). Improving the microbial safety of perishable food is currently a major preoccupation in the food industry (Vachon *et al*., 1991). Assurance of the quality of dairy products begins at the farms and ends in the hands of the consumers (Murphy *et al*., 2000). In order to improve the quality and to increase the shelf life of milk and milk products, it is necessary to eliminate sources of post production contamination and apply proper cleaning (Gruetmacher and Bradley, 1999).

Bacterial quality of raw milk is important for both industry and consumers, since high bacterial counts on the farm contribute to poor keeping quality and inferior products (Law, 1979). The number of bacteria depends upon the cleanliness and health of the animal and millers, cleanliness and sanitation of milking utensils, and the age and storage temperature of collected milk (Sartwell, 1977).

The heat treatment of milk prior to packaging for liquid consumption, or manufacture into milk based product, is an important critical control point to ensure that pathogenic organisms are killed. It also ensures that spoilage organisms are eliminated, or at least reduced in number, for optimum quality (IDF, 1994). Gruetzmacher and Bradley (1990) cited that factors that limit the shelf life of refrigerated pasteurized milk are the microbial quality of raw milk, time and temperature of pasteurization, presence and activity of post pasteurization contaminants, types and activity of pasteurization resistant microorganisms, and the storage temperature of milk after pasteurization. Post pasteurization contamination has received most of the attention and is considered to be the factor, which limits shelf life in the majority of cases (IDF, 1993).

Gruetmacher and Bradely (1999) reported that milk pasteurized at 85^0Cand milk heated to boiling temperature had revealed greater than 3000 and 10000 cfu/ml bacterial counts, respectively. (Padilha *et al*., 1998) analyzed 250 samples of pasteurized milk from various dairies; *Salmonella spp.* was not isolated while 32.8%, 24.0% and 12.4% of the samples contained coliform bacteria, fecal coliform and mesophilic bacteria, respectively.

The greatest risk for the distribution of quality milk is microbial contamination. Since

microorganisms are ubiquitous and milk also supports their growth, it can easily be contaminated and without adequate control, organisms become able to grow to that level which causes a risk to the product quality and its safety. Microbial spoilage continues to represent the greatest economic loss to the food industry. Likewise, micro-organisms are the main causes of food borne diseases.

Globally food safety has to be prioritized and in Nepal Good Manufacturing Practices (GMP) and other practices have to be initiated by dairies and in other hand actual condition is to be known, whether practices are sufficient or not by public health point of view. Milk and milk products are the vehicles for food and water borne diseases as well zoonotic diseases. We can know the tendency of post pasteurization contamination by conducting these types of research.

Although milk and dairy products are important components of a healthy diet if consumed unpasteurized, they also can present a health hazard due to possible contamination with pathogenic bacteria. These bacteria can originate even from clinically healthy animals from which milk is derived or from environmental contamination occurring during collection and storage of milk. This study has investigate on the microbiological quality of milk produced by small dairies in Kathamandu, which ultimately will be helpful in adopting measures against the possible health hazards that may occur by the consumption of milk products without being aware about its quality.

MATERIALS AND METHODS

The methodology of the research included site selection, collection of samples, transportation of the samples to the laboratory, enumeration of total viable bacteria, coliforms and yeast and molds. This study was completed during December 2008 to July 2009.

Four parts of Kathmandu district were selected for sampling, northern part (Balaju, Kapan area), southern part (Kirtipur, Balkhu area), eastern part (Bouddha, Koteshwor area) and western part (Naikap, Thankot area) respectively. Five dairies were selected randomly from each mentioned areas as sample sources. Altogether 40 samples of ready to sell fluid milk, two from each small dairy, were collected. The samples were carried in a cold ice box to the HICAST laboratory as soon as possible for analysis.

Physico-chemical analysis

Among the various physico-chemical parameters, pH and titratable acidity were observed in the obtained samples.

pH

pH of the collected milk samples was tested using digital pH meter available in the laboratory following the procedure explained in Laboratory handbook for Dairy industry NDDB/DSP 2001.

Titratable acidity

Titratable acidity of the samples was calculated by titrating the milk sample against standard sodium hydroxide solution (0.1N) as mentioned in the Laboratory handbook for Dairy industry NDDB/DSP 2001.

Microbiological analysis

Microbiological analysis of the samples included the works concerning with enumeration of microorganisms. Total plate count, coliform count, and yeast and mold count were the microbiological operations carried out on the collected samples. The major steps have been discussed below.

Enumeration

Enumeration simply implies count. The enumeration of the sample was done for total viable aerobic microorganisms, coliform bacteria and yeast and mold. Enumeration method is based on the assumption that the microbial cells present in a diluted sample mixed with an agar medium each forms visible, separated colonies. This is obtained by mixing a decimal dilution of the food sample homogenate with the medium. After incubation of the plates the number of bacteria per gm/ml of food sample is calculated. The enumeration and isolation was carried out by applying the following main steps and techniques.

Serial dilution technique

Serial dilution technique was applied prior to the pour plate technique to thin out the microbial population in the samples. Dilutions of the milk sample were prepared by a series of sterile quarter strength Ringer's solution. Each test tube contained 9ml QSRS. QSRS was sterilized at 15 lbs for 121^{o}C for 15 minutes.

Before the removal of test portion of any samples, sample pouches were shaken thoroughly and vigorously to mix the content of each pouches to ensure representative portions. Sample pouches were inverted at least 5 times to homogenize the sample. Then 1 ml of homogenate was pipetted out and added to the first dilution tube. This made the dilution of 10 fold. This dilution was shaken well and 1 ml was pipetted out and added to the next dilution tube. Similarly, the serial dilution proceeded as a series of ten folds to the required dilution.

Preparation of culture media

Four agar media were prepared for each sample. Plate count agar (PCA) was prepared for total viable count; Violet Red Bile Agar (VRBA) was prepared for coliform count and Potato dextrose agar (PDA) was prepared for yeast and mold count.

Required amount of media were weighed out and dissolved in distilled water by boiling. All the media then were autoclaved for 15 minutes at 15 lbs pressure and 121^{o}C temperature for sterilization. All the prepared media were kept in a water bath at 45^{o}C until use (Laboratory handbook for Dairy industry NDDB/DSP, 2001).In case of PDA; pH was adjusted to 3.5± 0.1 by the addition of 10% tartaric acid solution.

Pour plate method

Before pouring the medium, each plate was labeled with sample number, date of plating, medium and dilution of sample used. 1 ml of the required diluted sample was pipetted in the sterilized petri plate with the help of sterilized pipette. Then about 15 ml of liquefied medium at around 45^{o}C was introduced into each plate by lifting cover just high enough to insert pour medium. The sample dilution was thoroughly mixed by rotating the dish first in clockwise direction and then in anticlockwise direction and then tilting the dish vertically and horizontally. Having thus spread mixture evenly over bottom of plate was allowed to solidify on a leveled surface. Plates for coliform count were allowed additional 3-4 ml of plating medium, as an overlay to cover completely the surface of the solidified medium. It inhibits the surface colony formation (Laboratory handbook for Dairy industry NDDB/DSP 2001).

Incubation

After solidification of the media, plates were inverted and incubated. PCA plates for the total plate count were incubated at 32±1^{o}C for 48 hours. VRBA plates for coliform count were incubated at 45^{o}C for 24 to 48 hours. PDA Plates for the count of yeast and mold were incubated at 25^{o} C for 5 days (Laboratory handbook for Dairy industry NDDB/DSP 2001).

Controls and sterility of glassware, media and dilutions

To check the sterility of Ringer's solution Pipettes, Plates, and medium, the control Plates were poured for each sterilization lot of dilution blank and medium used. There was a minimum of one control plate for each medium.

Colony counting

All colonies on plates were counted after incubation period .Every sample of milk had two plates of different consequent dilutions for each medium. The dilutions of

the samples were selected that the total number of colonies on at least one plate would be 30 and 300 (APHA, 1967).

The count exceeding 300 colonies in 90 mm petri dish was reported as "Too numerous to count" (TNTC). All the plates from any sample having excessive spreader growth were reported as 'spreaders' (SPR) and plates showing no colonies were reported as "No growth" (NG).

Number of colony forming units per ml was calculated using the standard formula (Laboratory handbook for Dairy industry NDDB/DSP 2001).

Where, $\sum c$ = Total microbial cells in all plates

V = Volume of sample

n_1 = Number of plates in higher dilution

n_2 = Number of plates in adjacent lower dilution

d = Dilution factor (higher dilution)

Statistical analysis

Statistical analysis of the obtained data was carried out using MS Excel version 2007 and SPSS version 16. Measures of central tendency and one way analysis of variance (ANOVA) were used as the statistical tools for the analysis of obtained data.

RESULTS AND DISCUSSION

In this study, total of 20 small dairies in four different locations of Kathmandu district were selected. Various information regarding the industry, manpower, total production and hygienic condition were gathered by means of a questionnaire. In the meantime ready to sell fluid milk samples in loose packs were collected and brought to the laboratory of Himalayan College of Agricultural Sciences and Technology (HICAST) for processing of microbiological quality assessment. The various tests performed were Physico-chemical tests like pH and titratable acidity and microbiological tests like total plate count, total coliform count and total yeast and mold count. The whole work was carried out during December 2008 to June 2009.

Survey report

Status of small dairies

During the survey, the dairies were found to occupy spaces ranging from 150 square feet to 450 square feet the average area occupied being approximately 247.7 square feet (Table-1). The number of employees ranged from one to five with an average

of 2.6 persons per dairy. Regarding any authentic registration only one among the twenty dairies was found to be registered in the local authority (Kirtipur Dairy registered in Kirtipur municipality). Rests of the dairies were found running without any registration. 2 **Table 1:** Area coverage of small dairies

Area coverage (square feet)	No. of dairies	% out of total
<200	2	10
200-300	11	55
300-400	4	20
400-500	1	5
>500	2	10

Daily milk transaction

The total amount of milk handled by twenty dairies under study was found to be 3,945 liters per day. The lowest amount of milk handled by such small dairy was found to be 50 liters and the highest as 1,100 liters. The average amount of milk handled per dairy was found to be 197.25 liters per day. The average amount of milk sold as fluid milk by a dairy in one day was found to be 77 liters, the total milk sold as fluid milk being 1,540 liters. In the study 20% of the dairies were found to process less than 100 liters of milk. 40% of the dairies handled milk within the range 100 to 200 liters. Only 15% dairies were found to process milk in amount greater than 300 liters per day (Fig. 1).

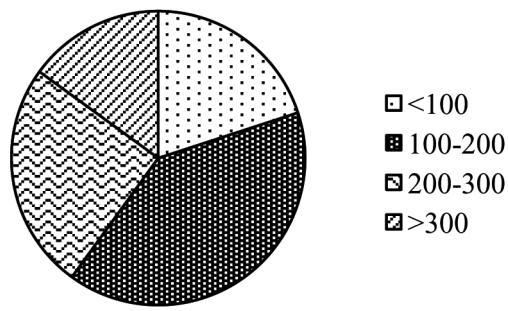

Fig. 1: Pie diagram showing daily milk transaction of the small dairies in liters per day.

Fluid milk to milk product ratio

The proportion of milk sold as fluid milk was found relatively lower than the milk used in manufacture of products. The average percentage of milk sold as fluid milk out of total milk collected was found to be 30.8%. During the study, some dairies

were found selling up to 76% of the collected milk as fluid milk while some were found to sell as least as 16%. Rest of the milk was found to be converted into various products, yoghurt being produced in the highest amount, desi butter in the second highest amount and paneer in the third highest amount. Normally it was found that the dairies handling less than 100 liters of milk per day used higher proportion of collected milk for the manufacture of products.

Training and experience of the dairy owners

Among the owners of twenty small dairies visited, only four (20%) were found to have acquired training on milk processing and dairy operation. Out of the four owners, two of them have got the training from Third livestock development project (TLDP) and rest of the two have got it from the dairy industries where they have previously worked.

Milk container

Black Polythene tank (the one commonly used as water reserve tank) was found to be the most common milk container used for storage and transportation purposes among the small dairies. Thirteen dairies out of 20 (65%) were found to use polythene containers along with aluminium cans. Seven dairies were found to use only tin and aluminium cans.

Hygienic condition of the dairy

During the survey visit to the concerned dairies, the hygienic condition of most of the dairies was seen to be in poor state. No dairy was found to follow the code of conduct regulated by Department of Food Technology and Quality Control. The floors were not smooth and clean as required. No workers were found to wear apron, caps, boots and gloves during handling and processing of milk. Only three among the 20 respondents were found to have knowledge of GMP and GHP even though proper application seemed to be lacking in their dairies.

Physico-chemical analysis of milk samples

Two physico-chemical parameters pH and titratable acidity were analyzed in the collected milk samples. The findings are discussed below.

Analysis of pH of the milk samples

The mean pH values of the milk samples from the four locations were found to be 6.67, 6.52, 6.60 and 6.67, respectively (Table.2). The highest pH was found to be 6.8 and the lowest 6.45.

The normal pH of fresh milk usually varies from 6.6 to 6.8 (De, 2005). Some samples of milk were found to have pH lower than this normal range. The pH of milk depends closely on temperature (Walstra et al., 2005). The pH of milk is found decreasing with increasing temperature as that of water. Fall in pH also may result due to increase in acidity as a result of microbial action. Here the milk samples of eastern part of Kathmandu were found to have lower pH compared to that of the other areas.

Analysis of titratable acidity of the milk samples

The milk with highest titratable acidity was found to contain 0.23% (as lactic acid) acidity and that with the lowest was found to contain 0.11%. The mean titratable acidity of the milk samples from the four locations was found to be 0.144, 0.188, 0.154 and 0.136, respectively (Table 2).

Table 2: pH and acidity of milk samples

Location	Sample Dairy.	pH			Acidity (% lactic acid)		
		Individual	Mean	SE	Individual	Mean	SE
Northern Part	A	6.66	6.67	0.058	0.13	0.14	0.013
	B	6.60			0.16		
	C	6.50			0.19		
	D	6.80			0.12		
	E	6.80			0.12		
Eastern Part	F	6.50	6.52	0.029	0.20	0.18	0.014
	G	6.63			0.14		
	H	6.50			0.19		
	I	6.45			0.23		
	J	6.52			0.18		
Southern Part	K	6.70	6.60	0.037	0.12	0.15	0.009
	L	6.62			0.15		
	M	6.58			0.16		
	N	6.60			0.16		
	O	6.50			0.18		
Western Part	P	6.80	6.67	0.032	0.11	0.13	0.010
	Q	6.70			0.12		
	R	6.67			0.13		
	S	6.58			0.17		
	T	6.62			0.15		

The natural acidity in milk is caused by the presence of casein, acid Phosphates, citrates etc. The higher the solid non fat content in milk, the higher the natural acidity and vice versa (De, 2005). The titratable acidity of fresh milk varies from 0.13 to 0.15 percent. Developed acidity is due to lactic acid, formed as a result of bacterial action on lactose in the milk. Here, the acidity of milk from eastern part was found comparatively higher than that of other parts. This may be due to development of lactic acid by microbial action on milk. Some acidity values were found even lower than the normal.

The higher pH values and lower titratable acidity values of such milk to some extent indicate the possible addition of caustic soda, probably for the preservative purpose, the fact does not have any proofs but is sometimes heard in the news. Or it may be due to some other reasons like heat treatment of milk which decreases acidity to some extent by freeing carbondioxide molecules (Walstra *et al*., 2005).

Microbiological analysis of milk samples

For the microbiological analysis, total plate count, coliform count and yeast and mold count were performed on the collected milk samples. The procedure applied for microbiological analysis was pour plate technique. The results of various counts are discussed below (Table 3, 4 and 5).

One limitation of the pour plate technique is that the only bacteria that can grow on the medium used and under the conditions of incubation provided will form colonies and will be counted. Another limitation is that each viable organism that is capable of growing under the culture conditions provided may not necessarily give rise to a single colony. The development of one colony from one cell can occur only when bacterial suspension is homogenous and no aggregates of cells are present; However if the cells have tendency to aggregate like cocci in clusters (*Staphylococci*), chains (*Streptococci*) or pairs (diplococci), the resulting count will be lower than the number of individual cells. For this reasons the counts are often reported as colony forming units (CFU) per milliliter rather than number of bacteria per milliliter (Laboratory handbook for Dairy industry NDDB/DSP 2001).

Total plate count of milk samples

The mean total plate counts in the four locations were found to be 2.25×10^6, 2.29×10^6, 2.36×10^6, 2.54×10^6cfu/ml, respectively (Table 3) Among all the samples the highest count was 4.27×10^6cfu/ml (6.63 log cfu/ml) and the lowest was 1.27×10^6cfu/ml (6.10 log cfu/ml).

Table 3: Total plate count of milk samples

Location	Sample Dairy.	Total Plate Count(TPC)					
		cfu/ml	Mean	SE	Log cfu/ml	Mean	SE
Northern part	A	1.81×10^6	2.25×10^6	5.20×10^5	6.25	6.31	0.08
	B	1.54×10^6			6.18		
	C	2.18×10^6			6.33		
	D	4.27×10^6			6.63		
	E	1.45×10^6			6.16		
Eastern Part	F	2.72×10^6	2.29×10^6	3.80×10^5	6.43	6.33	0.07
	G	1.72×10^6			6.23		
	H	2.27×10^6			6.35		
	I	1.27×10^6			6.10		
	J	3.45×10^6			6.53		
Southern Part	K	3.27×10^6	2.36×10^6	2.71×10^5	6.51	6.35	0.05
	L	1.45×10^6			6.16		
	M	2.00×10^6			6.30		
	N	2.54×10^6			6.40		
	O	2.54×10^6			6.40		
Western Part	P	1.81×10^6	2.54×10^6	3.04×10^5	6.25	6.39	0.06
	Q	3.00×10^6			6.47		
	R	2.09×10^6			6.32		
	S	3.27×10^6			6.51		
	T	2.54×10^6			6.40		

Total plate count is performed to determine the estimated number of total viable bacterial colonies. It does not indicate any particular group of organism. The results of total plate count to some extent resemble to that obtained by (Sapkota, 2004 and Shah, 2006). If we follow the Indian Standard (*IS*: *1479*, Part III, 1962), all the tested milk samples fall under "fair" grading (Table 4). The possible causes of high microbial load in the milk may be due to poor hygienic condition of the farm, milking utensils and milking practice. Lack of immediate chilling facility after milking also helps in increasing the microbial load.

Table 4: Bacteriological standards of milk

SPC /ml	**Grade**
Not exceeding 200,000	Very good
Between 200,000 and 1,000,000	Good
Between 1,000,000 and 5,000,000	Fair
Over 5,000,000	Poor

Coliform count of milk samples

All the samples showed positive results for coliform. The mean coliform counts in the four locations were found to be 2.23×10^2, 2.28×10^2, 2.54×10^2, 3.17×10^2cfu/ml, respectively (Table 5).Among all the twenty samples, the highest count was 4.27×10^2cfu /ml (2.63 log cfu/ml) and the lowest was 0.90×10^2cfu/ml (1.95 log cfu/ml).

Coliforms are the indicators of fecal or sewage contamination (Cominazzini, 1978). Coliform group of organisms are taken as indicator organism since they are found in large number and survive for long period, if present as well as can be detected easily in laboratory. Among them *E. coli* is the major indicator organism of milk and water sanitary quality. The presence of coliform bacteriain milk indicates poor sanitary condition of the dairy as well as possible presence of food borne pathogens in it. During the study each and every samples of milk were found to have coliform bacteria in significant number.

The results are similar to the findings of Sapkota, (2004) who has reported coliform count in the range of 2.59×10^4 to 4.4×10^7 in the raw milk of Kathmandu valley. DFTQC also has reported coliform counts greater than 2.4×10^3 in the substandard pasteurized milk samples (DFTQC, 2007/08). Adhilkary, (2006) in his study found coliform count as high as 6.9×10^5cfu /ml in raw milk of Kathmandu valley. These all above mentioned results show poor hygienic quality of milk in Kathmandu valley. The presence of coliform in milk might be due to poor hygienic condition of the farm, use of contaminated water, lack of cleaning and sanitation of the working area etc. Presence of coliforms in milk indicates that there may be presence of pathogens like *Salmonella*, *Shigella*, *Vibrio*spp, *Entamoebahistolytica*, *Giardialambia* and their cysts etc. which are of fecal origin (Jay, 1987).

Table 5: Coliform count of milk samples

Location	Sample Dairy.	Coliform Count					
		Cfu/ml	**Mean**	**SE**	**Log cfu/ml**	**Mean**	**SE**
Northern part	A	2.36×10^2	2.23×10^2	5.06×10^1	2.372	2.30	0.10
	B	1.81×10^2			2.257		
	C	4.00×10^2			2.602		
	D	2.09×10^2			2.320		
	E	0.90×10^2			1.954		
Eastern Part	F	1.36×10^2	2.28×10^2	3.82×10^1	2.133	2.33	0.07
	G	2.72×10^2			2.434		
	H	1.81×10^2			2.257		
	I	2.00×10^2			2.301		
	J	3.54×10^2			2.549		
Southern Part	K	3.27×10^2	2.54×10^2	4.00×10^1	2.514	2.38	0.05
	L	1.81×10^2			2.257		
	M	1.90×10^2			2.278		
	N	2.00×10^2			2.301		
	O	3.72×10^2			2.570		
Western Part	P	2.36×10^2	3.17×10^2	3.97×10^1	2.372	2.48	0.06
	Q	4.00×10^2			2.602		
	R	2.45×10^2			2.389		
	S	4.27×10^2			2.630		
	T	2.81×10^2			2.448		

Yeast and mold count of milk samples

The mean yeast mold counts in the four locations were found to be 6.324×10^4, 5.596×10^4, 5.504×10^4, 6.216×10^4cfu/ml, (Table 6). Among all the twenty samples, the highest count was found to be 8.000×10^4cfu/ml (4.903 log cfu/ml) and the lowest 3.450×10^4cfu/ml (4.538 log cfu/ml).

This finding is in agreement with the study done by Shah (2006) who found yeast and mold in the range of 10^4 to 10^6 in raw milk of Kathmandu valley. The major sources of yeast and molds in milk are the surrounding environment, skin of the milking animal, fodder etc.

Table 6: Yeast and Mold count of milk samples

Location	Sample Dairy	Yeast and Mold Count					
		Cfu /ml	**Mean**	**SE**	**Log cfu/ ml**	**Mean**	**SE**
Northern part	A	8.00×10^4	6.32×10^4	6.53×10^3	4.903	4.791	0.05
	B	5.36×10^4			4.729		
	C	4.45×10^4			4.648		
	D	7.45×10^4			4.872		
	E	6.36×10^4			4.803		
Eastern Part	F	5.36×10^4	5.59×10^4	2.73×10^3	4.729	4.745	0.02
	G	6.63×10^4			4.821		
	H	5.09×10^4			4.706		
	I	5.63×10^4			4.750		
	J	5.27×10^4			4.721		
Southern Part	K	7.36×10^4	5.50×10^4	4.10×10^3	4.867	4.726	0.03
	L	6.45×10^4			4.809		
	M	3.45×10^4			4.538		
	N	4.81×10^4			4.682		
	O	5.45×10^4			4.736		
Western Part	P	7.00×10^4	6.21×10^4	6.72×10^3	4.845	4.789	0.06
	Q	7.18×10^4			4.856		
	R	5.00×10^4			4.698		
	S	5.63×10^4			4.750		
	T	6.27×10^4			4.797		

Statistical Analysis

The analysis of variance (ANOVA) of pH and acidity of milk samples showed significant difference between samples of Eastern part with samples of Northern and Southern part respectively, at 5% level of significance ($p<0.05$). Regarding the microbiological quality of milk samples, no significant difference was found in total plate count, coliform count and yeast and mold count of the samples of all four parts ($P<0.05$) (Fig. 2).

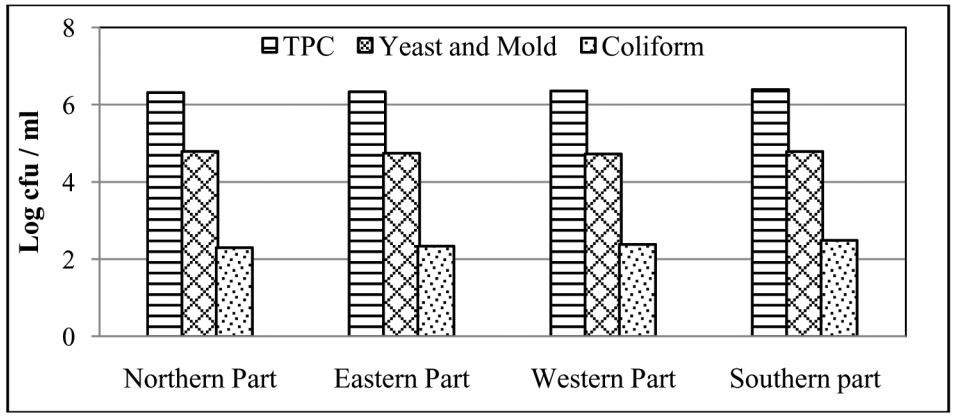

Fig. 2: Bar diagram showing total plate count, yeast and mold count and coliform count (log cfu/ml)

CONCLUSION

Microbiological quality of milk produced by small dairies in Kathmandu was evaluated in terms of total plate count, coliform count and yeast and mold count. The total plate count of the milk samples was found within the range of 1.272×10^6cfu/ml to 4.272×10^6cfu/ml, yeast and mold in the range of 3.450×10^4cfu/ml to 8.000×10^4cfu/ml and no sample was found free from coliform bacteria. The population of coliform bacteria was found within the range of 0.9×10^2cfu/ml to 4.27×10^2cfu/ml. The result revealed that the microbial load of the milk produced by such small dairies is relatively higher indicating their poor quality. Hence in order to maintain overall quality of milk good hygienic practice, good manufacturing practices and other recommended code of conducts should be implemented.

SUGGESTIONS

The study on microbiological quality of milk produced from small dairies indicated poor hygienic quality of the milk. In one hand, the milk from such small dairies has helped in fulfilling the increasing milk demand of the valley and on the other hand such contaminated milk could be detrimental to consumer health. So, considering the role of small dairies in fulfilling the market demands, there is urgent need to improve their milk quality to safeguard the consumer health. In order to improve the microbiological quality of milk produced by small dairies, following suggestions have been made;

Focus should be given to cleanliness and sanitization of farms, milking utensils, milking persons, dairy workers and processing equipments. Maintaining cold chain of milk from the farm till distribution to the consumers is necessary. Pricing system

based on the microbiological quality of milk should be brought into practice to encourage the farmer for hygienic milk production. Mechanism should be developed for proper management of the small dairies as cottage industries and routine quality monitoring of their products. Proper training and technical support to small dairy entrepreneurs should be provided focusing on good manufacturing practice. Code of conduct for farmers and processing plants should be implemented strictly. Timely revision and effective implementation of the food act is necessary from concerned authority. Consumers should be aware about the quality of milk they are consuming. Good Veterinary Practices (GVP) and Good Agriculture Practices (GAP) should be encouraged to the farmer's level. Comprehensive quality policy for milk and milk products at national level should be launched focusing upon quality assurance system rather than quality control system.

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Nepalese Vet. J. 32:44-49

Hospital Based Retrospective Study of Clinical Mastitis in Dairy Livestock of Western Chitwan, Nepal

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ABSTRACT

The mastitis is one of the major causes for low milk production in dairy animals. Specifically, focusing on major infectious causes, comparative infection in four teats, and associated animal risk factors of mastitis, hospital based retrospective study was conducted. The milk samples were first taken for California Mastitis Test (CMT) and then subsequently for culture and bacterial identification. Most of the dairy animals were found to be suffered from mastitis in first parity (n=45, 43%) followed by second parity (n=25; 24%), fourth (n=15; 14%), and then third (n=9; 8.7%). Incidence of mastitis is more within first month of parturition (n=47, 45.6%). Around 43% (n=45) of the positive mastitis cases of animals with 1-6 month and very few (n=11, 10.7%) mastitis cases were of more than 6 months from parturition. Regarding infection, in CMT test, highly thick reaction (+++) were observed in left fore quarter (n=15, 16.85%), then in left hind (n=7, 10.29%), right fore (n=6, 8.6%) and right hind (n=5, 7.2%). Similarly in the same test, moderately thick reaction were highest in left hind (n =13, 14.60%), left fore quarter (n=11, 16.17%), right fore quarter (n=10, 14.4%) and right hind quqrter (n=5, 7.2%). In the cultural examination, most dominant cause of infection was coliform (n=48), followed by Staphylococcal (n=7) and Streptococcal infection (n=2). Coliform were most common cause of infection (n=37) in left fore quarter, right fore quarter (n=40) and right hind quarter. Routine milk test through CMT at farm level, farmer's awareness on mastitis and immediate treatment as far as possible could be of potential steps to enhance milk production.

Key words: Hospital, Mastitis, CMT, Coliform, Retrospective study, Chitwan

INTRODUCTION

Animal husbandry is one of the integral components of rural economy in Nepal where it provides employment and income-generating opportunities. The mastitis is one of the major causes for low milk production in dairy livestock of Chitwan. Livestock Production, one of the major components of Nepalese mixed farming

system contribute 31% in the national Agricultural Gross Domestic Product (AGDP) (CBS, 2009). Buffalo milk production contributes nearly 72% (1,031,500 MT) and cattle milk contributes nearly 28% (413,919 MT) in the fiscal year 2008/09.Mastitis is one among the top three threats faced by the farmers in terms of economic loss, our cattle and buffaloes could definitely not stay apart. The cost of clinical mastitis has also been estimated to be $107 US per clinical episode with over 70% of the cost associated with decreased milk production and milk withheld from the market, over 20% with drugs, veterinary costs and replacement costs, and the remainder with labor (DeGraves and Fetrow, 1993).In Nepal, according to Ng *et al*., (2010) largest proportion of losses in milk results from decreased milk production i.e., Rs. 4287 or USD 63 per buffalo per lactation. Milk loss was found 11% of average total lactation yield. Expenditure in medicine accounts 34% of the total treatment and management cost. In most countries, surveys in dairy herds indicate that prevalence of infection of mastitis pathogens is about 50% of cows and quarter infection rate of about 25% (Radostits *et al.,* 2009). Thus, this specifically focuses on study of clinical mastitis from milk samples and retrospectively study of the animal associated risk factor for incidence of them in dairy cattle and buffaloes.

MATERIALS AND METHODS

With the objective to study major infectious causes of mastitis, comparative infection in different teats, animal associated risk factors for mastitis, the hospital based retrospective study was conducted in 2012 at Veterinary Teaching Hospital. Numbers of clinical mastitis related cases are brought to veterinary teaching hospital (VTH) daily which are then undertaken for investigation under suggestion of veterinary doctor. All the required information regarding the case and samples are gathered during sample collection. All the information regarding parity, age, sex, course of infection, preliminary treatment were recorded. The milk samples were first taken for California Mastitis Test (CMT) and then subsequently for culture and bacterial identification. In CMT, thick (+), moderately thick (++), highly thick (+++) were assigned based on thickness after reaction for positive cases. The milk samples were undertaken for identification of coliform, streptococcal and staphylococcal infection through isolation and identification (Quinn *et al.,* 1998). The data were analyzed for fiscal year 2012/13 available in mastitis laboratory of Department of Veterinary Medicine, and Veterinary Teaching Hospital, Agriculture and Forestry University. The descriptive statistics are described through frequency and percent while the associations are described through chi-square test. The probability values below 0.05 are considered significant.

RESULTS

A total of 103 samples were analyzed and the results were discussed. Of the total samples, 56.3% (n= 58) were of cattle and rest 43.7% (n= 45) of buffalo. Among cattle (n=58), 53% (n=31) were from Jersey cross, 29% (n=17) were from Holstein Friesian cross and 18% (n=10) were from local cattle. Similarly for that of buffalo, 71% (n= 32) samples were from Murrah cross, 29% (n=13) were from local buffaloes. Many of the dairy animals were found to be suffered from mastitis in first parity (n=45, 43%) followed by second parity (n=25; 24%), fourth (n=15; 14%), and then third (n=9; 8.7%). Many of dairy animals were found to be suffered from mastitis with in first month of parturition (n=47, 45.6%). Around 43% (n=45) of the positive mastitis cases were from animals with 1-6 month of parity and very few (n=11, 10.7%) mastitis cases were from more than 6 months of parturition. The most of the milk samples were from left fore quarter (n=89) followed by right fore (n=69), right hind (n=69) and left hind (n=68). Regarding infection, in CMT test, highly thick reaction (+++) were observed in left fore quarter (n=15, 16.85%), then in left hind (n=7, 10.29%), right fore (n=6, 8.6%) and right hind (n=5, 7.2%). Similarly in the same test, moderately thick reaction were highest in left hind (n =13, 14.60%), left fore (n=11, 16.17%), right fore (n=10, 14.4%) and right hind (n=5, 7.2%) (Table1).

Table 1: Status of clinical mastitis in different quarters of udder

Species	**Status of infection**	**Quarter of Udder**			
		Left fore	**Left hind**	**Right fore**	**Right hind**
Buffalo (n=45)	+++	4	2	1	1
	++	5	5	4	2
	+	15	11	11	9
Cattle (n=58)	+++	11	5	5	4
	++	8	6	6	3
	+	13	13	12	17

+++ = High infection, ++ = Moderate infection, + = Slight infection

Further laboratory investigation was conducted through cultural examination of milk samples immediately after CMT. In this study, most dominant cause of infection was coliform (n=48), followed by Staphylococcal (n=7) and Streptococcal infection (n=2). On left hind quarter, coliform were most common cause of infection (n=37), and then staphylococcal infection (n=3) and streptococcus (n=1). On right fore

quarter, coliform were most common cause of infection (n=40) with staphylococcal and streptococcus infection. The infection is similar right hind quarter. The most common cause of infection was coliform (n=35) followed by streptococcus (n=2) and staphylococcal infection (n=2).

DISCUSSION

In our study, many of the dairy animals were found to be suffered from mastitis in first parity (n=45, 43%) followed by second parity (n=25; 24%), fourth (n=15; 14%), and then third (n=9; 8.7%). The high incidence of clinical mastitis in first parity is supported by the reports of Radostits *et al.,* (2009).Similarly in a study by Khanal and Pandit (2012) in cattle and buffalo of Lamjung, the prevalence of mastitis was higher among the dairy animals in late lactation (58.8%) compared to that in early lactation (41.3%) which is contrast to this finding where the mastitis was highest in first parity. Dhakal and Tiwari (1992) found that left hind quarters were the mostly affected (34.2%) followed by right hind (31.6%) in cattle which is contrast to our finding but it is supported by Thilager *et al.*, (1992) who reported the higher incidence of mastitis in left fore quarters. This finding was in line as reported by Khanal and Pandit (2013). Jha *et al.,* (1993) reported higher (33.9%) in right fore quarter followed by left fore quarter (28.6%) in buffaloes which is also contrast to our finding.Coliform mastitis was the most frequent type followed by Staphylococcal mastitis in all breeds of cattle and buffaloes in Chitwan (Dhakal *et al.,* 2002) which is in similar line of finding from our study. However, this finding contrast with the finding of Balakrishnan *et al.,* (2004) that reported *Staphylococcus species* being the predominant pathogens followed by *Coliform species* involved in his bovine mastitis study. Similar results were obtained by Dhote *et al*., (1999) in their study on sub-clinical mastitis in cows. Mandial *et al.,* (1999) found that *Staphylococcus species* are the most common organism in mastitis of cows followed by *Streptococcus species* and *E. coli*. Some other study regarding mastitis, coagulate negative staphylococcus and *Coliforms* are predominant in clinical mastitis as reported by Dhakal *et al.,* (2007). Upadhyaya (2008) reported *Staphylococcus* sp. (17.5%) as dominant isolate followed by *E.coli* (14.1%), *Streptococcus* sp. (7.5%) and *Klebsiella* sp. (3.3%) respectively. On the quarter basis, RF quarter has highest number of bacterial growth followed by Left Hind, Right Hind and Left Forerespectively.

CONCLUSION

The common cause of infection were bacterial mostly the coliform infection. Routine milk test through CMT at farm level, farmer's awareness on risk factors of mastitis and immediate treatment as far as possible could be of potential steps to reduce mastitis and enhance milk production in Nepal.

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Climate Sensitivity of Gastrointestinal Nematode Infection of Goats in Nepal and Required Management Approach

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ABSTRACT

Gastrointestinal nematode (GIN) infection constitutes the most important health problem of goats in Nepal for limiting their productivity and in some cases their survival. A study conducted for a year (February 2013-January, 2014) to determine the prevalence and magnitude of GIN infection of goats at twelve different sites in three different eco regions (mountainous, mid hill and terai) of the country, showed that the infection was very closely associated with the rain fall pattern, with higher prevalence during the wet monsoon months. The magnitude of infection as reflected by fecal egg counts (eggs per gram of feces) was significantly different ($p<0.05$) between the three eco-regions with highest level of infection in the terai region followed by mid hills and mountains, reflecting the association with warmer climatic conditions at the lower altitude regions. The closer association of climatic factors with the prevalence and magnitude of GIN infection necessitates the need to adopt the treatment and control strategies according to the season of infection, which implies that the treatment of the goats should be carried out during wet monsoon months, so that the infection in the animals could be minimized and losses from infection could be avoided with consequent improvement in productivity of goats.

Key words: Gastrointestinal nematode, Infection, Goats, Nepal, Climate, Relationship

INTRODUCTION

In Nepal, goats are raised in all parts of the country under different climatic and management systems mostly under subsistence production system. Productivity of the animals is low due to various limiting factors and infection of gastrointestinal nematodes constitutes an important constraint for reducing their productivity (Joshi, 1995). Studies on infection of gastrointestinal nematodes (GIN) in goats and their effect on production and productivity has been scattered and location specific (Shrestha, 1994; Shrestha *et al.*, 1990; Kadariya and Joshi, 1994; Joshi, 2000)

without studying the overall situation of the country. Hence, a clear understanding on the disease situation and its relationship with climatic factors was not known for the different eco-zones and regions of the country. Hence, the present study was undertaken to evaluate the pattern of infection of GIN in goats, its seasonality and climatic relationship in all three eco-zones (tropical plains of Terai, semi-tropical mid hills and temperate mountain regions) and geographical regions (eastern, central, mid and far western) of the country, so that a clear understanding on GIN infection in goat population of the country could be known and a rational approach for its management could be recommended.

MATERIALS AND METHODS

Site Selection

Twelve sites were selected at different locations of the country representing the different ecological and geographical regions of the country. The location and characteristics of each site is presented as Table 1.

Table 1: Location and altitude of selected sites:

Eco-zones	Eastern	Altitude (masl)	Central/ Western	Altitude (masl)	Mid Western	Altitude (masl)	Far-west	Altitude (masl)
Terai	Sunsari	200	Bara	140	Bardiya	200	Kanchanpur	250
Hills	Dhankuta	1200	Lamjung	1100	Surkhet	1200	Baitadi	1200
Mountain	Dhankuta	2200	Rasuwa	2400	Jumla	2400		
			Mustang	2400				

Among the selected sites in the mountain range, Mustang site represented the dry temperate climate, while the other sites at this eco-zone represented wet temperate climate, where as the sites in the Terai were below 250 meter above sea level (masl) with tropical climate and the mid hill sites at an altitude at around 1100-1200 masl had sub-tropical climatic conditions.

Sample collection and analysis

Fecal samples were collected per rectum from the representative goat population (between 35-50 samples) between February 2013 to January 2014 from all selected sites in the zipped polythene bags individually at bi-monthly intervals, preserved by few drops of 10% formaldehyde and transported to the laboratories for fecal egg count. Fecal egg counts were carried out by Modified Mc Master method (MAFF, 1984). The total number of fecal samples collected and examined is presented as Table 2.

Table 2: Number of fecal samples collected and examined

Eco-zone	District/site	Altitude (masl)	Number of fecal samples collected and examined	Samples examined/ eco-zone
Mountain	Dhankuta (Murtidhunga)	2200	488	1168
	Rasuwa (Gatlang)	2400	211	
	Mustang (Seng)	2400	308	
	Jumla (Dipalgaon)	2400	161	
Hills	Dhankuta (Dhankuta bazaar)	1200	300	947
	Lamjung (Gaon sahar)	1100	149	
	Surkhet (Gadi)	1200	206	
	Baitadi (Baskot, Meloli)	1200	292	
Terai	Sunsari (Madhesa)	200	300	1026
	Bara (Maheshpur)	140	187	
	Bardiya (Mainapokhar)	200	261	
	Kanchanpur (Suda)	250	278	
Total			**3141**	

Except for Rasuwa (Gatlang) goats were managed under the sedentary management system in small flocks, in which they were housed during the nights and grazed on community grazing areas during the day, while at Rasuwa (Gatlang), they were managed under the migratory management system.

Climate data analysis

Climate data (maximum and minimum temperature and rain fall) for the selected sites were represented by the climate data of the nearest metereological station of the Department of Hydrology and Meteorology (DHM). Climate data were obtained for the selected met stations of the DHM for the past 30 years and trend on climatic pattern and changes were analyzed using two types of analysis on three climatic variables. The stations were pooled in three different ecological zone based on their location. The mean annual climatic data of corresponding station of respective year of individual ecological zone (Mountain, Hill and Terai) were then averaged and used to analyze the trend of climatic variability. Both Mann-Kendall trend test and regression analysis was performed on these data for individual eco zone for all three climatic variables

RESULTS AND DISCUSSION

Analysis of fecal samples for six consecutive times at two monthly intervals showed that except for Mustang and Jumla samples, where no prevalence of GIN infection in goats was recorded, the prevalence rate between other sites varied between 24–60% (Fig. 1). By pooling the data for eco-zone prevalence, the prevalence rate at mountain sites was considerably lower than the hill and Terai sites, while there was no difference between hills and Terai regions (Fig. 2). The lower prevalence at mountain sites could be explained as no infection was recorded in goats at Mustang and Jumla sites, which could be due to the arid temperate climate of Mustang and Jumla, with limited possibility of the continuation of parasitic life-cycle and their transmission, which clearly reflects the association and sensitivity of climatic factors for the transmission of gastrointestinal parasites in goats.

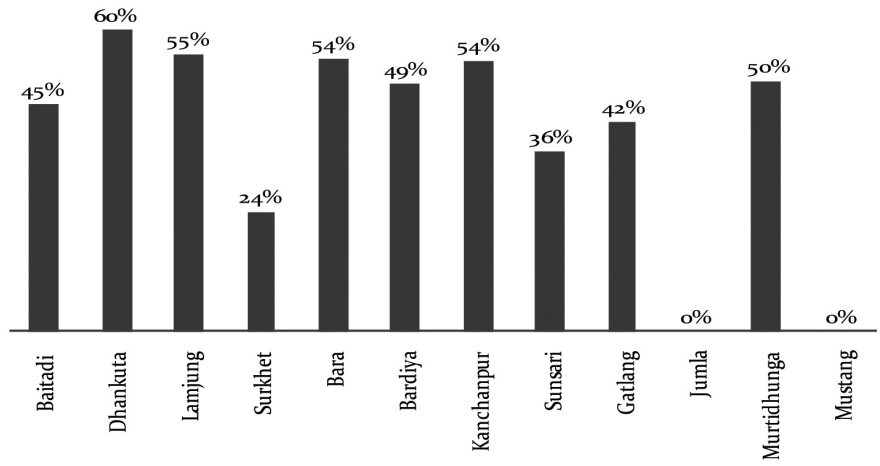

Fig. 1: Prevalence of gastrointestinal nematode infection at different sites

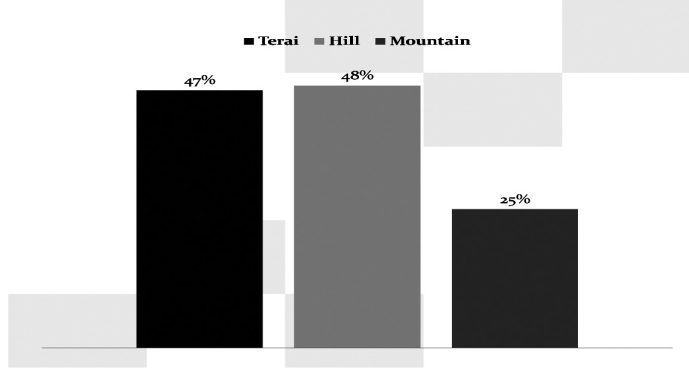

Fig. 2: Mean prevalence of GIN in goats at different eco-zones

The magnitude of GIN infection as reflected by the fecal egg counts (eggs per gram of feces) of the goats from three eco-zones was also significantly ($p<0.05$) lower in the goats of the mountain region than that of the goats from hills and terai region (Fig. 3). Two distinct peaks in fecal egg counts, the first during April-May and the second during August-September was recorded in the goats of hills and Terai region, while no such trend was recorded in the goats from Mountain region. The significantly lower fecal egg counts could be attributed to cooler and drier environment of mountain region, reflecting the close association of parasite infection and moist warmer temperatures of hills and Terai regions of the country.

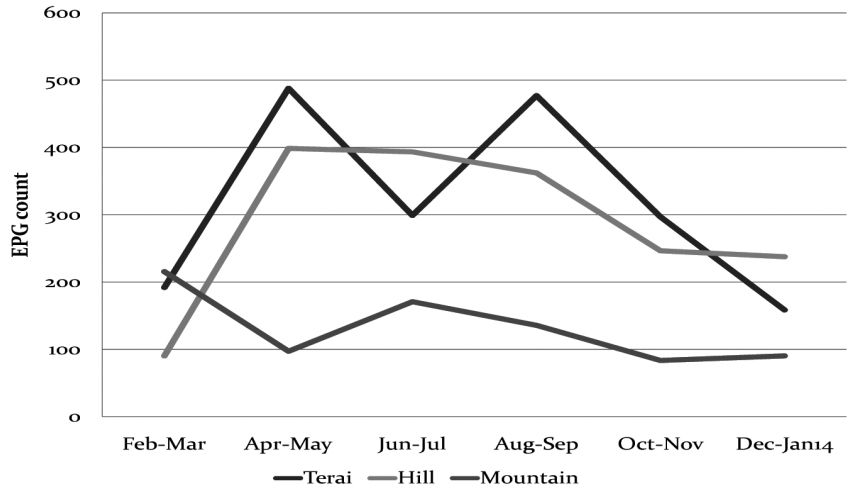

Fig. 3: Mean fecal egg counts of goats from three eco-zones

While the first peak in fecal egg counts (EPG) could be attributed to pre-monsoon rain and increased pasture infection, the second peak could be related to the monsoon rain and increased precipitation leading to suitable environment for the development and transmission of parasite larvae. With the end of monsoon rain and beginning of winter months from November onward, the level and intensity of pasture infection decreases which was reflected in low fecal egg counts during the winter and drier summer months.

Climate data analysis

While the precipitation trend in Nepal is almost constant between the years with more than 80% of rainfall occurring during the monsoon months (June to September), analysis of rain fall pattern by Mann-Kendall test in three eco-zones during past 30 years shows that the precipitation has been decreasing at the rate of 10.98mm per year in mountain eco zone, while in the hill and terai eco-zones there has not been any significant change in precipitation trend (Fig. 4).

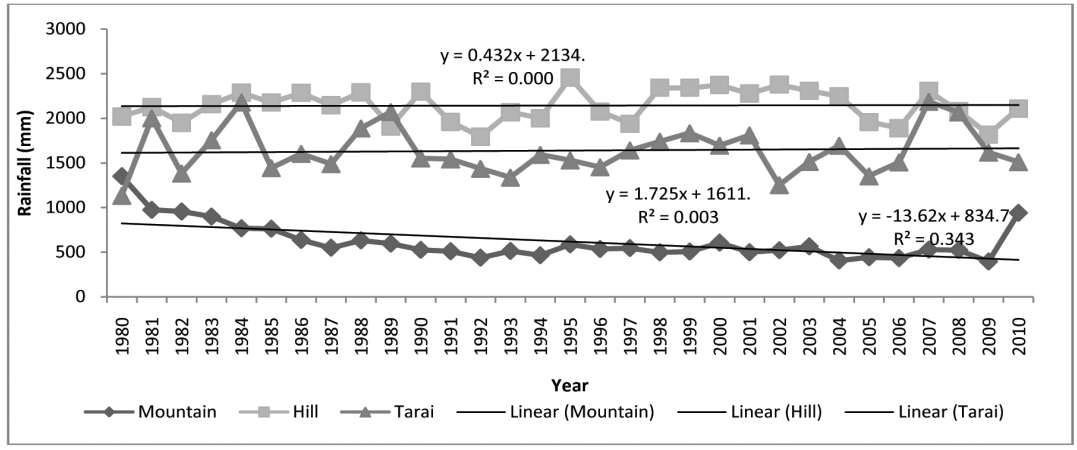

Fig. 4: Mean precipitation trend during 1980-2010 in three eco-zones

The mean maximum and mean minimum temperature at three eco-zones during 1980-2010 showed that the annual mean maximum temperature of the Terai, hills and mountains 30^0C, 23^0C and 18^0C respectively (Fig. 5), while the annual mean minimum temperature in these eco-zones was 18^0C, 13^0C and 5^0C respectively for the Terai, hills and mountains respectively (Fig. 6). Goldsmith (1981) has reported a decrease in 5.5 ^{0}C with every 1000 meter rise in altitude, which was also reflected in the climatic variation recorded in the present study. The mean annual maximum temperature in the mountains and hill eco-zone showed an increase in maximum temperature by 0.068°C per annum and 0.069°C per annum respectively whereas, in terai eco-zone there was no any specific trend in this parameter, while the annual mean minimum temperature, in mountain eco zone did not show any significant trend, whereas in hill and terai eco-zones, there was an increase of 0.033°C and 0.026°C per annum respectively.

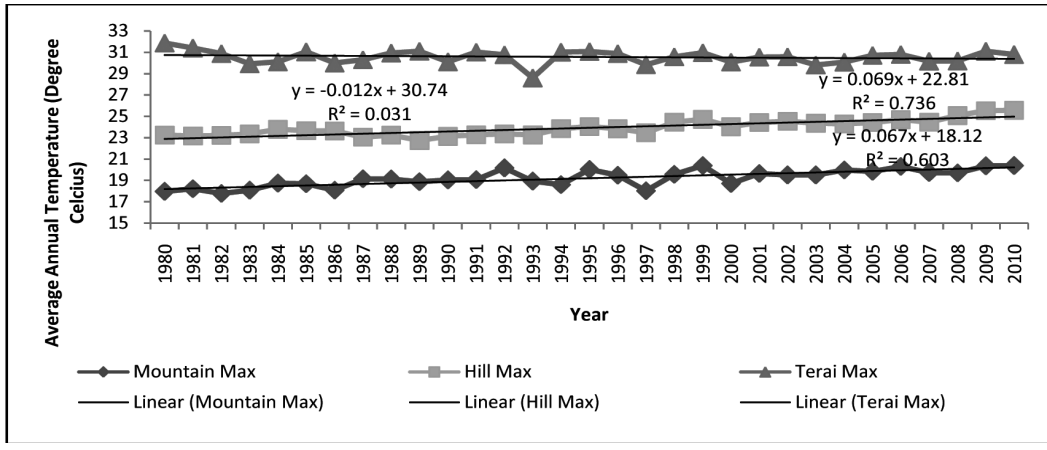

Fig. 5: Annual mean maximum temperature at three eco-zones during 1980-2010

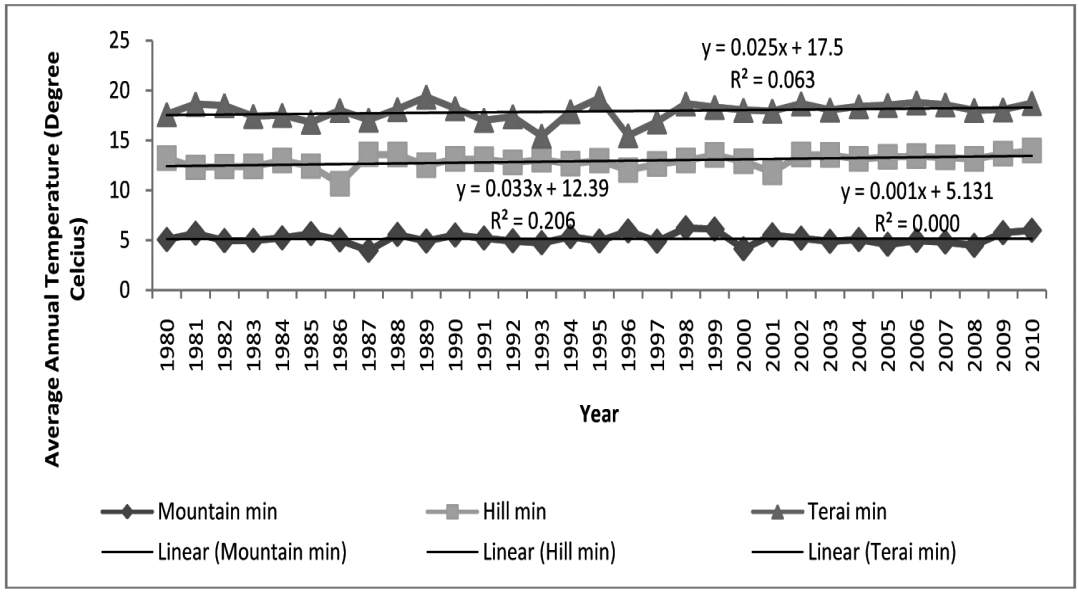

Fig. 6: Annual mean minimum temperature at three eco-zones during 1980-2010

Climatic sensitivity of GIN infection

Infection of gastrointestinal nematode in goats showed some distinct climatic association in three eco-zones of Nepal. The elevated peaks in mean fecal egg counts (EPG) from the months of April-May to September-October in the Terai and hill eco-zones showed distinct association of monsoon precipitation factor along with the warmer temperature during these months. This association was however, not so obvious at the mountain eco-zone, perhaps due to lower mean temperature range at this eco-zones. Crofton (1965) reported that critical temperature for the egg development of *Trichostrongylus axei*, *T. vitrinus*, and *H.contortus* to be 8-9°C with adequate precipitation, and as this temperature was present in the Terai and hill eco-zones of the country throughout the year for continuation of life cycle and precipitation being the only constraint except during the pre-monsoon and monsoon season, the continuation of life cycle and parasite transmission could only be possible during monsoon and pre monsoon season only. However, at the mountain eco-zone, although the mean maximum is above 10°C, the annual mean minimum temperature is around 5 °C, which indicates that limitation of temperature might be a critical factor for the development and transmission of parasites during some months of year. In addition, availability of precipitation only during the monsoon months also limits the transmission of nematode parasites at the mountain eco-zone as well. The lower

prevalence and magnitude of GIN in goats in the mountain eco-zone might be due to the lower temperature than at the hills and Terai eco-zones. It was also due to the arid climatic condition of some of the selected sites, which influenced the overall mean for the mountain eco-zone. It was however evident that in Nepal, precipitation has been the dominating climatic factor and transmission and magnitude of GIN infection is directly related to rain fall and rain fall pattern in the country, which clearly reflect the sensitivity of GIN infection in goats with the climatic factors.

Management of GIN infection in Nepal

Among the various approaches studied and adopted for GIN management in goats in many countries of the world, such as rational use of anthelmintics, rotational grazing using the clean pastures, development of resistant breeds and use of non chemical substances as anthelmintics, the rational use of chemical anthelmintics and exploration on non chemical substances for anthelmintic use are the only possible options available for Nepalese farmers. As evidenced by various studies, infection of gastrointestinal nematode parasites in goats is limited to wet summer months in almost all parts of the country, the rational approach for GIN management in Nepal should be based on minimizing the infection and reduce the effects of infection as much as possible. It must however be noted that the effective anthelmintics can only eliminate the existing infection within the animals but it could not prevent the incoming infection. Hence, it is important that the animals should be treated in such a way that they could be maintained as clean as possible during the peak infection period. It could be possible if the goats be treated at monthly intervals during the wet summer months only and there would not be any necessity of treating these animals during the dry winter and summer months.

ACKNOWLEDGEMENTS

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Prevalence of Helminth Parasites in Goats of Nuwakot District

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ABSTRACT

A study was conducted to find out the prevalence of the helminth parasites in goats of Thulokhola watershed area of Nuwakot district. Altogether 129 faecal samples were brought from different village development committee (VDCs) (Ratmate, Duipipal and Jiling) of Nuwakot and were examined for helminth parasites in the Parasitology Laboratory of Animal Health Research Division (AHRD), Nepal Agricultural Research Council (NARC). The samples were examined qualitatively by sedimentation technique and quantitatively by McMaster counting method for identification of helminths. Out of 129 samples, 107 (82.95%) samples were found positive for one or other type of helminth parasites where 22 (17.05%) samples were negative. Prevalence of nematode parasites were highest (82.17%) while trematode showed a lowest prevalence (3.87%). The parasites identified were Strongyle sp. (82.17%) (Trichostrongylus sp., Ostertagia sp., Oesophagostomum sp., Haemonchus sp. and Chabertia sp.), Trichuris sp. (13.95%), Moniezia sp. (7.75%) and Fasciola sp. (3.87%). The prevalence of helminths infections was 87.5% and 77.19% in the age group of above 6 months and below 6 months, respectively. Ratmate showed the highest prevalence (85.71%) while Jiling showed a lowest prevalence (81.25%) and prevalence percentage in Duipipal was (83.33%). Among three VDCs, mid altitude showed the highest prevalence (88.89%) while high altitude showed a lowest prevalence (77.08%) followed by 84.44% in low altitude. The mean fecal egg counts of the goat was significantly higher in March (297.7%) and lower in February (830.23%) followed by January (195.4%). The mean fecal egg count was higher (291.7%) in adult and lower in young (147.4%).

INTRODUCTION

In Nepal, the total goat population is estimated to be about 8,844,172 heads which contributes 20% of total meat production of the country (CBS, 2010). Goats are multipurpose animals and are mainly kept for meat, milk, fiber (mohair, cashmere) and skin. In addition, they also provide manure for maintaining soil fertility. Goats are used as pack animals and are especially important among the pastoralists or

farmers keeping animals under transhumance migratory management system. Goat milk is used for the production of soft cheese due to the nature of protein present in it. The goat milk is also prescribed for the persons having allergic reaction to cow milk because it contains an antiallergic factor. The goats are mainly raised and looked after by women and children and are kept as Pewa (personal property) and income from the sale of goat is spent by them.

In Nepal, there are many problems of goat production, among them diseases and parasites are most important factor reducing goat production and productivity. Among the parasites, nematodes are most commonly identified in a fecal examination. Infection with gastrointestinal nematodes is regarded as one of the important factor causing productivity loss. Singh *et al.*, (1973) reported that *Haemonchus, Oesophagostomum, Trichostrongylus, Bunostomum, Strongyloides, Nematodirus, and Chabertia* species appear in varying degrees in the gastrointestinal worm complex in fecal samples of large and small ruminants collected in the Kathmandu valley.

Trematodes commonly known as flukes and these include Fasciola sp., Schistosoma sp. and Paramphistomum sp. Fasciolosis is considered to be widely spread among goats in Nepal. Fasciola gigantica is considered to be the main species causing fasciolosis in goats. Lohani and Jaeckle (1981) identified Fasciola gigantica, Fasciola hepatica and intermediate forms in water buffalo and goats slaughtered in Tansen, Palpa (Thakur and Thakuri, 1992).

Cestodes are also seen in goats of Nepal. Cestodes like *Monezia sp*., *Coenurus cerebralis*, *Cysticercus tenuicollis* and hydatid cyst are found in goats and sheep. Cestodes found in gut are acquired by eating contaminated food and water.

MATERIALS AND METHODS

Description of the study site

This study was conducted in the 3 VDCs (Ratmate, Duipipal and Jiling) of Thulokhola watershed in Nuwakot district in the central Nepal. The Thulokhola watershed is a minor tributary of the Trishuli River. The lowest elevation of the Thulokhola watershed is less than 440 m above sea level (asl) at the confluence of the Trishuli river and the highest elevation is 1648 m asl.

Sample size

Of 2000 goat population in study area, a total of 129 faecal samples of female goats were collected during January to March as their health status was very poor. Laboratory work was performed at Animal Health Research Division (AHRD), Khumaltar, Lalitpur (Table 1).

Table 1: Number of samples

Altitude	**No. of samples**	**Area (VDCs)**	**No. of samples**
Mid	36	Ratmate	21
High	48	Duipipal	60
Low	45	JIling	48
Total	129		129

Method of sampling

Random sampling method was used to collect faecal sample. Two goats were selected from each house hold in three VDCs representing large number of household to be included in the sampling.

Collection of sample

Samples were collected from rectum manually, kept in zip-lock plastic bag with grips containing 10% formalin. Each plastic bag was labeled clearly with animal identification, date and place of collection.

Qualitative fecal examination

The samples was examined qualitatively by sedimentation technique as per (Soulsby, 1976)

Differential sedimentation

The majority of trematode eggs are too large and heavy to float reliably in the flotation fluids normally used for nematode eggs. They do however sink rapidly to the bottom of a fecal/water suspension and this is the basis of the fecal sedimentation technique. So, Sedimentation method is most appropriate for examination of heavier egg such as fluke's eggs. The procedures are as follows; an approximately 3gm feces was weighed. Feces was homogenized with water using mortar and pestle. The suspension was passed through a coarse mesh sieve and the debris was discarded. The filtrate was then allowed to stand for 5 minutes in a beaker. The supernatant was removed and sediment was resuspended with water (till the suspension becomes clear). The suspension was then allowed to sediment for next 5 minutes. The supernatant was drawn off. Few drops of methylene blue was added which stains fecal particle deep blue leaving trematodes egg unstained. A drop of stained sediment was placed on clear slide, covered with cover slip. The fecal smear was examined using a compound microscope.

Quantitative fecal examination

Quantitative investigation was done by egg per gram count (EPG) to know the number of parasites in the animal. The fecal egg count (FEC) was performed to enumerate the number of parasitic eggs excreted per gram of feces (EPG). The Mc Master technique as described by (Urquhart et al., 1987) was used to detect the EPG in the samples.

By Mc Master counting technique

Three gram of feces was weighed. Feces were homogenized with water (42ml) using mortar and pestle. The suspension was passed through a coarse mesh sieve and the debris was discarded. Then 15 ml filtrate was taken in the centrifuge tube. The tube was centrifuged at 2,000 rpm for two minutes. Supernatant was discarded. Sediment was agitated and tube was filled with flotation solution (NaCl) to previous level.. Then, the fluid in the centrifuge tube was mixed well by a pipette and enough fluid (0.15 ml) was drawn from the supernatant to fill the chamber of the Mc Master slide. Care was adopted to avoid air bubbles. Filled McMaster chamber was left to rest on the table for 3-5 minutes before counting (min. 3 minutes to allow all eggs to flotate and max 10 minutes, as some eggs might be discarded in the flotation fluid). The number of total eggs within the chamber was counted under the microscope (10X), and the number of eggs per gm of faces was calculated by multiplying the number of eggs by 100.

RESULTS AND DISCUSSION

Management status

Mixed farming system had been practiced in Nuwakot district as in most of the hill districts of Nepal. Farmers in the lower (less than 440 m asl) and mid altitude keep buffalo, cattle and goats whereas farmers in the upper altitude (1648 m asl) raise largely goats and cattle.

Most of the farmers adopt the partial grazing system i.e grazing their animals in community forest and also some farmers practiced cut and carry system of grasses. Natural and cultivated green grasses are the prime source of forages in rainy season while fodder trees are the major source of animal feed in the winter.

Farmers are treating their livestock without any plan due to lack of information on epidemiological pattern of internal parasites. They refer for treatment when they find some symptoms of endoparasitism like diarrhoeic faeces, debilitated body condition and less body weight gain.

Percentage of positive and negative cases

Out of 129 faecal samples, 107 (82.95%) samples were found positive for one or other type of helminth parasites where 22 (17.05%) samples were negative (Fig.1).

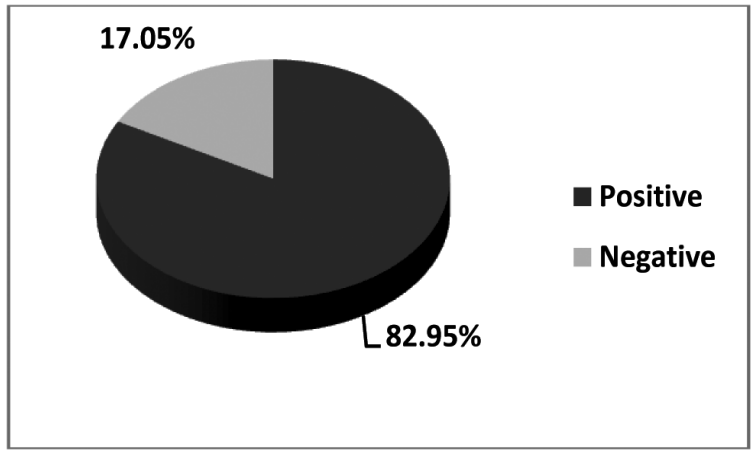

Fig. 1: Percentage of positive and negative cases

Prevalence of helminths parasites in goats of Thulokhola VDC of Nuwakot was higher (82.95%) than the studies of Parajuli (2007) and Kushwaha (2000) who had found 81.53% and 44% prevalence, respectively.

Overall prevalence of helminths

Out of 129 goat fecal samples examined, prevalence of nematode parasites were highest (82.17%) followed by cestode (7.75%) and trematode (3.87%). The most common parasites encountered were Strongyle sp. *(82.17%) (Trichostrongylus* sp, *Ostertagia* sp.*, Oesophagostomum* sp.*, Haemonchus* sp. *and Chabertia* sp.*), Trichuris* sp. (13.95%)**,** *Moniezia sp*. (7.75%) and *Fasciola* sp. *(3.87%)*.

Thakuri *et al*., 1994 reported 34% prevalence of nematode in Dhankuta district. The prevalence reported in this study is very high or even more than double as compare to Thakuri. Both Dhankuta and Nuwakot have similar altitude and also more or less management practices but difference in time of data recording i.e data recorded by Thakuri was more than fifteen years ago this indicates that prevalence of parasites have been increasing, this may be possibly due to change in climate.

Age-wise prevalence of parasites

For the purpose of this study, the ages of the goats were classified into two categories namely, young (0-6 months) and adult (> 6 months). The prevalence in each group are presented in Fig. 2.

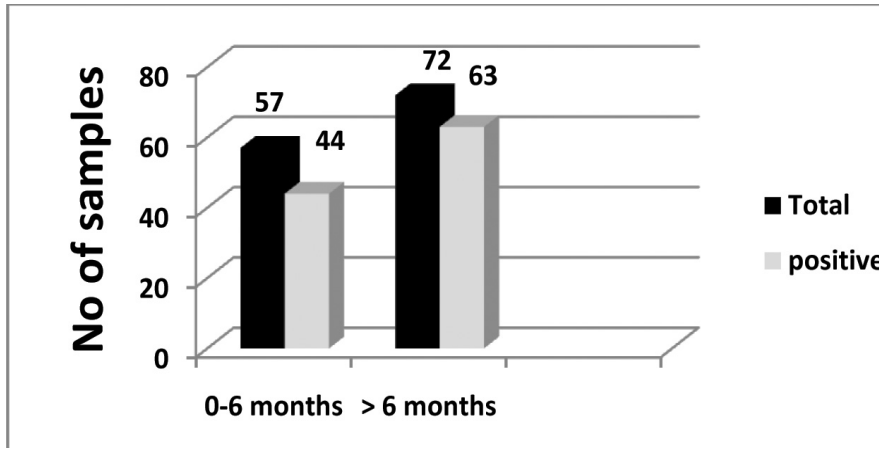

Fig. 2: Age-wise distribution of the parasitic infestation in goats

The prevalence of helminths infections in absolute figures reflects a higher occurrence in the (> 6 months) age group (87.5%) than (0-6 months) age group (77.19%). These findings indicate that adult goats get more infection than young goats. This is contrast with the findings of Boomker *et al.,* (1994) who found that the prevalence was inversely related to age. Young goats below 2 months of age are generally not taken to the forest for grazing and the low worm burdens could be attributed to a diet consisting mainly milk and only small amounts of vegetation containing infective larvae.

Area-wise prevalence of parasites

Ratmate showed the highest prevalence (85.71%) while Jiling showed a lowest prevalence (81.25%) and prevalence percentage in duipipal was (83.33%) (Fig. 3).

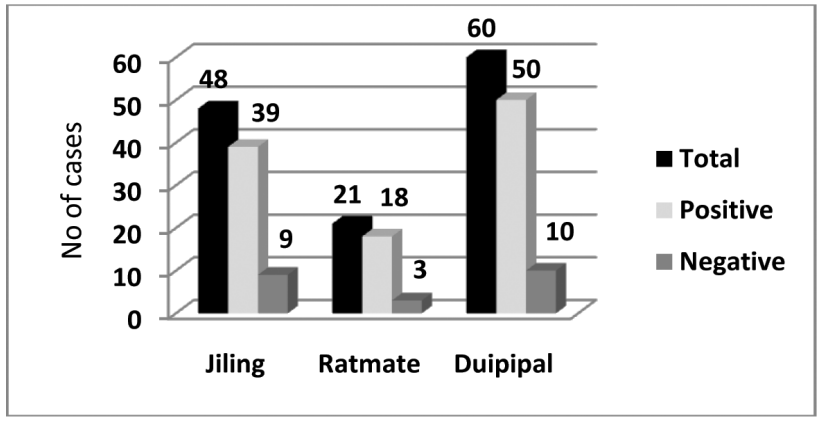

Fig. 3: Area-wise distribution of the parasitic infestation in goats

Strongyle sp. was most common parasites found in all areas. No trematode parasite was found in Duipipal area. Ratmate showed the highest prevalence. It may be due to long deworming interval, grazing in same pasture and ignorance in goat health management by the farmers. These factors might be the reasons for high prevalence in this area. Comparatively, Jiling showed the lowest number positive cases. This may due to cut and carry method of feed is practiced in this area.

Altitude- wise prevalence of parasites

Mid altitude showed the highest prevalence (88.89%) while High altitude showed a lowest prevalence (77.08%) followed by 84.44% in low altitude (Fig. 4). The prevalence percent in mid and low altitude was quite similar whereas prevalence in high altitude is considerable low. This may be possibly due to cold or low temperature in high altitude which may not be favourable for the multiplication of the parasites. The prevalence in mid and low altitude is high which favours the growth and multiplication of parasites. Secondly density of goat population is high in low and mid altitude and shares common pasture land which might favour the increase of prevalence in this area.

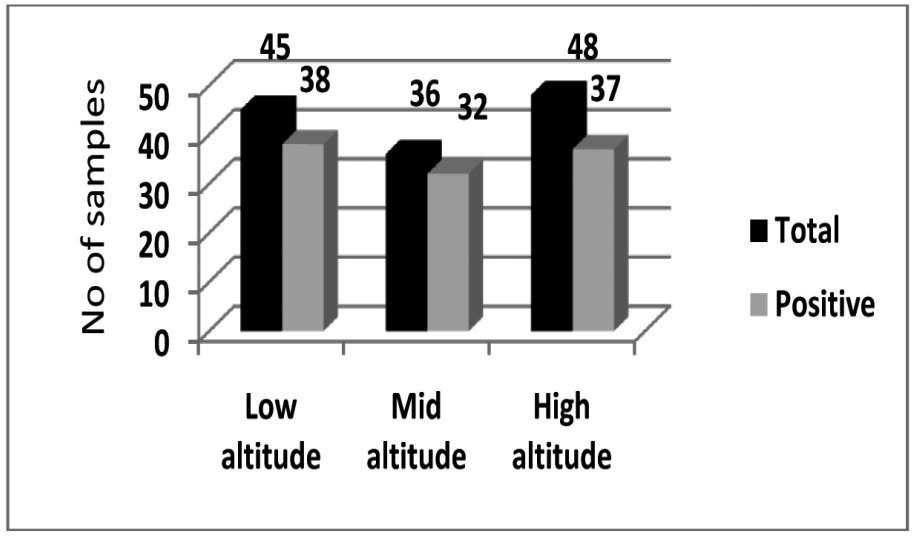

Fig. 4: Altitude-wise distribution of the parasitic infestation in goat

Month-wise Fecal egg counts in goats

The mean fecal egg counts (EPG) of the parasites in the goat during different months is presented in Table 2.

Table 2: Month-wise EPG in goats

Months	**Sample size (n)**	**EPG**
January	43	195.35
February	43	190.69
March	43	297.67
Total	129	

The mean fecal egg counts of the goat was significantly higher in March (297.67) and lower in February (190.69).

The temperature during January, February and March varied. March exhibited higher temperature (23.99°C) and approximately 90% relative humidity while February had faced comparatively lower temperature than March i.e. 20.2°C.

The difference in amount of temperature and average annual rainfall might be stated for the higher EPG during March and lower in February. This result is similar with the epidemiological study by Martin et al., 1988. He stated, warm wet conditions provide a very suitable environment for the survival and multiplication of parasites that can serve as a vector of disease. In this study also, month of March revealed warm conditions as comparison to February.

Age-wise fecal egg counts in goats

The mean fecal egg counts (EPG) of the parasites in the goats of different age groups is presented in Table 3.

Table 3: Age-wise EPG in goats

Age (Months)	**Sample size (n)**	**EPG**
Young (0-6)	57	147.37
Adult (> 6)	72	291.67
Total	129	

The mean fecal egg count of the goat was higher in adult and lower in young goats. The above finding may be due to young goats below two months of age are not taken in the grazing fields which lower the chances of getting infection in the young goats. Similarly, previous infection during young age persisted for long period and adult parasites produces more number of eggs which was evident in adult goats. Likewise deworming is generally not practiced in the area.

CONCLUSION

The prevalence of helminths parasites in goats of Thulokhola VDC of Nuwakot were very high (82.95%). Prevalence of nematode parasites were highest (82.17%) while trematode showed a lowest prevalence (3.87%). The present study showed that major helminths parasites belonging to genera *Strongyle* sp. *(82.17%), Trichuris* sp. (13.95%)**,** *Moniezia* sp. (7.75%) and *Fasciola* sp. *(3.87%)*. As goats are raised in common pasture land, not regularly dewormed against parasites and have poor body condition due to less feed availability which makes susceptible to the parasitic infestation or prone to get more infection. Most of the above mentioned genera of parasites are highly pathogenic species of goats. The infection of these parasites will certainly have great impact on health, production and productivity of goats. Heavy infection of these parasites can cause even sudden death of the goat.

Ratmate showed the highest prevalence while Jiling showed a lowest prevalence. Long deworming interval, repeated grazing in same pasture and ignorance in goat health management by the farmers, etc might be the reasoned for high prevalence. The prevalence of helminths infections is absolute figures reflects a higher occurrence in the (> 6 months) age group (87.5%) and (0-6 months) age group (77.19%) having the least. These findings indicate that adult goats are more susceptible than young goats. This may be due to young goats below two months of age are generally not taken in to the forest for grazing.

The prevalence percent in mid and low altitude was quite similar whereas prevalence in high altitude is considerable low. The mean fecal egg counts of the goats was significantly higher in March (297.67%) and lower in February (190.69%). The difference in amount of temperature might be stated for the higher EPG during March and lower in February.

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Study on Quality Parameters of Semen and Artificial Insemination in Goat

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ABSTRACT

A study was carried out in National Livestock Breeding Centre Pokhara to determine the effects of processing techniques on the quality of semen from Jamunapari, Barbari and Boer bucks. The volume of the semen obtained from Jamunapari,Barbari and Boer were 1.5ml, 2ml and 1.2 ml, respectively and the color varying from milky white to creamy white. The semen motility percentage was almost similar for Jamunapari and Barbari but lower for Boer. The morphological defects of curved tail in Jamunapari, Barbari and Boer 100% were 64%, 66% and 60%, respectively; straight tail were 36%, 34% and 40% respectively; the live spermatozoa were 72%, 70% and 67%, respectively; dead spermatozoa were 28%, 30% and 33%, respectively; morphology were 16%, 18% and 24% respectively and concentration were 40, 34 and 38 millions spermatozoa per straw, respectively. For artificial insemination; out of 129, 113 (87.6%) of pure Boer 4 (3.1%) of Barbari and 12 (9.3%) of Jamunapari buck semen were used for AI purpose. A total of 129 goats (32.6%) Khari, (64.4%) Khari x Jamunapari, (0.8%) Saanen cross and (1.6%) Terai were artificially inseminated in terai and mid hills districts of Nepal under this study. The kidding percentages were 41.86%, which means, AI may be a solution for farmers wanting to produce cross breed goats. AI can also contribute to a faster improvement of the local breed.

INTRODUCTION

Concerning with the increasing demand of animal products basically meat and dairy products, qualitative rearing of animals at commercial level has immense value. Analysis of semen parameters can give an authentic source of operational procedure that will enhance both quality & quantity of semen. It is important to remember that there are many different techniques which will give satisfactory semen quality for AI. So, frozen semen is used for AI as the easy and cheap way of insemination on large scale. Large number of AI can be done in animals by using synchronization. Although, the technology of producing semen and production of skilled AI technician have

improved conception rate in goat. Farmers are not aware about the breeding system in goat. So, that production performance is going down day by day by unnecessary breeding with local indigenous buck.

The most important parameters pertaining to fertility are the number of spermatozoa inseminated and their motility (Castellini and Lattaioli, 1999). Differences in laboratory methodologies can introduce substantial variations in the evaluation of sperm parameters (sperm counts, motility and morphology) (WHO, 1999). Semen which is stored for up to 7 days, the sooner liquid-stored semen is used for insemination, the better the results obtained (Vishwanath and Shannon, 1997). Semen volume was more than 1.5 ml (Tuli and Holtz, 1995; Sundararaman *et al*., 2007). For frozen-thawed semen, progressive sperm cell motility of 54% was reported (Tuli and Holtz, 1995). Goat fresh sperm cell motility generally ranges from 80 to 90% (Gacitua and Arav, 2005). However, Purdy (2006) reported no or low recovery rates (0 to 18%) of motile sperm cells post-thaw in goats.

The effect of collection frequency on semen characteristics is considerable and must be recorded in detail. Two ejaculates collected once a week (in a period of at least 15 min) give good semen production results (Bencheikh, 1995, Moce *et al*., 2000). A daily constant 16L: 8D light program increases sperm production (qualitative and quantitative aspects) compared with a shorter light duration (8L: 16D, Theau-clement *et al*., 1994). Day length has an effect on reproduction in the buck and the doe. During the fall, the endocrine system also increases levels of the sex hormones, testosterone and luteinizing hormone (Ritar, 1990).

The volume of the ejaculate will be generally between 0.5 and 1.5 ml. Blokhuis (1962) estimated the average volume from a series of collections to range between 0.25 and 5.0 ml with ideal volume not to exceed 2 ml. Smith (1978) reported the average buck semen volume to be between 0.5 and 1.0 ml; however, averages of 0.85, 0.82, and 0.68 ml have been reported by Huat (1973), Patel (1967), and Eaton and Simons (1952), respectively.

The color of buck semen ranges from a creamy white color to yellow. Red coloring of the sample indicates the presence of blood. Yellow watery colors may indicate urine in the ejaculate. A black or dark sample may indicate debris such as dirt or manure contaminating the sample due to an un-cleansed prepuce or the presence of pus Patel (1967) and Eaton and Simons (1952).

Post thaw motility

Motility of spermatozoa has been extensively used as a parameter to assess viability of such cells (Guraya, 1987). The motility of spermatozoa is required for fertility.

Motility itself is not an indication of fertility and it is more often an expression of physical activity. Sperm cell motility of 72% (Tuli and Holtz, 1995), sperm cell concentration of greater than 3 billion sperm cells (Motlomelo *et al.,* 2002). Swelling causes changes in both cell size and shape that can be evaluated using a phase contrast microscope (Cabrita *et al.,* 1999). Coiling of the tail begins at the distal end of the tail and proceeds toward the mid-piece and head as the osmotic pressure of the suspending media is lowered (Jeyendran *et al.,* 1984).

The concentration of buck sperm reported to be necessary for a satisfactory fertilization rate is 40 million sperm per ml when fresh semen is used and 120 million motile sperm when frozen semen is used (Corteelet *et al.,* 1975). Semen put up at a concentration of 120 million or 60 million per 0.5 cc straw and having a 50% post-thaw survival rate yielded suitable fertilization. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot *et al*., 1997).

The percentage of morphological abnormalities in the semen of below average and poorly fertile bucks may be 10-15%, and 15%+, respectively (Eaton and Simmons, 1952; Huat, 1973; Ott, 1978; and Patel, 1967). The morphological characteristics of spermatozoa are influenced by several factors including the genetic make-up and physiological stage of the animal, nutrition, season, climatic factors, and disease (Dana et al., 2000; Dowsett and Knott, 1996; Barth and Oko, 1989).

The main objective of this study included the quality parameters of semen and implication of artificial insemination in goat which included the motility of semen, study the live and dead spermatozoa, study the morphology of semen, determine the HOSS test, assess the concentration of semen and evaluation of the conception rate in goat

MATERIALS AND METHODS

Site of Study

The study was conducted in NLBC, Pokhara and Surkhet district. The fresh semen of different breeds was collected at Bandipur Goat Research Farm of NARC. The semen of Jamunapari, pure Boer and Barbari were collected by using artificial vagina method. The artificial inseminations of goats were done in Kaski and Surkhet districts.

Semen collection

Semen samples were collected from Boer, Barbari and Jamunapari bucks of Bandipur, Tahanu and diluter (AndroMed 200ml, minitube international-Ref: 13503/0200) was used for the sample preservation and was carried to the lab of NLBC, Pokhara.

Methods of sampling

Collected semen sample was processed by using automated computerized IS4 and Genomax filling, sealing and printing machines made of IMV France. The particular machine produced 15000 straws per hour. The straws were placed into straw racks and refrigerated at the temperature 0±5 ^{0}C for 4 hours. Then, all the straws were frozen slowly up to the temperature -140 ^{0}C by using automated computerized bio-freezer, (IMV, France). Finally, the straws were transferred to the liquid nitrogen container, having temperature of -196 ^{0}C.

Sample handling procedure

A sample of frozen semen straw was taken out of the liquid nitrogen container. Without exposing the straw at room temperature for more than 4-5 seconds it was thawed in a water bath at 37oC for 35-40 seconds. It was wiped with tissue paper for avoiding contamination. With the help of sterile scissors the end of straw was cut on single lock part and the insemination of the doe's were done by transcervical method by using speculum and artificial insemination gun. The estrous goats (heat) were identified by using teaser bucks.

Data from laboratory examination and AI recorded data was inserted into SPSS. The data were analyzed statistically using SPSS 21.0.

Methodology of microscopic examination

Post thaw motility (Fresh semen)

A drop of semen was placed on the grease free clean pre warmed glass slide (37^{0}C) with micropipette and a cover slip applied on the sample to avoiding formation of air bubbles. The slide is then examined under microscope at 10X magnification. Five widely space fields are examined to estimate the percentage of motile cells.

Post thaw viability (Frozen semen)

The frozen straw is thawed for 35-40 seconds at 37^{0}C. Incubation of semen at 37^{0}C in water bath and then evaluation of motility is done three times per hour of incubation. Samples showing higher percentage of motile spermatozoa for longer periods of incubation at 37^{0}C are considered as better samples in terms of viability of spermatozoa.

Hypo Osmotic Swelling Test (HOST)

Thawing straw cut and pour semen on tube (37^{0}C), mix with 1ml of hypo-osmotic media solution with 100 micron micropipette and incubate for 20 minutes. A drop of mixture on pre-warm clean glass slide with cover slip and examine on microscope

with 20x magnification. 100 cells count in different fields. 60%-70% are good semen with tail curved which was counted HOS positive and other HOS negative. HOS responsive cell (%) = HOS positive/total cell count*100 for 30 minutes.

Sperm concentration per dose of semen

The diluted semen taken to haemocytometer slide with cover glass without any bubbles examine under low power objectives after 5 minutes. The numbers of sperms are count in the four corner chambers and one middle chamber (16 x 5 = 80 small chambers). Estimated of sperm concentration: a) Sperm concentration = Number of spermatozoa counted X multiplication factor X the dilution factor. b) Total sperm count = Sperm concentration X semen volume. c) Multiplication factor = 50,000 for spermatozoa counted in small square E1, E2, E3, E4 and E5. d) Dilution factor = 20 for 0.1 ml in 39.9 ml (Source: NLBC, Pokhara)

Morphological studies of spermatozoa

Morphological studies Type of abnormalities: Head, mid-piece, tail, proximal droplets. On sterilized borosil glass tube 5ml of buffer formal saline and 1-2 drops of near semen is add and incubate at 37^0C for 10-20 minutes. Sample drop is kept on glass slide, cover with cover slip and examine under oil immersion objective. 200 sperms should count from different fields and types of sperm abnormalities will tabulate. Sperm abnormalities and maximum allowed frequency in normal buck semen are: a) Head abnormalities: Young buck 10%: Old buck 20%, b) Mid-pieces abnormalities 55, c) Tail abnormalities 5%, d) proximal and distal droplets 5%. Semen should not be used if the sample contains more than 20% abnormalities.

Live and dead spermatozoa

Three drops of 5% Eosin and 5 drops of 10% Nigrosine were taken and mixed in a test tube in water bath at 340C. Two drops of stain and a small drop of semen are mixed in a pre-warm slide and mixed gently. Two smears were prepared, allowed to dry in the air. Random fields (diagonally) are counted over the slides to obtain. Under oil immersion 100 sperms counted, live and dead sperm ratio was calculated. Bucks having more than 20% dead sperms in neat semen were advised for sexual rest and correction.

RESULTS

The volume of the semen obtained from Jamunapari, Barbari and Boer were 1.5ml, 2ml and 1.2 ml, respectively (Table 1) and the color varying from milky white to creamy white. The motility percentage was almost similar for Jamunapari and Barbari but lower for Boer (Table 2).

Table 1: Volume and color of Jamunapari, Barbari and Boer buck (28.06.2013 A.D.)

	Jamunapari	**Barbari**	**Boer**
Volume	1.5ml	2ml	1.2ml
Color	Milky white	Creamy white	Milky white

Table 2: Total sperm motility and movement at different time interval of Jamunapari, Barbari and Boer buck (28.06.2013 A.D.)

Breed	**Jamunapari**		**Barbari**		**Boer 100%**	
	Motility %	**Movement**	**Motility %**	**Movement**	**Motility %**	**Movement**
Fresh semen	80%	Very fast	80%	Very fast	75%	Very fast
Fz-0hr	50%	Fast	50%	Fast	45%	Fast
Fz-1hr	40%	Good	40%	Good	35%	Good
Fz-2hr	25%	Fair	30%	Fair	20%	Poor
Fz-3hr	15%	Poor	15%	Poor	10%	Very poor

The morphological defects of tail in Jamunapari, Barbari and Boer 100% were 64%, 66% and 60%, respectively; straight tail were 36%, 34% and 40%, respectively; the live spermatozoa were 72%, 70% and 67%, respectively; dead spermatozoa were 28%, 30% and 33%, respectively; morphology were 16%, 18% and 24%, respectively and concentration were 40, 34 and 38 in term of million spermatozoa per dose, respectively, the sperm concentrations of Jamunapari, Barbari and Boer buck is presented in Table 3.

Table 3: Sperm concentration of Jamunapari, Barbari and Boer buck (05.07.2013 A.D.)

Breed	**Jamunapari**	**Barbari**	**Boer 100%**
Concentration/dose	$40x10^6$	$34x10^6$	$38x10^6$

The total number of goats was 129, out of 129 goats the number of parturient were 54. As per the calculation the total kidding percentage were 41.86% (Table 4)

Table 4 Total numbers of parturient and not-parturient goats

	Frequency	**Percent**	**Cumulative percent**
Parturient	54	41.86	41.86
Non-Parturient	75	58.14	100.0
Total	129	100.0	

CONCLUSION

This study was done to determine the semen quality from Boer, Barbari and Jamunapari bucks with the view on improving conception rates after AI with frozen-thawed semen. Seminal quality parameters were used to evaluate the effect of freeze-thawing procedure on goat sperm characteristics, and to relate possible changes in sperm parameters to cryopreservation.

The semen collection, processing and artificial insemination were done in goat for the first time in Nepal and it was successfully done. The AI was done from Jamunapari, Barbari and Boer buck's semen straw. With the application of AI, the inbreeding defects can be reduced in the goats. The major implication of AI in goat is to produce the elite bucks for nucleus herds and commercialization of the goat rearing.

Motility, morphology, HOSS test, live/dead and concentration of spermatozoa was estimated under a microscope. The correlation between semen quality and parturition rates in inseminated does was also investigated.

The percentage of kidding was 41.86%, which means, AI may be a solution for farmers who want to produce crossbreed goats. AI can also contribute to a faster improvement of the local breed.

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Community Initiative for Genetic Improvements in Goats of Ladavir, Sindhuli

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ABSTRACT

The purpose of this study was to establish Goat Resource Village to produce genetically superior seed goats for multiplication in other parts of Nepal through selection practice in initiation of community members at Ladavir VDC, Sindhuli. The study was started in April 2012 with 821 base flock of breeding goats/nannies in 272 households, all being member from self helped groups of Heifer Nepal. Each nanny was provided with the production performance record card and community members were trained to fill the record cards appropriately. All the information regarding the production performances of the nannies and kids mentioned in the card were entered in the software "Access database program". For the selection of the does, 50% selection intensity was applied on top performing does on the basis of three months litter weight/doe. In the same way, for the selection of male kids and female kids as seed animals, 20% and 50% selection intensity respectively was applied on top performing kids at five months body weight gain as per the report generated by the software. Out of 821 goats available initially in base flock, 218 were selected and rest were culled and replaced again. Till March 2014, the community members have been able to maintain production performance record cards of 1146 goats in 426 households. The parameters analyzed at 12 months interval shows that average daily weight gain increased from 81.90 ± 22.38 g to 89.82 ± 21.79 g in male kids and 72.14 ± 13.80 g to 81.21 ± 18.02 g in female kids; average five months body weight gain increased from 14.12 ± 3.39 kg to 15.46 ± 3.52 kg in male kids and 12.53 ± 2.06 kg to 14.14 ± 2.68 kg in female kids; and average three months litter weight/doe increased from 10.93 ± 4.14 kg to 12.13 ± 4.38 kg in first kidding goats and 13.43 ± 5.42 kg to 14.70 ± 6.10 kg above first kidding goats. This significantly increased figure reflects that selection and culling practice in the flocks has improved the breed performances as the selection continues. Within the study period, 60 male and 105 female genetically superior seed goats were produced and marketed to various districts of central and eastern development region as well as within village for its further multiplication through the social entrepreneur women cooperative

established by the community itself. Production and marketing of superior quality seed animals across the communities around the countries proved goat farming in the establishment of the Goat Resource Village through selection practice within breed in turn to increase productivity of the goats.

Key words: Cooperative, Goat Resource Village, Record keeping, Seed goats, Selection

INTRODUCTION

In fiscal year 2011/12, Nepal had 9.55 million of goat populations. Despite various efforts in the past productivity has not been increased significantly to reduce the import and in same fiscal year Nepal imported 1,181,428 live goats worth NRs 991,536,306 for meat purposes from India only and this trend is increasing every year (MoAD, 2012). For self-sufficiency in meat, the smallholder goat production system must be efficient in terms of productivity and competitive in terms of marketing. Even though improvement in the management (feeding, health care etc.) is the first step that needs to be taken to improve productivity, more can be gained by improving the genetic potential of the animal (ESGPIP, 2008). Thus genetic improvement is a critically important activity to enhance productivity by ensuring availability of improved breeds. We don't have continuous, structural and established genetic improvement programs therefore chance of negative selection prevails in native goats of Nepal. Therefore significant genetic gain is achievable in the flocks through selection that have wide variation in production parameters. Selection is the silent force which decides certain preferred parents in a population to produce more desirable offspring in the next generation. But, selecting superior quality goats scientifically in the community for enhancing growth rate and meat qualities has not been yet established in Nepal due to difficulty in data recording and its management of the individual goat performances of the village flocks.

Pedigree recording and genetic selection in the village goat flocks of smallholder farmers have been deemed infeasible by researchers and development workers due to uncontrolled village breeding practices and this was found to be overcome by selection practice through community initiation managed by village cooperative (Solomon *et al.,* 2014). Hence this demands the establishment of performance record keeping of individual goats in village flocks and community based selection as a tool of technical intervention for improvement of breeds of native goat flocks for gradual permanent genetic gain.

Thus this study aims to establish Goat Resource Village to produce genetically superior seed goats of increased productivity for its further multiplication in the participation of the community members through cooperative.

MATERIALS AND METHODS

Study Area

The study area included Ladavir Village Development Committee of Sindhuli District which borders Kavereplanchok, Ramechhap, and Okhaldhunga in the North; Sarlahi, Mahottari, Dhanusha, and Siraha in the South; Udaypur in the East, and Makawanpur in the West. The proposed site is around 30 km north from the East-West highway and has much potential for goat farming due to adequate availability of forage and fodder to sustain existing goat population with enough area available to extend the forage and fodder production to expand goat farming.

Goat production system in village

Farmers are raising goats of Jamunapari cross (cross between Jamunapari and indigenous Khari breed) suitable for that area. The majority of the farmers rear three or even more nannies, managed under stall-fed system. Heifer' previous activities had formed foundation in goat husbandry through "Improved Animal Management" training and moreover, basically through Farmers' Field School where in effect of improved management versus traditional management in goat productivity was compared and demonstrated to community members. Seeing better results in improved practice, the majority of the goat raisers here, since then, adopted the improved goat husbandry practice under stall fed system. To avoid inbreeding and maintain breeding sex ratio of 1:30, farmers had 15 superior quality bucks. On top of that, Heifer supported 28 more bucks of Jamunapari crosses with the increase in number of does later from the Mahuli Goat Resource Center, Saptari.

Identification of goat and its record card registration

Initially a total of 821 breeding goats of Jamunapari crosses (nannies above 5 months and under tenth kidding) were identified in 272 households and increased to 1146 goats in 426 households from April 2012 to March 2014. All were tagged and registered with record card being maintained in the file for each household.

Training

All households included in the project were trained on "Goat performances record keeping and breed improvement" training where they learned about the production record card maintenance, use of weighing balance and procedure of data entry in the card. Basically technical aspects and importance on the selection for breed improvement with respect to production performances was highlighted with a view to establish goat resource village.

Record card maintenance

Record card keeping was carried out continuously by the member participants themselves after being trained to fill the cards. Information included in the card were owner's name and address, history of the goat (goat name, tag number, name of the breed, and age of the goat, goat's parental name or identification, date of first service) and monthly measure of body weight from birth to five months age and in every respective kidding. Besides, it also included the information regarding the date of service/conception and date of parturition of every kidding; marketing date, date of death of kids and does and tag number of buck as well. At least two weighing balances per SHG and record cards to each individual goat were provided by Heifer Nepal to measure the weights of the kids in order to fill the performance record card.

Data Entry in Software

Performances record data of every individual and their new born were entered in the "Access Database Program" software developed by Himalayan IT, New Baneshwar, Kathmandu, Nepal and provided by Heifer Nepal. This software can compute the mean ± sd. of average daily body weight gain, body weight gain at five months, and three month litter weight/doe. These results were computed and analyzed as and when required.

Goat resource village management

A Social Entrepreneur Women Cooperative "Prayash Samajik UdhyamiMahila Sahakari Sanstha Ltd. Ladavir, Sindhuli" established by the members of SHG supervise the implementation of the goat resource center management, basically in the collection and interpretation of the recorded data with respect to genetic improvements. Cooperative also supervises the technical aspects for the production of seed animals that is to be marketed, like setting selection cut off value for the year imposing selection intensity in the population, observations of general health and body configurations, and analysis of inbreeding status from the record card. Achievements or outcomes from the selection programme were shared and discussed with members of the SHGs or cooperative in periodic meetings held every quarter with a view to help individuals decide to select the best one and cull the poor performing goats and replace with superior ones. After the completion of the project, to sustain the breed improvement programme or goat resource village the same cooperative will further undertake all the necessary activities accordingly.

Computation and Data Analysis

Results were computed for the initial two year of the project duration from April 2012 to March 2014

For selection of the nannies/does from base flock

Cut off value of the three month litter weight/doe of the total population was determined by 50 % selection intensity and the nannies above cut off value were selected for further breeding and rest were culled. However the does above the tenth kidding were not included in the selection. Culled ones were replaced to maintain the flock size.

For selection and marketing of seed animals

Cut off value at five months body weight gain was determined by 50% selection intensity in female kids and 20% selection intensity in male kids for the selection of the offspring. Kids above cut off value were kept under further investigation for more two or three months by the Cooperative and the best one in every aspect for breeding were verified and recommended to be marketed as seed animal. Every male kid below the cut off value at five months or kids even above the cut off value that were not fit for breeding in any respect were castrated and female kids were slaughtered for meat or culled out from the village flocks.

For the analysis of the genetic improvement of the flock

Yearly change in mean ± sd. of three months litter weight gain per doe, average daily weight gain and five months body weight gain were determined for the analysis of the genetic improvement of the flock

Data of three months litter weight/doe of an individual and the five months body weight gain of kids analyzed by the frequency distribution curve being computed in MS Excel. Change in mean ± sd. of average daily weight gain, average five month body weight gain of the kids, and average three month litter weight per doe in one year interval is presented in the table as per the results generated by the "Access database program" software.

RESULTS

Selection of the nannies from the base flock

115 nannies experienced the first kidding with an average three month litter weight/doe of 12.13 kg. Imposing 50% selection intensity in these nannies, cut off value of three month litter weight was found to be 11.18 kg. Based on this value, 58 were selected, however, 11 (10.00%) were below the mean population but also selected for further breeding assuming that these individuals could perform better second kidding onwards. The rest 57 nannies below the cut off value were culled out from village flocks or slaughtered (Fig. 1).

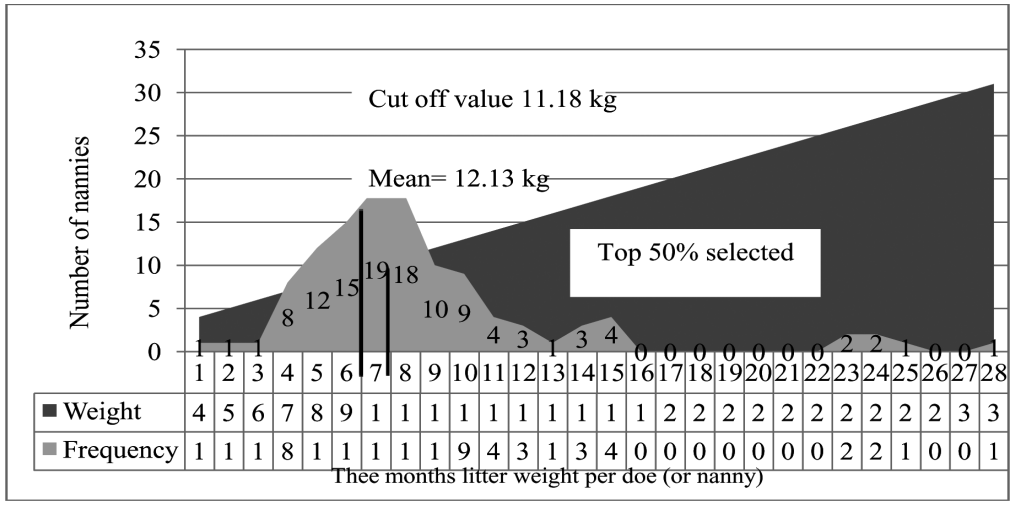


Fig. 1: Frequency distribution of nannies at first kidding

Similarly, 320 nannies experienced different kidding stages from second to tenth kidding with an average three month litter weight/doe of 14.70 kg. Imposing 50% selection intensity in these nannies, cut off value of three month litter weight was found to be 13 kg. Above this cut off value 160 (50.00%) were selected, however, 32 (10.00%) were below the mean population but also selected for further breeding with assumption that these individual could perform better next kidding onwards and rest 160 nannies below cut off value culled out from village flocks or slaughtered (Fig. 2).

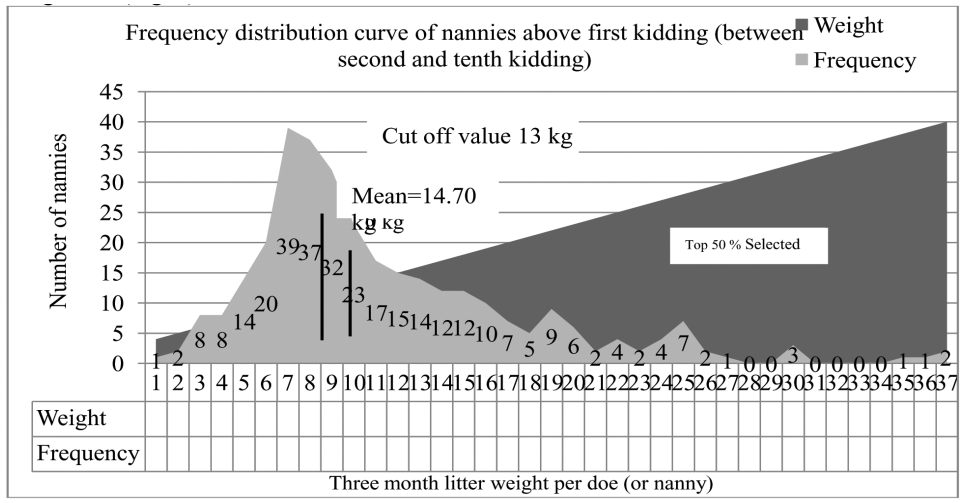


Fig. 2: shows the selection of the nannies at above first kidding from Ladavir Goat Resource Village, Sindhuli, Nepal.

Out of the initial 821 base flocks in the first year of the project there were 435 births. And out of 435 goats, 218 goats were selected on the basis of the cut off value of three months litter weight per doe and rest 217 were culled and replaced by new breeding goats to maintain the flock size. In this way selection tool was applied at the Ladavir Goat Resource Village to improve the performances of the base flock.

Selection and marketing management of the produced seed animals

Selection of seed animal

Average weight at five months age of 211 female kids from 435 births was found to be 14.14±2.68 kg. Imposing 50% selection intensity, cut off value was found to be 14 kg which is close to the mean value. 113 female kids above the cut off value were selected to be verified as seed animals for further multiplication (Fig. 3).

Average weight at five months age of 301 male kids from 435 births was found to be 15.46 ±3.52 kg. Imposing 20% selection intensity, cut off value was found to be 18 kg. Hence 73 male kids were found above the cut off value and were selected to be verified as seed animal for further multiplication (Figure 4).

Marketing status of the produced seed animal

Seed animals produced at Ladavir Goat Resource Village are evaluated and marketed at predetermined price through Social Entrepreneur Women Cooperative "Prayash Samajik Udhyami Mahila Sahakari Sanstha Ltd. Ladavir, Sindhuli".

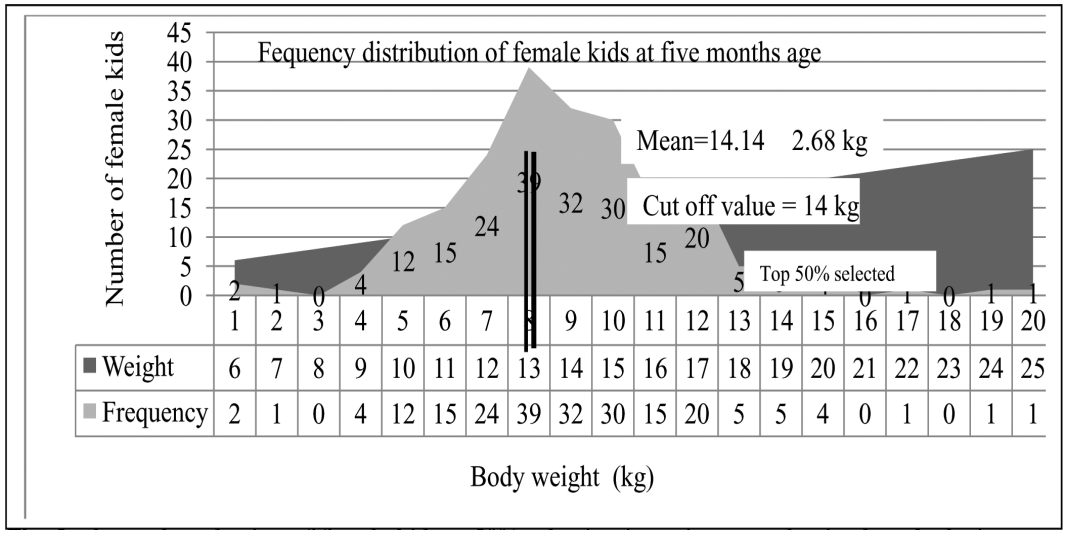
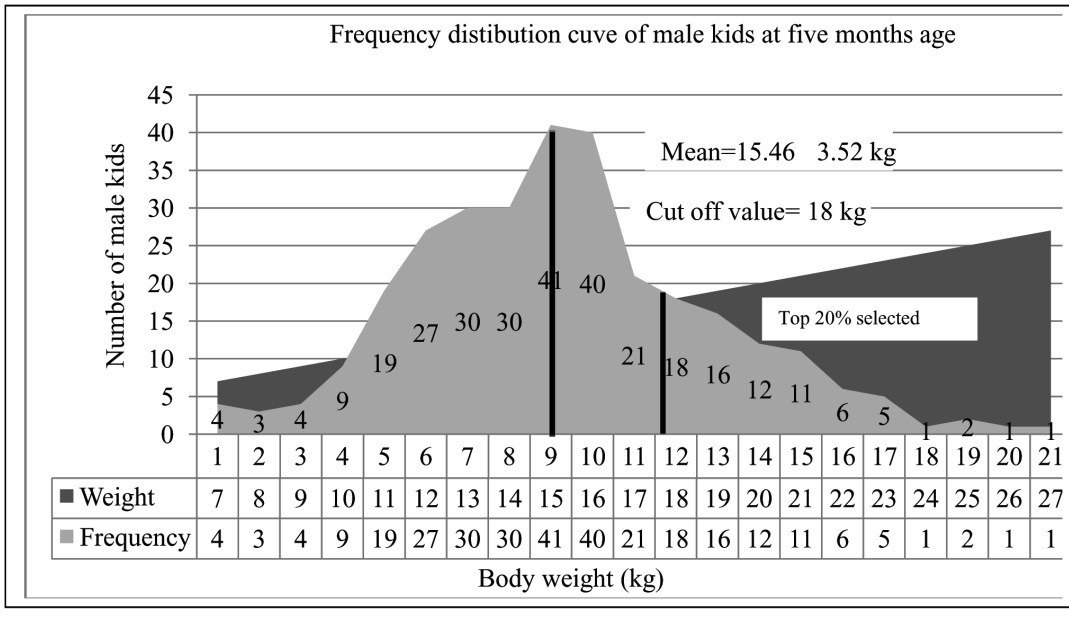


Fig. 3: shows the selection of female kids at 50% selection intensity as seed animals at Ladavir Goat Resource Village, Sindhuli.

By the second year of the selection practice in village goat flocks, the cooperative produced and sold 60 out of 73 male kids above its cut off value and 105 out of 113 female kids above its cut off value as seed/breeding animal to various districts of Central and Eastern development region and within the villages as well for further multiplication (Fig 4).

Generally at Ladavir, farmers used to sell goats for meat irrespective of their quality at the rate of NRs. 350/kg for castrated goats and at NRs. 250/kg for female kids or does. Before, they had not realized the importance of the seed animals from the breeding and marketing point of view. Cooperative initiated to collect the seed animals as per the record card performances from the community members at appreciating price of NRs. 450 for male and NRs. 350 for female per kg live body weight. Since then, smallholder farmers have been benefiting more from goat enterprise by selective selling of their superior quality goats as seed animal and non quality as meat animal. This increased price for the seed animal motivated the smallholder goat raisers to keep their best kids to be marketed as seed animal rather than to sell every goat as meat animal.

Finally project was able to change marketing status and benefited community more than previous from the goat enterprise. This ultimately encouraged community members to get involved in genetic improvement of goats.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Weight	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Frequency	4	3	4	9	19	27	30	30	41	40	21	18	16	12	11	6	5	1	2	1	1

Fig. 4: shows the selection of male kids at 20% selection intensity as seed animals at Ladavir, Goat Resource Village, Sindhuli,

Analysis of the genetic improvement of goat flocks

Breed improvement analysis was interpreted by the comparison of the change in the value of the parameters of the average daily weight gain, average five month body weight gain and three month litter weight per doe at one year interval. Average daily weight gain till five months in one year interval increased from 81.90 ± 22.38 g to 88.67 ± 21.93 g in male kids and from 72.14 ± 13.80 g to 81.21 ± 18.02 g in female kids and similarly, average five month body weight gain found to increased from 14.12 ± 3.39 kg to 15.46 ± 3.52 kg in male kids and 12.53 ± 2.06 kg to 14.14 ± 2.68 kg in female kids. Again in the same time period three month litter weight/doe also increased from 10.93 ± 4.14 kg to 12.13 ± 4.38 in first kidding does and from 13.43 ± 5.42 kg to 14.70 ± 6.10 kg above first kidding between second and tenth kidding (Table 1). Hence, increased figures for every parameter by one year interval in Table 1 signify improving performance of nannies in the village flocks to produce superior quality progeny due to continuation of selection practice.

Table 1: shows breed improvement of the does as per improving performances of their progeny at Ladavir Goat Resource Village, Sindhuli, Nepal.

Parameters	First 12 month (April 12-March 13)	Second 12 month (April 13-March 14)	Remarks/Change
1. Average daily weight gain till five months (g)			
i) Male kids	81.90 ± 22.38	88.67 ± 21.93	Increased
ii) Female kids	72.14 ± 13.80	81.21 ± 18.02	,,
2. Average weight at five months (kg)			
i) Male kids	14.12 ± 3.39	15.46 ± 3.52	Increased
ii) Female kids	12.53 ± 2.06	14.14 ± 2.68	,,
3. Average three months litter weight per doe (kg)			
i) At first kidding	10.93 ± 4.14	12.13 ± 4.38	Increased
ii) Above first kidding	13.43 ± 5.42	14.70 ± 6.10	,,

DISCUSSION

In the project study of genetic gain in selected Khari goats over generations in eastern hills of Nepal (Neopane and Pokharel, 2010) average six month body weight of single born kids at the site was 11.9±0.214 kg whereas for multiple born kids it was 10.7±0.239 kg which is very less in comparison to this project study where even female kids of Jamunapari crosses attained average weight of 14.14±2.68 kg at five month (Fig. 3 and Table 1). Similarly, the body weights of Khari and Sinhal at six month age reported by Agriculture Research Station (Goat) Bandipur, Tanahu was found to be 11.02 ± 4.31 kg and 14.03 ± 3.12 kg respectively and that of 50 % Jamunapari (Jamunapari X Khari) and 50% Boer (Boer X Khari) was 14.69 ± 4.60 kg and 17.85 ± 4.36 kg respectively (ARS, 2067/68). Results of the growth performances of Jamunapari crosses under this project area are better than the performances of the Khari and Sinhal while more or less similar to performances of 50 % Jamunapari and 50% Boer at Agriculture Research Station,, Bandipur. Hence, different study reveals growth performances of Jamunapari crosses or crossbred goats are better than the indigenous Khari and Sinhal.

In the similar study, growth performances of the crossbred male kids of Boer (Pure Boer X Khari) at five month age was 16.05 ± 0.23 kg and that of female kid s was 15.5 ± 0.25 kg (Adhikari *et al*; 2013). This result is not in accordance with the result of project study in first year but tends to be similar with the result of second year progress (Table 1). The average daily weight gain of the boer crossbred goat have been found between 100 to 200 gm in the farmers' field (Pandey, 2008) which is less than the Jamunapari crossbred goats as revealed in this study (Table 1). However, average daily weight gain has increased in the second year of the project in comparison to the first year indicating selection and culling practices of the does in village flocks based on their three month litter weight/doe improved the growth performances (Table 1).

Technical Bulletin on genetic improvement of sheep and goat at village level published by the Ethiopia Sheep and Goat Productivity Programme (ESGPIP), Ethiopia describes the guidelines for the genetic improvement of sheep and goats at village level flocks by implementing the simple measures of record keeping and selection practices in flocks at smallholder producer with its management by cooperative concept (ESGPIP, 2008 and ESGPIP, 2011) as carried out by this project. Hence the output of the project suggest that genetic exploration and improvement of our existing goat breed can be done simply through continuous selection practice over years to enhance the productivity rather than wandering for new introduction and distribution of exotic breeds and not practicing the selection process.

However selection programme of the goat breed should be area specific and within the breeds like Khari in mid hills, Jamunapari crosses in Siwaliks, lower hills and Terai region otherwise due to lack of adaptation productivity is retarded (Oli, 1987).

CONCLUSION

Selection practice within the breed can be adopted through participation of the community members for the production of the seed animals and to improve the genetic performances of the village goat flocks. Hence genetic potential of native Nepalese breed of area specific can be explored and improved significantly for the goat productivity enhancement instead of introducing a new exotic breed. Production and marketing of superior quality seed animals through selection practice proved goat farming to be a more profitable business, which motivated the community in the establishment of the Goat Resource Village.

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Management Practices and Major Health Problems in Small Scale Pig Farms of Chitwan Valley, Nepal

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ABSTRACT

Pig farming is important mean of income generation, food security and poverty alleviation in Nepal. With the objective to assess pig management system, and major health problems which cause direct loss by piglet mortality and indirect loss by poor feed efficiency, six pig pocket area of Chitwan valley were selected where pig farms were selected on convenient sampling basis. At least 33 pig farms were selected from each site totaling 212. The most common source of food was rice and wheat bran. The fermented alcohol by-product (60%) and hotel remnant (45%) were usual feeding cheapest source. Around two-third of the farms (64%, n=136) suffered some sorts of health problems in pigs among which the most common was diarrhea and vomiting (n=46, 21.7%). Farmers' perception on major problems was obtained on value indexing basis which showed skin diseases the most prominent followed by diarrhea, respiratory problems, loss of body weight, injury, anorexia, vomiting, fever, sudden death and abortion in female. The most common skin diseases were scabies, allergy, mild mange, ringworm and folliculitis. Better understanding of health problems and practices to mitigate these problems could open the door for commercialization of pig husbandry in Nepal.

Key words: Piggery, Health problems, Piglet, Mortality, Small scale farm, Chitwan

INTRODUCTION

Pig rearing is one important mean of income generation, food security and poverty alleviation in Nepal. The pork is popular at present condition in Nepal and the production volume was increased to 18 thousand ton in 2010 (DLS, 2010) compared to 15 thousand ton in 2004. Generally, two far rowing per sow per year with litter size of 8–12 is obtained but farmers are unable to harvest all piglets born alive. They bear a heavy loss due to piglet mortality before they are weaned. According to Dyck and Swierstra (1987), there are various causes of early piglet mortality among which starvation and crushing were considered important. The NAHMS, India in

comprehensive research revealed crushing, starvation, scouring, and respiratory diseases as major problems. Similarly, there are several diversified causes for early piglet mortality in Nepalese context too. It is immensely important to find dominant causes of piglet mortality. The research hence was carried out with the objective to assess prevailing management practices and major health problems that cause significant losses in pig directly by piglet mortality from birth to weaning or indirectly through poor feed conversion ratio.

MATERIALS AND METHODS

Site and pig farms selection

Ten different pig production VDCs were identified based on secondary information and among them six were selected randomly. They were viz: Mukunpur, Bharatpur, Jugedi, Shaktikhor, Gitanagar and Sharadanagar. The information regarding pig farms in the selected sites were collected from livestock service office, and livestock service center. Using farm mapping with assistance from local pig farmer, list of swine farms in the community was assembled. More than 33 pig farms from each site were selected totaling 212 farms.

Data collection and analysis

Primary source of information was collected through semi structured questionnaire survey, focus group discussion (FGD) and key informant interview (KII). Secondary information were collected government reports, university reports and journals. Prior to the field survey, a preliminary study was carried out to become familiar with different features of the study area such as pig farming community and farming practices, veterinary technicians, agrovet owners, nearby veterinary service centers that helps in designing sampling framework and questionnaire preparation. The questionnaire was prepared, pretested and necessary modulation was taken. The questionnaire focused on some major variables such as farm characteristics, day to day farm management, feed and feeding and major health problems. The obtained data were summarized and displayed in tabular form with frequency and percentage. Weighted mean was employed to rank health problems. The associations were studied using Chi-square test. The p value less than 0.05 were considered significant.

RESULT

The housing

The objective of pig husbandry was found different among the farmers. Around half of farmers (48%, n=102) were involved in production, many of them (39%, n=82) were in both production and breeding whereas very few were (13%, n= 28) breeding

only. All the farmers had in-house farming system. The major sources of piglet were from nearby private farms and their own farms raising since long ago. Almost all the farms (99%, n= 209) had cemented floors but most of them were not scientific. Around 10% of the farmers perceived that they couldn't manage sufficient space for their pigs and piglets.

Nutrition management

All pigs farms provided concentrate ration to their pigs where wheat and rice based ration was common. All the farms provided food for more than one time in a day (Table 1). More than half of the respondents (60%, n= 127) prepare alcohol and use the by-product as major ration source for their pigs. Very few farmers were found to provide commercial ration (6%, n=13) whereas near 45% of the farmers (n= 95) mostly depended on hotel remnants for swine ration.

Table 1: Ration management of pig (N=212)

Activities	Aptitude	No of pig farm (%)
Times of food provision	Two times in a day	194 (91)
	Three times in a day	12 (6)
	Four times in a day	6(3)
Major concentrate as ration	Rice bran	70 (33)
	Wheat bran	30 (14)
	Mixed	112 (53)
Alcohol by-product as ration	Yes	127(60)
	No	85(40)
Hotel by-product as ration	Yes	95(45)
	No	117(55)
Commercial swine feed for daily ration	Yes	13 (6)
	No	199(94)
Separate drinking water and feed compartment	Yes	201(95)
	No	11(5)

Farmers' knowledge on pig health and disease

Farmers suffer losses due to mortality and illness in their piglets and pigs. The pigs are suffering from different kinds of diseases which directly or indirectly lead to losses on farm. Around two-third of the respondents (64%, n=136) mentioned some sorts of diseases in their farms. The details are in Table 2.

Table 2: Different kinds of disease condition in pigs

Disease Condition	No of respondents (%)
HH suffering no disease outbreak	76(36)
Diarrhea	20 (9)
Diarrhea and vomiting but no death	46 (22)
Bloody diarrhea	4(2)
Diarrhea and death	19 (9)
High fever	1(0.5)
High fever and death	1(0.5)
High fever, diarrhea and death	13(6)
Paralysis	4(2)
Pneumonia	6(3)
Staphylococcal infection	4(2)
Erysepalothrix	6(3)
Sudden death	9(4)
Death due to unknown cause	10 (5)

For this, through focal group discussion, ten most common health problems in the farms were sorted by farmers. These problems were loss of weight, anorexia, breathing problems, skin problems, diarrhea, vomiting, injury, crushing. The ranking of these problems were made based on weighted mean value (Table 3).

Table 3: Farmers' perception of major health problems in pigs (Total farms N=212)

Major problems	Total value	Weighted mean value	Rank
Skin problems	193.980	0.915	I
Diarrhea	188.680	0.890	II
Respiratory problems	172.144	0.812	III
Loss of weight	136.952	0.646	IV
Injury	120.628	0.569	V
Anorexia	117.660	0.555	VI
Vomiting	94.764	0.447	VII
Fever	90.100	0.425	VIII
Sudden death	74.624	0.352	IX
Abortion	57.664	0.272	X

All the farmers didn't vaccinate their pigs against different probable outbreaks. About 96% (n=204) of the farmers vaccinated their pigs against diseases. The most of the farmers vaccinated against swine fever (90.2%, n= 204) and Foot and Mouth Disease (84%, n= 179). None of the farmers reported external parasites but near about one third of them (28%, n= 60) thought that their pigs had internal parasites although 90% of them dewormed their pigs in regular basis (every six month). During observation also, our veterinary team did not noticed any external parasites. However, on farm basis, almost 90% of farms had some sorts of skin diseases in pigs (Table 4).

Table 4: Major skin diseases in pig farms (N=212)

Various skin diseases	**No of Pig farms (%)**
Farms without skin disease	22 (10)
Scabies	98(46)
Allergic skin reaction	39(18)
Mild mange	32(15)
Ringworm	17(8)
Folliculitis and Macule	4(2)

DISCUSSION

The major ration in small scale pig farming includes wheat and rice bran, which considered the cheapest source. Most of the farmers provide the food for two times a day and most of the ration is mixed of rice and wheat bran. More than half of the respondents prepare alcohol and use the by-product as major ration source for their pigs. In this sense, there is strong positive correlation between pig farming and alcohol preparation. Similar finding was reported by (Lekule and Kyvsgaard, 2003) where small scale pig farmers mainly rural areas of South East Asia fed their pigs mostly brewery byproducts, or waste products like food remnants. The housing system is diversified as free range, tertiary and confinement, which is contrast to this finding in Nepal that almost all the farmers had similar type of housing and feeding system. In small scale pig farming, the nutritional management is usually through use of locally available feed ingredients. Our finding is in the line of finding by (Rekwot *et al.,* 2003) from Nigeria where the feeds of small farms included kitchen waste, food remnant, bran from cereal crops like maize, rice, millet, sorghum, vegetables and kitchen wastes.

Regarding knowledge on diseases of pigs, about two-third of farmers suffered losses in pig production due to different kinds of illness in pigs. The pigs during their growth period manifested different clinical symptoms. Among which scouring and vomiting without death was the most common (21.7%, n=46) followed by scouring but no vomiting (9%, n=19) and high fever with scouring and death (6.1%, n=13). The scouring was the most common problem in pigs. Our finding agrees with the finding of (Ajala *et al.,* 2007) where the diarrhea, cough, helminthosis, skin diseases were the major health problems in small scale piggery farming. Paterson *et al.,* (2001) from Bolivia reported internal parasitic infestation as major problem which usually has indirect impact on diarrhea. However, mastitis in sow was not reported in our study.

Not all but most of the farmers had vaccinated their pig against major outbreaks like FMD and Swine Fever. This is consistent with the finding from (Ajala *et al.* 2007).Our finding reveals that 90% (n=190) of pig farms had at least one of the skin diseases; the most dominant were scabies, allergic skin, and mange. (Matos *et al.,* 2011) from Mozambique reported mixed infection of mange, ticks, fleas, lice as common skin problems in pigs with sarcoptic mange in large proportion of pigs (25%). The dominancy of skin diseases might be because of poor shed management and ignorance on early treatment in Nepalese context. One important finding of this research was ranking of major health problems in pig husbandry at farm level. The most common problem that farmers ranked first was skin problems. The other common problems ranking second, third, fourth and fifth respectively were scouring, respiratory problems, improper growth and crushing (Table 3). According (Wabacha *et al*., 2004), the major cause of pre weaning piglet mortality were diarrhea, skin necrosis, pruritus. These findings strengthen our finding because the research showed diarrhea and skin problems as major health problems associated with pig production.

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Status and Characterization of Highly Pathogenic Avian Influenza Virus Subtype H5N1 in Nepal

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ABSTRACT

Avian influenza (AI) is a contagious respiratory disease caused by type A influenza virus. Regular outbreaks of H5N1 avian influenza virus have occurred in South Asia since its first detection in India and Pakistan during 2006. The first outbreak of avian Influenza H5N1 was in 2009 in Nepal. From 2009-2011, the outbreaks of AI were due to clades 2.2 and 2.3.2 H5N1 virus. In 2012-2013 clades of 2.3.2.1 and 2.3.2.1a were detected. During this period 737 samples from different bird species were analysed by Polymerase chain reaction (PCR) from different regions of Nepal. Extraction of RNA from samples and their reverse transcriptase (RT) PCR reaction were performed as par the protocols provided with commercial kits from Qiagen and Invitrogen respectively. During the test RT- PCR and rRT-PCR techniques were used. Final results were interpreted by performing agarose gel electrophoresis in RT-PCR and observing cycle threshold (CT) value in rRT- PCR. Among all the samples, 192 turned out to be positive for H5. Molecular characterization and phylogenic analysis was performed at Veterinary Laboratory Agency (VLA) in UK and High Security Animal Disease Laboratory (HSADL) Anandanagar Bhopal, India. The results obtained from VLA, UK and HASDL, Bhopal revealed that HPAI H5N1 Virus clades of 2.3.2.1 and 2.3.2.1a were circulating in Nepal and causing heavy economic loss in the country.

INTRODUCTION

The highly pathogenic avian influenza (HPAI) subtype H5N1 virus first appeared in 1996 in Geese in Guangdong, China, and continues to circulate in the poultry population in many countries, mainly in Asia subsequently infecting wild birds or domestic poultry with sporadic zoonotic transmission to humans and raised pandemic concern (Alexander, 2000).

The clade 2.2 H5N1 virus that caused widespread outbreaks in wild birds of Qingha Lake in China subsequently spread westwards to the Middle East and south Asia,

Europe and Africa in 2006–2007 and got established in the poultry populations of some countries of Asia and Africa (Li *et al.,* 2011). During 2010 clade 2.3.2 detected in wild birds in Hong Kong, Japan, Russia and Mongolia (Gilbert *et al.,* 2012; Reid *et.al.*, 2011).

In January 2009, the first outbreak of HPAI subtype H5N1 (clade 2.2) occurred in Jhapa district of Nepal. In subsequent years many outbreaks occurred in the different districts of Nepal and the clade was similar to that found in India, Bangladesh, Bhutan (OIE 2010; Nagarajan et *al.,* 2012; Mondal et *al.,* 2013; CVL, 2009 - 2011).

The first introduction of clade 2.3.2 of H5N1 virus in South Asia was reported in Nepal in 2010 (OIE, 2010 ; Reid SM *et al*., 2011; Sedai D *et .al.,* 2012). Similarly, India reported the first introduction of the clade 2.3.2 of H5N1 virus in 2011 (Nagarajan *et al.,* 2012). In 2011 and 2012 HPAI H5N1 Virus 2.3.2.1 clade was circulating in different parts of Nepal which was closely related to clades from Mongolia, Rumania, Bulgaria (WHO/OIE/FAO 2014; Sedai D *et al.,* 2012; Gilbert M *et al*., 2012; Atanaska Marinova-Petkova *et al.,* 2014). From 2012- 2013 July, the outbreaks of H5N1 in Nepal were due to a combination of clade 2.3.2.1 and 2.3.2.1a. Phylogenetic analysis showed the similar lineage of clade of previous isolates of Nepal HPAI virus (H5N1) clade. No any human case was found due to HPAI subtype H5N1 in Nepal (Sedai *et al.,* 2012)

During July 2011 to July 2013 outbreaks of HPAI (H5N1) occurred in poultry in 19 districts of the country. Different species of birds affected were commercial chicken, commercial layers chicken, backyard chicken, Parent stocks chickens, pigeons, Giriraj chickens, ducks, and crows.

MATERIALS AND METHODS

Sources of sample and sampling Method

The samples were collected after the postmortem examination of dead birds received from farmers in Central Veterinary Laboratory (CVL) and from the field, Regional Veterinary Laboratories (RVL), National Avian Laboratory (NAL) and District Livestock Services Offices (DLSO). The submitted samples were dead birds, tracheal/ cloacal swabs and fresh fecal from backyard, commercial broiler, commercial layers chicken, ducks, pigeon and wild birds.

During 2011-2012 and 2012-2013 A.D. a total of 6662 and 6786 tracheal and cloacal

swabs, serum and fecal samples were received from different districts, NAL and RVLS for Avian Influenza diagnosis. Initially the tracheal and cloacal swabs samples were screened by using Rapid Flu A Test Kits (Anigen/ Synbiotic, USA) as recommended by the manufacturer. All Flu A positive samples and 10 % negative samples in rapid test and samples from high mortality cases (Approx. 737 samples) were tested by Reverse Transcription Polymerase Chain Reaction (RT-PCR & r RT-PCR) method.

Identification by RT-PCR and real time RT-PCR

During July 2011 to July 2013, altogether 737 Flu A positive and 10% Flu A negative samples from different districts of Nepal from different bird species were analyzed by using both Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Real Time PCR (r RT-PCR) methods by using OIE Reference Laboratory, Australian Animal Health Laboratory (AAHL), Geelong, protocol for the detection of virus. One step RT-PCR and real time RT-PCR for identification of Type A influenza virus and H5 subtype was carried out as previously described (Pasick J, 2008).

Viral RNA was extracted from samples by using Qiagen Viral RNeasy ® Mini Kit (Cat. No. 74106) as recommended by the manufacturers protocol.

Super Script™ III One- Step RT-PCR system with Platinum® Taq DNA polymerase, (Cat. No. 12574-026) Invitrogen master mix kit used for detection of influenza type A virus and sub type H5 in RT PCR method whereas SuperScriptIII Platinum One_Step Quantitative RT-PCR System with ROX Invitrogen Cat No. 11732-020 master mix kit was used for the detection of Flu A virus and sub type H5N1. Master Mix was prepared according to the protocol provided by OIE Reference Laboratory, Australian Animal Health Laboratory (AAHL), Geelong, Australia.

Further confirmation of HPAI Virus H5N1, sequencing and characterization of HPAI Virus H5N1 were done in Veterinary Laboratory Agency (VLA) in UK and High Security Animal Disease Laboratory (HSADL) Anandanagar Bhopal, India.

Thermo cycling

For Conventional RT PCR Thermocycler Biometra T personal model / Master Cycler Personal, Eppendorf was used for production of cDNA and its amplification. Each tube containing 25 µl of reaction mixture was subjected to RT PCR.

For One Step RT- PCR Invitrogen kit, cycling condition was carried out according to AAHL protocol. After Thermo cycling, Agarose gel electrophoresis of the amplified product was done.

In Real Time RT-PCR, Applied Bio System (ABI), 7500 model was used and cycling profile was 50^0C for 15 min, 95^0C for 2 min, and 40 cycles of 95^0 C for 10 sec, 60^0 C for 30 sec.

Agarose Gel Electrophoresis

1X TAE Buffer (TAE Buffer, 40X (Molecular Biology Grade) Cat No. V4281, Promega) was used to prepare 2 % agarose gel (Agarose Molecular Biology Grade, Cat. No. 75510-019, Invitrogen) and added 1% Ethidium Bromide Solution (10mg/ml) (Molecular Biological Grade, Cat. No. H5041: Promega, USA) to the heated gel.

A total of 10 µl of PCR product was added to loading buffer Blue (6X TriTrack ™ DNA Loading Dye, Catalog no.: R1161, Fermentas) , mixed and loaded on a 2 % agarose gel and run at 120 volts for 30 minutes in Biometra (Standard Power Pack P25). After electrophoresis DNA bands were visualized in Gel documentation system (Dark Hood DH-40).

Laboratory diagnosis of HPAI virus using RT PCR and r RT PCR was carried out at Central Veterinary Laboratory, Kathmandu whereas further molecular characterization and phylogenic analysis were performed at Veterinary Laboratory Agency (VLA) UK and High Security Animal Disease Laboratory (HSADL) Anandanagar Bhopal, India. All these outbreaks were controlled by culling and killing of birds followed by strict quarantine and biosecurity.

RESULT

PCR

Samples were loaded in each well as indicated by their specified number. Positive control (H5N1 Inactivated Antigen, VLA) and negative control (RNase free water) were also run along with the samples. Positive control showed a band at level equivalent to 150bp and none of the band in negative control.

A total of 358 (2011-2012) and 379 (2012-13) samples were tested by RT-PCR and rRt-PCR techniques for H5 and H5N1 out of which 71(19.83%) and 121 (31.93%) were found positive for HPAI H5 and H5N1 respectively (CVL , 2011-2012 & 2012-13).

Virus Sequences and Clades

According to the VLA, Weybridge, UK, the clades of Nepal Virus were 2.3.2.1 and 2.3.2.1a.

DISCUSSION

Avian Influenza virus subtype H5N1 can cause high mortality of poultry and direct economic losses. Small genetic changes are known to occur in influenza A viruses, including those that may affect humans or animals. The emergence of the H5N1 virus, such as clade 2.3.2.1 is one of such genetic mutations taking place as part of the natural evolution of the virus (OIE, 2011; OIE, 2013).

The study showed that the first outbreak of avian influenza H5N1 was in 2009 in Nepal with the clade of 2.2. The first introduction of clade 2.3.2 HPAI virus H5N1 in Nepal occurred in February 2010 and was the first event in South Asia. Till the end of 2012, HPAI H5N1 of clade 2.3.2.1 was circulating in HPAI outbreaks of Nepal (Sedai et al., 2012). Further characterization of the clade 2.3.2.1 has resulted to be clade 2.3.2.1a (OIE, 2013).

Avian Influenza virus of clade 2.3.2 had reported in domestic poultry in India (Nagarajan et al., 2012). HPAI virus H5N1 2.3.2 had repeatedly detected in wild birds in Hong Kong, Japan, Russia and Mangolia and that clade might have established in migratory birds (Gilbert et al. 2012; Reid et al., 2011).

Continued co-circulation of different subclades of HPAI virus H5N1 may be adapted in the domesticated poultry in Nepal and other countries of South Asia. The first outbreaks of H5N1 in chicken farms in Hong Kong were reported in March, April and May 1997 (Sims *et al.,* 2005). In China the peak of the Asian H5N1 epidemic occurred in winter months (Li *et al.,* 2004), possibly as a result of the better survival of the virus in low temperatures, combined with the increased movement and trade in poultry associated with winter festivals. Similarly in Nepal there was high incidence of outbreaks in winter seasons in previous years because of migration of wild birds. However, the outbreaks of avian influenza had detected throughout the year in Nepal irrespective of weather with high incidence during March to April (CVL, 2012 and 2013).

CONCLUSION

Nepal experienced the incidence of HPAI subtype H5N1 virus with the clades of 2.3.2.1 and 2.3.2.1a during the period of 2012-2013 July. Subsequently various districts of Nepal faced the disease problem and it caused the heavy economic loss to the country. Finally, the disease was successfully controlled by containment and destruction of the contaminated material.

In conclusion, H5N1 viruses will continue to circulate in Asia and mutate and the emergence of new reassortant H5N1 viruses. However, the implementation of

effective control measures, which is well under way in the country, coupled with enhanced programmes for the surveillance and timely diagnosis of infection, will help to reduce the risks to poultry and human health. It is also recommended maintaining the effective biosecurity and quarantine controls to reduce this risk. Lastly, Nepal needs a sustained commitment of resources for the disease diagnosis and technical support and guidance from the national and international institutions.

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Prevalence of Enterococci in In-Ova Vaccinated and in Unvaccinated Dead Developing Chicken Embryos and Pathogenicity study of *Enterococcus Faecalis* in Embryos

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ABSTRACT

A total of 428 chicken embryos of 36 batches from five different sources were examined to detect the causative agent of embryonic death of chicken embryos where 53.3% of the embryos found to be infected with microorganisms and among them 62.9% of the embryos were infected with Enterococci where 38.4% were pure Enterococci and 24.5% mixed with other bacteria. While reproducing disease by dipping and swabbing with overnight culture of Enterococcus faecalis; 27.5% in dipping, 12.5% pointed end swabbing and 10% in broad end swabbing of embryos were dead and Enterococcus faecalis was re-isolated from more than 90% of dead embryos. Although there was prevalence of five different species of Enterococci from the un-piped embryos but E. faecalis (77.0%) was major followed by E. faecium (16.4%). Motile Enterococci E. gallinarum and E. casseliflavus were not prevalent in in-ova unvaccinated dead embryos. Exposed surface area of an egg shell with E. faecalis and number of bacteria to be attached and penetrated is important factors to cause pathogenesis.

Key words: Air sac, dipping, embryos, lethal, swabbing, and un-piped.

INTRODUCTION

Enterococcus faecalis was previously known as *Streptococcus faecalis* is an inhabitant of the intestinal tracts of humans and many other animals, including the chicken (Murray, 1990). This bacterium has been described as a commensal or opportunistic, gram-positive, facultative anaerobe. When it inadvertently enters circulation, it can cause endocarditis, as well as urinary, intra-abdominal and pelvic infection (Murray, 1990). In this study major isolates from the dead chicken embryos were *E. faecalis*. Thus, *E. faecalis* seemed to be logical choice to study the embryo pathogenicity. Enterococal pathogenicity study has become a hot issue since it has been considered as nosocomial infection causing organism in-between 1970

to 1980 in human. Before that it was considered as innocuous organism. A little has been known regarding pathogenicity in animal, that's why still in animal it is considered as a putative organism. In human hospital Enterococci have emerged as one of the leading clinical challenges due to multiple drug resistance. Many multiple drug resistance isolates have been isolated and identified from poultry feces, meat (Simjee *et al.*, 2002) and other poultry environments. Pathogenicity in poultry as an agent causing endocarditis (Huycke and Gilmore, 1995), pulmonary hypertension syndrome (Tankson *et al.,* 2001), bacteremia and encephalomalacia (Cardona *et al.*, 1993, and Randall *et al.,* 1993), focal necrosis of the brain of chick (Devriese *et al., 1995*), hepatic granuloma in turkey (Hernandez *et al*., 1972) have been detected. Among Enterococci some acquired traits like antibiotic resistance determinants; cytolysin, gelatinase, aggregation substance, extracellular super-oxide production, and Enterococcal surface proteins which are related to cause disease or enhance to cause disease. *E. faecalis* is found to be more pathogenic than *E. faecium* as all these traits are available in *E. faecalis* and only antibiotic resistance determinants and aggregation substance rarely in *E. faecium* (Jett *et al.*, 1994). In this study we have isolated and identified embryo-pathogenic Enterococci (62.9 %) from un-piped dead embryos, which was the major cause of embryonic death of chicken embryos. Heart and lungs of broilers do not have a permanent bacterial flora (Takson *et al.*, 2001); however, 41 different bacteria were found in these organs at various times before, during and after hatching and *E. faecalis* was isolated in more chicks and at more times than any of other 40 transient bacteria.

The incubation period of avian eggs may be influenced by the behavior of the incubating parent (Ricklefs and Smeraski, 1983, Briskie and Sealy, 1990), thermal properties of the nest (Schaeffer 1980; Ricklefs and Smeraski, 1983), and physical properties of the egg itself such as mass and shell thickness (O'Connor, 1984). Interspecific variation in incubation periods also occurs independently of variables such as egg mass, likely reflecting species-specific differences in rates of embryonic growth and development (Ricklefs, 1993). Longer incubation periods may reduce the amount of time a young bird has to fledge, molt, or accumulate fat reserves for migration, as well as extending its period of vulnerability to nest predators (Perrins, 1977). Therefore, short incubation periods are thought to be desirable than delayed hatching for most bird species. Delayed hatching could be one of the biggest problems in commercial hatchery because waiting to be hatched hampers another coming batch, ultimately hampers whole business and cause big economic loss.

MATERIALS AND METHODS

Case study

A total of 428 un-piped embryos of 36 batches from 5 different sources including specific pathogen free (SPF Valo, Germany) embryos were examined for identifying causative organism. In the study; 248 (57.9%) of the embryos form layers, 38.3% from broiler and 3.7% were from SPF. Among the embryos from layers; 234 (94.4%) embryos were in-ova vaccinated against Mareks' disease. Embryos from broilers, some layers and SPF were not in-ova vaccinated.

Isolation and identification

Un-piped embryos were received from different parent stock farms. Only intact embryos were selected for the bacterial and fungal isolation. Embryos were cleaned with 70% ethanol externally twice before taking sample. Embryos were opened with flamed sterilized forceps and sample were taken inserting autoclaved cotton bud in-to the embryos. Sample was inoculated in three different medium plates e.g. Brain Heart Infusion Agar (BHIA) (Difco), Enterococcosel Agar (EA) (Difco) and Sabouraud Dextrose Agar (SDA) (Difco) to observe the different types of bacteria as well as fungi. Inoculated media plates were incubated at 37^0C for 24 hours for BHIA, up to 72 hours for EA and for minimum seven days at room temperature for SDA.

Black pigmented colonies on EA were compared with simultaneously inoculated on BHIA and SDA plates to differentiate pure and mix culture. Moist circular and black pigmented colonies from EA were tested with conventional test e.g. Gram stain, catalase test and growth in presence of 6.5% salt in Brain Heart Infusion (BHI) broth (Difco) to identify as genus Enterococcus. *Enterococcus species* were identified with the help of conventional sugar fermentation test by Facklam and Collin (1989), as well as Vitek (BioMerieux) GPI card. Motility of the Enterococci was tested by using MIO (Difco) medium. Biochemical tests of several suspected different Enterococci colonies were tested separately from each positive sample plates to differentiate mixed Enterococcal infection. Presences of colonies other than Enterococci on same plate were considered as mixed infection.

Pathological lesions in embryo infected with Enterococci

A total of 42 mature embryos from four different batches were opened to observe pathological lesions. Shell membrane, yolk sac, liver, gallbladder, heart, kidney and overall embryos situation were observed for pathological lesions.

Producing of pathogenesis with *Enterococcus faecalis*:

Missing Confirmation of contamination free experimental embryos for experiment Freshly laid fertilized eggs were received from a commercial hatchery. Fertilized eggs were selected for experiment, discarding abnormal misshaped eggs. Ten percent of the fertilized eggs, selected for experiment were randomly selected for checking contamination. Fertilized eggs were cleaned with 70% ethanol twice in five minutes interval. Sampling was done from each and every selected fertilized eggs with sterilized cotton bud breaking the shell with flamed sterilized forceps. Samples were inoculated in blood agar and incubated for overnight. Any bacterial growth in blood agar was considered as contamination and the whole lot of fertilized eggs was discarded. If there was no contamination in tested fertilized eggs, then all lots were taken as free from contamination.

Dipping of Fertilized eggs in overnight culture broth of *E. faecalis*

Experimental fertilized eggs were cleaned with 70% ethanol twice in five minutes interval before dipping. A total of 40 zero day embryos (fertilized eggs) were dipped in overnight culture of *E. faecalis* about 20 seconds. Other 20 embryos were kept as control dipping only in culture medium. Before the dipping both *E. faecalis* broth and culture medium were warmed at 37^0C to avoid chilling effect to the embryos. Inoculated embryos were incubated at 100^0F and 55 to 60% of humidity (PN Incubator and Hatchers, Korea).

Swabbing of fertilized eggs (zero day embryos) at broad end and pointed end with overnight culture broth of *E. faecalis*

Forty, day old embryos were swabbed with overnight culture broth of *E. faecalis* at broad end and 40 other were swabbed at pointed end about an area of 20 cm^2. Twenty control day old embryos for each group were treated with same manner with sterile broth medium at broad and pointed end. Embryos were incubated at 100^0F and 55 to 60% of humidity (PN Incubator and Hatchers, Korea) after treatment. Candling was done everyday after nine days of incubation. Re-isolation of *E. faecalis* (experimental strain) from dead embryos was done and confirmed with conventional biochemical test and GPI Vitek (BioMerieux) card system.

RESULTS

Frequency of Enterococcal infection in un-piped embryos

A total of 53.3 % were infected out of 428 un-piped embryos examined for bacterial and fungal infection. Among the infected embryos 62.9 % were infected with *Enterococus spp* where 38.4 % purely Enterococci followed by 24.5 % mixed with

Enterococci and 25.8 % were other bacteria.

Enterococcus faecalis and *E. faecium* were the major Enterococcal isolates from the un-piped embryos. In overall; *E. faecalis* represented 77.0%, followed by *E. faecium* with 16.4 %. Among others; *E. gallinarum*, *E. columbae* and *E. raffinosus* were 3.3 %, 2.5 %, and 0.8 % respectively (Fig. 1). A single colony of *Aspergillus spp* was detected from one embryo. Only *E. faecalis* and *E. faecium* were the Enterococci prevalent in embryos which were not vaccinated in-ova(Table 1). But altogether five species of Enterococci were isolated from in-ova vaccinated un-piped embryos.

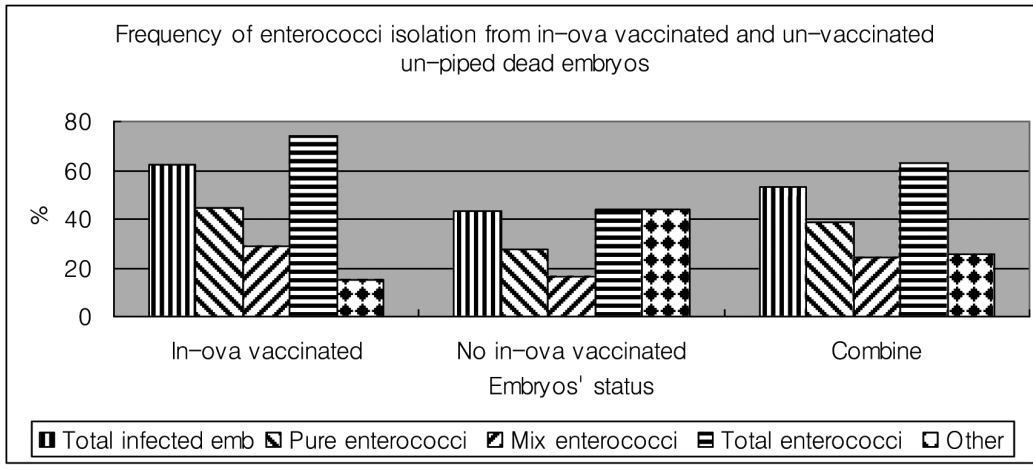

Fig. 1: Frequency of Enterococcal infection in in-ova vaccinated and un-vaccinated dead chicken embryos during incubation.

Table 1: Mortality of developing embryos due to *E. faecalis* infection when its shell wall was artificially contaminated by dipping and swabbing with *E. faecalis* broth just before incubation at zero day age.

Groups	**Sample size (embryos)**	**Nos of embryos died**	**Mortality %**	**Bacterial recovery %**
Dipping	40	11	27.5	90.9
Pointed end swabbing	40	5	12.5	100
Broad end swabbing	40	4	10	100
Dipping control	20	1@	5	0
Pointed end swabbing control	20	0		0
Broad end swabbing control	20	1@	5	0

Note: @ indicates undetected developing embryo (infertile egg)

Pathological lesions in dead embryos

Pathological lesions detected from the Enterococcal infected dead embryos (Table 2) from the case study were severe yolk sac infection, gall bladder swollen, rotten shell membrane, nephritis, necrotic foci with slight enlarged liver, fowl smell, blood clots in heart and swollen head. Lesions were similar in the experimental dead embryos inoculated with *E. faecalis*. Yolk sac infection, slight hepatomegali, nephritis and rotten shell membrane were severe in experimental dead embryos.

Table 2: Pathological lesions observed in embryos in different four batches

Shell membrane rotten	Swollen head	Necrotic foci in liver	Clot in heart	Kidney hemorrhage	Gall bladder swelling	Fowl smell of embryos	Yolksac infection
27/42 (64%)	21/42 (50%)	25/42 (62%)	23/42 (55%)	23/42 (54%)	28/42 (65%)	24/42 (58%)	31/42 (74%)

Reproducing pathogenicity

A total of 27.5% (11/40) of the embryos were killed when zero day old embryos were dipped in over night culture broth of *E. faecalis* and 90.9% (10/11) of dead embryos were positive with inoculated *E. faecalis*; in contrast 5.0% (1/20) embryos was dead (Table 1) and no microorganism was detected from the same dead embryo. Developing embryo was also not detected from the same, so considered as infertile egg. Embryo was unable to detect from one embryo among 11 dead embryos in the treated group was also considered as infertile egg.

Only 12.5% (5/40) of embryos were found dead when it was swabbed at pointed end of zero day embryos where 80.0% (4/5) were late embryonic death and only 20.0% (1/5) was early embryonic death. All the dead embryos were positive for inoculated *E. faecalis*. There was no mortality of embryos from the control group.

A total of 10.0% (4/40) embryos were killed when broad end of zero days' embryos was swabbed with *E. faecalis* broth and 100% (4/4) dead embryos were positive for inoculated *E. faecalis*. Five percent (1/20) embryo mortality was detected in control group where no microorganism was detected from dead embryo and was late embryonic death.

DISCUSSION

Case study showed significance difference in prevalence of *Enterococci species* between in-ova vaccinated and un-vaccinated chicken embryos. Motile Enterococci

were not detected from un-vaccinated chicken embryos. This result supports the penetrability of *E. faecalis* and *E. faecium* through egg shell and indicates that motile Enterococci were contaminants during or after in-ova vaccination.

Although the hatching eggs/embryonated eggs are having physical and chemical barrier to protect egg from invading microorganism but Enterococci with its hardy nature and able to tolerate high level of salt, bile, and even high temperature for longer time can stay on shell and can penetrate the shell membrane and enters into egg or embryo. Enterococci producing O2 are better adapted physiologically to utilize limited resources in the environment. As it can withstand and grow in high range of pH, it can grow in albumen that has pH around 9.2 and in allantoic fluid. It has been also observed that Enterococci possess the ability to survive within professional phagocytes (Gentry-Weeks *et al.,* 1998) that is due to aggregation substance, which helps in adherence, entry and survival in macrophages (Su *et al.,* 1991). Lysozyme poses broad spectrum antibacterial action against gram negative and gram positive bacteria (Ibrahim *et al*., 2001) and lysozyme constitutes approximately 3.5% of hen egg white (Osuga and Feeney, 1977). So the egg has enough amount of lysozyme to protect bacterial invasion in it, but Enterococci being a gram positive bacteria but it shows lysozyme resistant traits and gets entry into the eggs and causes pathogenicity leading ultimately about 30-40% death of developing embryos.

The finding of yolk sac infection 74.0% in dead chicken embryos in this study co-relates with Jean E. Sander *et al.*, (1998) who described significantly greater yolk weight in chicks injected with *E. faecalis* intra-yolk sac route. Cardona *et al.,* (1993) reported a field case of coagulative necrosis in liver and retained greenish yolk with *E. hirae* infection in 4-10 days old leghorn chicks. Majority of *E. faecalis* produce superoxide (O2) (Huycke *et al.*, 1996) and the membrane damaging effect of oxygen radicals may potentiate the ability of the organism to translocate across the weakened epithelial barrier. In this study it is noted that 64.0% of embryos were having rotten shell membrane, could be the effect of oxygen radical produced by *E. faecalis*.

From this study it is known that *Enterococci faecalis* which could enter in to the embryo in the early age even if they were in less number were able to kill embryos. During the time of laying eggs are hot and the pores in eggs are bigger in size as well as when it is cooling the negative pressure inside of egg can pull the things around the pores inside. There is enough chance of fecal contamination of egg in the cloacae and easily can enter inside.

CONCLUSION

In-ova vaccinated embryos were more contaminated with various microorganism where Enterococcal infection is exceptionally high and with the finding of dipping and swabbing; conclusion of this study is that exposed surface area of chicken embryo is important to cause the degree of pathogenicity ultimately leading to the death of embryo during incubation.

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A Case Report on *Erysipelas* in a Broiler Chicken Flock

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ABSTRACT

Erysipelothrix rhusiopathiae (formerly E. insidiosa) is a small gram-positive rod that causes erysipelas mainly in swine and turkeys and less frequently in other birds and humans. The major symptoms in chickens are weakness, depression, diarrhea and sudden death and gross lesions are related to septicemia including petechial hemorrhages and congestion in various tissues. From a broiler farm experiencing stunted growth of about 30% of chickens; dead birds were consigned for diagnosis. Gross lesions were general congestion, petechial hemorrhages in internal organs, especially kidney, and pneumonia. From lung sample non-hemolytic dewy, pinpoint colonies were isolated on sheep blood agar. The isolated bacterium was Gram-positive rod and catalase negative. According to the result of Vitek GPI card it was identified as E. rhusiopathiae by 99% accuracy. Isolated ER strain could tolerate 10% bile but unable to tolerate 40% and it is non-fermenter of most of the sugar. Therefore, we report here the first case of erysipelas in broiler chickens in Korea. Because erysipelas is important in food safety and public health, further study for prevalence of erysipelas in chicken flocks is required in the near future.

Key words*:* Erysipelothrix rhusiopathy, Grampositive rod, Broiler Breeder, Plate agglutination, Pin-point colony

Introduction

Erysipelothrix rhusiopathiae is a causal agent of swine erysipelas, which is of economic importance in the swine industry by virtue of causing acute septicemia, chronic arthritis, and endocarditis. However, the pathogene has worldwide distribution and may also infect different vertebrates including human (Brooke and Riley 1999). Erysipelas has been described in turkeys, ducks, emus and laying hens (Dhillon *et al*., 1980, Griffiths & Buller 1991 and Mutalib *et al*., 1993). A report of *Erysipelothrix spp*. isolated from 15.7% of skin samples & 1.9% from throat samples of broiler chickens (Seahorn 1989) has been reported. No reports have been available about erysipelas infection in poultry in Korea and also very little is known about the erysipelas in broiler chicken. Takahashi *et al*., 2000; described about 5.5% of

chicken sera were positive against *E. rhusiopathiae* in serological survey in Japan. Erysipelas of fish in south Kazakhstan (Sinev and Shaifullin 1969), cardiovascular lesion in sheep (Chineme *et al.*, 1973) due to erysipelas; erysipelas in guinea fowl (Baker & Westwood 1971); erysipelas outbreak in Broiler breeder flock (Borland 1970); outbreak of erysipelas in pheasant poults (Bygrave 1971). Erysipelas in quail (Nakazawa *et al.*, 1998); outbreak of erysipelas in farm geese (Gunning and Morton 1988) are the reports available about outbreak and isolation of ER. Route of infection is not clearly known but it would be possible entry of organism from abrasion of skin or GI mucosa as arthropod *D. gallinae* is a potential vector to transmit disease (Chirico *et al*., 2003).

MATERIALS AND METHODS

Case report

Eight dead Ross broiler chickens with the history of about 30% of chicken with stunted growth without significant mortality were submitted in Avian Disease Lab (ADL) of College of Veterinary Medicine, Seoul National University. During the post mortem examination, severe pneumonia was detected in all the birds with white necrotic foci on liver (mild) in six, focal congestion of pancreas in five, severe atrophy and hardening of BF in five, pinpoint hem in spleen in five, hemorrhages on eye lid and trachea in four, and hemorrhages in cecal tonsil in three were detected. Sample from liver and lungs were taken for bacteria isolation. Brillient Green Agar (Difco) with Novobiocin, (BGN), MacConkey (MAC) (Difco), and Blood Agar (BA) from Komed were used for bacteria isolation, and Gram staining, catalase test and GPI Vitek card were used for bacteria identification. Embryo inoculation and PCR was performed with specific primer to diagnose Newcastle disease (ND) infectious bursal disease (IBD), avian influenza (AI) and infectious bronchitis (IB). Serological survey of broiler breeder & broiler was done from 58 serum samples submitted to ADL.

Growth pattern of ER with different % of FBS in BHI

Three ml each 10, 7.5, 5, 2, 1 & 0% fetal bovine serum (FBS) supplied BHI broth was taken in 5 ml plastic tube mixed with 100µl of three hours incubated ER broth and incubated at 37^0C for 24 hours. The 24 hours incubated ER broth with Different percentage of FBS was serially diluted with phosphate buffer solution (PBS) up to 10^{-4} dilution. Hundred micro liter of 10^{-4} dilution of each broth culture were spread on three BHIA plates individually. The plates were incubated for 48 hours and counted colony forming unit and identified growth pattern in FBS.

Antibiotic susceptibility test of isolate was performed according NLCCS standard by disc diffusion method.

RESULTS AND DISCUSSION

Bacteria isolation

Two out of four lung samples inoculated in BA showed very small non-hemolytic, catalase negative and Gram positive rods that were further tested with GPI Vitek card & diagnosed as 99% *Erysipelothrix rhusiopathiae* (ER). No growth was seen in BGN and MAC. ER organism was very hard to grow when transferred into brain heart infusion agar (BHIA, Difco), in 24 hours incubated at 37^0C and small pinpoint like colonies observed in 48 hours of incubation. Isolated ER strain could tolerate 10% bile but unable to tolerate 40% & it is non-fermenter of most of the sugar (Table 1). We report this case as **first report in Korea** as there is no report available about prevalence of *Erysipelothrix rhusiopathiae* in broiler in Korea.

Table 1: Biochemical properties of isolated ER with Vitek GPI card.

BT	Result	BT	Result	BT	Result	BT	Result	BT	Result
PB	-ve	6%NaCl	-ve	10% Bile	+ve	40%Ble	-ve	Esculin	-ve
Tet R	-ve	Urea	-ve	Arginine	-ve	Dextrose	-ve	Lactose	-ve
Mannitol	-ve	Raffinose	-ve	Salicin	-ve	Sorbose	-ve	Sucrose	-ve
Trehalose	-ve	Arabinose	-ve	Pyruvate	-ve	Melibiose	+ve	Melezitose	-ve
Cellulose	-ve	Ribose	-ve	Xylose	-ve	Catalase	-ve	Hemolysis	-ve

Note: BT = Biochemical Test, -ve = Negative, +ve = Positive, PB = Peptone Base, Tet R=Tetrazoliun Red

Tests for virus detection

Embryo inoculation

Four 11 days chicken embryos were inoculated in chorioallantoic sac with 200µl of 0.2µm filter filtrate of tissue homogenate. There was no embryo death till 6 days of post inoculation & from all the embryos allantoic fluid was negative for hem agglutination with chicken RBC which indicates absence of embryo-pathogenic & hem-agglutinating viruses.

Polymerase chain reaction (PCR)

PCR was run using specific primer to diagnose ND, AI, IB & IBD was found to be negative.

Growth pattern of organism

BHI supplied with 5% FBS found to be the best followed with 2% FBS in BHI to grow ER in this experiment. Growth of ER in 0, 1, and 10% of FBS in BHI was

almost the similar. The result indicates that FBS may also contain some bacterial growth inhibitor that may show their effect when they are in sufficient amount (Fig. 1).

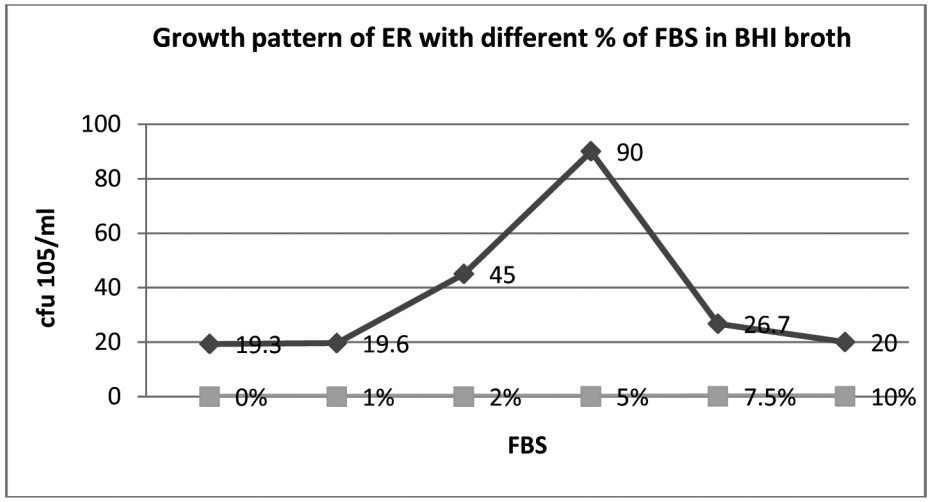

Fig. 1: Growth pattern of ER with different % of FBS in BHI broth.

Serological survey

A total of 5.2% (3/58) serum samples from seven broiler breeder & broiler farm were found positive when tested with plate agglutination test using antigen prepared from isolated ER which resembles the finding of Takahashi *et al.,* 2000, in Japan.

Antibiotic susceptibility test

Antibiotic susceptibility test by disc diffusion method was done using 10 different antibiotics according to the guideline provided by NLCCS. Among the antibiotics ER found to be susceptible with chloramphenicol, gentamicin, amikacin and penicillin, and resistance with tetracycline, erythromycin, colistin, kanamicin, erythromycin & vancomycin.

CONCLUSION

Erysipelothrix rhusiopathiae is also a pathogenic bacterium causing economic losses in broiler farming and is a first report in Korea. In serological survey; 5.2% of serum was found positive in plate agglutination test. ER is very better grown in BHI broth if it is supplied with serum 5% of FBS. Isolated ER was resistant to tetracycline, erythromycin, colistin, kanamicin, erythromycin, and vancomycin, and sensitive with chloramphenicol, gentamicin, amikacin and penicillin.

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Effects of Yeast Culture (*Saccharomyces cerevisiae*) Supplementation on Growth Performance, Immunomodulation and Intestinal Morphology in Broiler Chicken

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ABSTRACT

A study was conducted to investigate the effects of yeast culture (Saccharomyces cerevisiae) supplementation on growth performance, immune functions and intestinal morphology of broiler chicken at Livestock Farm, Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan during 1st October to 14th November, 2012. Nine days old Hubbard (F15) chicks (n= 320) were divided into five treatments with four replications, having sixteen birds in each, in completely randomized design (CRD). Chicks were raised in deep litter system and fed standard diet, having antibiotic as growth promoter, and yeast culture (YC) @ 0, 1, 2, 3 and 4 g/kg in treatments T1, T2, T3, T4 and T5 respectively from 10th to 44th days of age. Results of the present study showed that the inclusion of YC in the diet of chicks significantly (P <0.01) increased the body weight gain, and decreased the feed intake and FCR as the level of YC increased up to 3gm/kg of feed. Chicks fed with YC 4gm/kg of feed showed similar performance (P >0.01) as chicks fed with YC 3gm/kg of feed. Chicks fed with YC 3gm / kg of feed (T4) had the highest cumulative body weight (2508.41 gm vs 2403.57 gm), and the lowest feed intake (4337.58 gm vs 4470.92 gm) and FCR (1.87 vs 2.02) during experimental period. There was no significant (p>0.05) effect of YC supplementation on carcass characteristics of broiler chicken. Similarly, YC inclusion significantly (P < 0.01) increased chicks antibody titer to NDV, serum protein and serum globulin as the level of YC increased up to 3 gm/kg of feed. Chicks fed with YC 4 gm/kg of feed showed similar values (P >0.01) as chicks fed with YC 3gm/kg of feed. Serum albumin to globulin ratios (P >0.05) were in decreasing order as level of YC increased. Likewise, significantly (P <0.01) increased mucosal microvillus height and crypt depth of duodenum and jejunum were observed as the level of YC increased up to 3 gm/kg of feed and chicks fed with YC 4 gm/kg of feed showed the similar result as chicks fed with YC 3gm/kg of feed. Microvillus height to crypt depth ratios were in increasing pattern as the level of YC increased. The results of this study indicate that dietary supplementation of YC at 3gm/kg with standard diet improved performance, immune functions and intestinal morphology of broiler chicken.

***Key words**:* Yeast culture (*Saccharomyces cerevisiae*), growth performance, immune functions, cumulative body weight, antibody titer, serum protein, serum globulin, microvillus, crypt depth and intestinal morphology.

INTRODUCTION

Poultry farming is an important enterprise among livestock enterprises of Nepal because it contributes about 8.3% of the livestock GDP and 4% of the Agricultural GDP of the country (DLS, 2009/10). Among poultry farming, broiler poultry shares around 81% of the total poultry population of Nepal and its farming is increasing throughout the country faster than layers farming because of quick return and low investment (MoAD, 2011). Total meat production of the country is estimated about 277625 MT., out of which 36.1 thousand MT. (13.03 %) is from poultry during fiscal year 2010/11(MoAD, 2011). It is a potential tool to fight against malnutrition and unemployment problem (FAO, 2012). In addition, it provides environment friendly organic manure.

Although the poultry sector is growing faster and our country has become self sustainable in poultry products, the sector is now facing some problems like high cost of production, low performance and high mortality. High cost of production is due to low feed efficiency and high feed cost. Efficiency in feeding has been the major concern in raising poultry, as nutrition and feeding cost 65 to 75% cost of production (Paryad, 2008). Similarly, low performance is due to unhealthy intestine which result in decrease digestion and absorption of nutrients. Likewise, high mortality is due to the outbreak of diseases because of low immunity and unhealthy chicks. Researchers supplemented antimicrobials and other natural products, such as probiotics, yeast and yeast derived preparation to animal feeds as the growth promoter to solve the above problems (Muihead, 1992). In Nepalese context, most of the commercial pellet feed manufacturers use antibiotics as a growth promoter considering its lower cost compare to probiotics but still the problems do exist. Therefore, a remedy is needed which is suitable to use with antibiotic containing pellet feed and help to mitigate the above problems faced by poultry growers.

Yeast and yeast-derived preparation are inexpensive feed supplements compared to probiotics and have some major impact on animal performance and feed efficiency (Dawson, 2001). Yeasts are fungi and so it has resistant to anti-bacterial agents (Auclair, 2003). A yeast culture (YC) is a yeast-fermented feed additive that contains both live and dead yeast cells (*Saccharomyces cerevisiae)* and media on which it was grown on (Linn and Raeth-Knight, 2006). It is dried in such a manner as to

preserve the fermentation activity of yeasts. It increase the body weight gain and feed efficiency because it is a great source of naturally occurring B-vitamins and disaccharidase enzymes which enhance digestion of fiber, protein, fats and minerals (Buts *et al*., 1994). It also improves intestinal mucosal microvilli height that helps to increase the secretion of digestive enzymes and absorption of nutrients (Gao *et al*., 2008). Likewise, it has ability to competitively inhibit pathogenic bacteria and promote the growth of beneficial bacteria, thus improve the intestinal health (Gedek, 1989; Brugier and Patte, 1975).). Similarly, its cell wall contains glucans as immune-amplifier and mannon oligosaccharide (MOS) as toxin binder (Auclair, 2000; Stanley *et al*., 1993 and Castagliulo *et al.*, 1996).

Considering the beneficial effects of yeast culture and its suitability to use with antibiotics, application of yeast culture with commercial pellet feed to alleviate the above problems in Nepalese contest is justifiable. However, there are still conflicting reports on the beneficial effects of yeast inclusion in poultry diets. Hayat *et al*., (1993) suggested that the beneficial effects of *Saccharomyces* dried yeast in feed may be influenced by the bird's genome and recommended for further studies. In spite of series of studies on yeast inclusion in poultry diets, no one has come out with the specific effect of YC in different levels with antibiotics containing feed on performance, immune function and intestinal health of broiler chicks. Therefore, this study was conducted to evaluate the some effects of yeast culture supplementation with diets to Hubbard (F15) broiler chicken.

MATERIALS AND METHODS

Birds, housing and feeding

An experiment with 320 day old Hubbard (F15) chicks was conducted at Livestock Farm, Institute of Agriculture and Animal Science (IAAS), Rampur, Chitwan during 1st October to 14th November, 2012. Brooding was done in electric brooder till 9th day of age and then chicks (n= 320) were divided into five treatments with four replications, having sixteen birds in each, in completely randomized design (CRD). Chicks were allocated in a deep litter system and fed standard diet contains antibiotic as growth promote , and YC @ 0, 1, 2, 3 and 4 g/kg in treatments T1, T2, T3, T4 and T5, respectively from 10th to 44th days of age. Yeast culture (*S.* ***cerevisiae*****)**, trade name Provisacc (manufactured by Vetcare Pvt. Ltd. India.), contains 5000 million colony forming unit (CFU). Ingredients and the calculated composition of the experimental diets are shown in Table 1 and 2. Vaccination against HPS (day5), ND (day 7 and 28) and IBD (day 14 and 21) were done.

Table 1: Composition of the experimental (standard) diets fed to the birds during starting (1 – 9 days), growing (10-21days) and finishing periods (22-42 days).

Ingredients	**Starter-Bo (kg) (1-9days)**	**Grower-B1 (kg) (10-21days)**	**Finisher -B2 (kg) (22- 44days)**
Maize	615	650	650
Rice polish	18	38	58.5
Soya meal	290	235	215
MBM	68.5	69	69
DCP	1.5	2.6	2.60
Salt	2.5	2.5	2.50
Lysin	2.0	1	0.70
Methionine	2.5	1.9	1.7
Total	1000.00	1000.00	1000.00

Table 2: Calculated nutrient composition of experimental (standard) diet

Nutrients	**Starter (Bo)**	**Grower (B1)**	**Finisher (B2)**
ME (Kcal/kg)	2950	3000	3100
CP %	22	20	19
CF %	4	4.25	4.50
Crude fat %	1.2	1.1	1
Ca %	1.1	1	1
Av. P %	0.5	0.5	0.5

Feed additives used in feed: Mineral mixture, vitamin mixture, anticoccidial drugs (Madhuramycine), multi-enzyme, liver tonic, toxin binder (toxfin) and antibiotics (Bacitracine@20 gm / ton in Bo, Nitrofuazone @20 gm / ton in B1, verginiamycine @100 gm / ton in B2). Doses of feed non-antibiotics feed additives were adjusted according to manufacturer's indication.

Observation reordered

Daily and weekly feed consumed and weekly and cumulative live weights were recorded. Feed conversation ratio (FCR) was calculated at weekly interval by using following formulae:

Feed conversion ratio (FCR) = Feed intake (g) / Weight gain (g)

Dressing percentage and carcass characteristics

On 41 days of age, one bird was taken randomly from each plot i.e. four bird from each treatment, slaughtered by cervical dislocation, weighed and data were recorded like: live weight, dressed wt., chest meat wt., leg piece wt., liver wt. etc. Dressing percentage was calculated as:

$$\text{Dressing percentage} = \frac{\text{Dressed wt of birds (Kg)} \times 100}{\text{Live weight of birds (Kg)}}$$

Blood and tissue samples collection

Blood samples were taken on 27th and 41th days of age from wing vein to determine the serum antibody titer to NDV, total protein, albumin, globulin and albumin: globulin ratio. On the 41th day one bird form each experimental unit was slaughtered by cervical dislocation. A piece of sample (1.5cm) from duodenum (medial portion) and jejunum (initial portion) were taken and preserved in 10% buffered formalin for histopathology.

Laboratory analysis

The antibodies titre against NDV was determined by Haemaglutination Inhibition (HI) test, using NDV- LaSota (Commercial available live, freeze dried, lentogenic F- mild strain ND vaccine: Indovax $^{®,}$ batch no F1212, 500 doses) antigen by beta procedure utilizing 8HA units of virus and 1% suspension of fresh chicken RBC making serial two fold dilution of serum in antigen, as per the method described by Allan and Gough (1974). Similarly, serum protein and albumin were determined by Biuret and BCG methods described reagent kit provided by manufacturer: (Crest Biosystem, India).

Histological slides were prepared from samples of mid-duodenum and initial part of jejunum. Microscopic observation was done by light microscope (Olympus) at 10X lens with help of stage micrometer (one line reading =0.01mm). This reading was converted into micro meter (μm) by multiplying with 1000.

Statistical methods and data analysis

The data obtained from feeding trial were recorded in MS –Excel 2007. The data were analyzed for significant test using MSTAT program, version 1.3, Michigan State University, USA. ANOVA was used to test the treatment differences. Comparison between means was done by LSD at 5% and 1% level of significance whenever necessary.

RESULTS AND DISCUSSION

Growth performance

The average cumulative body weight, feed intake, feed conversion ratio and carcass characteristics of broiler chicks fed different levels of yeast culture (YC) are presented in Table 3. presents Results showed that the inclusion of YC (*S. cerevisiae)* significantly ($p<0.01$) increased the body weight from second week onwards and significantly ($p <0.01$) decreased the feed intake and FCR. on day 44 (i.e. 5th week) chicks fed with YC 3 gm / kg of feed had the highest body weight (2508.41 gm vs 2403.57gm) followed by chicks fed with YC 4, 2, 1 and 0 gm / kg of feed but chicks fed with YC 3 and 4 gm / kg of feed had the similar body weight ($p >0.01$). Cumulative feed consumption decreased as the level of YC increased from 0 to 4 gm / kg of feed. On day 44, the highest cumulative feed consumption was observed in chicks fed standard diet only, followed by chicks fed with YC 1, 2, 3 and 4 gm / kg of feed but chicks fed with YC 2, 3 and 4 gm / kg of feed had similar feed consumption ($p>0.01$).

Table 3: Effect of yeast culture supplementation with feed on growth performance of broiler chicken

Yeast culture supplementation (gm / kg of diet)							
Item	**0**	**1**	**2**	**3**	**4**	**CV %**	**Sig.**
Cumulative body wt. (gm / bird)							
Day 0	36 .00	36. 00	36.00	36.00	36.00		ns
Day 9	190.70	189.53	189.37	190.70	189.37	1.21	ns
Day 16	436.25	436.01	439.06	441.25	447.26	1.74	ns
Day 23	835.00^{c}	840.70^{c}	847.26$^{b\ c}$	860.07^{ab}	862.42^{a}	1.16	**

Day 30	1318.58 [c]	1341.33 [b]	1356.41 [ab]	1370.66 [a]	1368.75 [a]	0.80	**
Day 37	1860.75 [c]	1887.16 [b]	1902.28 [b]	1937.33 [a]	1937.33 [a]	0.84	**
Day 44	2403.57[d]	2439.73[c]	2464.64[b]	2508.41[a]	2508.21[a]	0.46	**
Feed consumption (gm / bird)							
[1]Day 10 - 44	4470.92[a]	4383.83[b]	4353.69[c]	4337.58 [c]	4331.12 [c]	0.42	**
[2]Day 1- 44	4670.92 [a]	4583.83 [b]	4553.69 [c]	4537.58 [c]	4531.12 [c]		
FCR							
Day 10 – 44	2.020 [a]	1.948[b]	1.913[c]	1.871[d]	1.867 [d]	0.69	**
Day 1- 44	1.97 [a]	1.90 [b]	1.875 [c]	1.835 [d]	1.833 [d]	0.70	**
Carcass characteristics at 41 days of age							
Pre-slaughter wt.	1940.00[c]	2170.00[ab]	2195.00[ab]	2257.00 [a]	2077.00[bc]	4.45	**
Dressing %	76.73	77.88	78.66	78.74	78.83	2.28	ns
Chest piece (%)	28.29	27.52	27.74	27 .00	29.15	6.84	ns
Leg piece (%)	28.06	29.06	28.21	28.02	28.38	7.41	ns
Wing piece (%)	11.72	11.40	11.27	11.51	12.00	6.79	ns
Liver (%)	29.15	2.83	3.03	2.90	3.01	3.39	ns

Means within the same row with different superscripts are significantly different ($P<0.01$). ns= Non significant.

[1] Experiment period, [2] Whole life period (feed consumed till 9th days of age, 200 gm per bird, was added in experiment period)

The overall FCR decreased as the level of YC increased from 0 to 4 gm /kg of feed. Chicks fed standard diet only had the highest overall FCR followed by chicks fed with YC 1, 2, 3 and 4 gm / kg of feed but chicks fed with YC 3 and 4 gm / kg of feed had similar FCR ($P>0.01$) during experimental period as well as whole life period.

Dressing percentage increased as the level of YC supplementation increased though there was non significant ($P>0.05$) difference among treatments. The highest dressing percentage (78.83 %) was obtained in chicks fed with YC 4 gm / per kg of feed followed by chicks fed with YC 3, 2, 1 and 0 gm / per kg of feed. Similar result was obtained in carcass characteristics (i.e. chest piece %, Leg piece %, Wing piece %, Liver %).

The results indicate that YC is an important natural growth promoters and its supplementation with feed, contains antibiotics (Nitrofurazone in grower and Verginiamycin in finisher) as growth promoter, improved the growth performance of broiler chicken. The obtained results confirmed the previous findings of several researchers (Gao *et al.,* 2008; Spring *et al.,* 2000; Zang *et al.,* 2005; Van Heugten *et al*., 2003; Mohamed *et al*., -2008 etc.) who found that yeast supplementation improved the growth performance.

Yeast culture contains yeast cells as well as metabolites such as peptides, organic acids, oligosaccharides, aminoacids, flavor and aroma substances, and possibly some unidentified growth factors, which have been proposed to produce beneficial performance responses in animal production (Gao *et al.*, 2008). Similarly, Van Heugten *et al.,* (2003) observed that live yeast supplementation had a positive effect on nursery pig performance when diets contained growth-promoting Cu, Zn, and antibiotics; whereas no positive effect was found in yeast-treated pigs fed antibacterial-free diets. Likewise, Mohamed *et al*., (2008) stated that addition of MOS (component of cell wall of yeast), enramycin or the combination of both did slightly improve ($P>0.05$) body weight gain by about 2% compared to the control diet. He also stated that in some studies in which certain antibiotics were used in combination with MOS, synergistic beneficial effects on broiler live performance were observed compared to antibiotic alone. Virginiamycin + MOS, gave significant improvement in feed conversion (Mathis, 2000); bacitracin-MD and virginiamycin shuttle program + MOS, improved feed conversion (Sefton *et al*., 2002). Blake *et al.*

(2006) indicated that the combination of MOS and Bacitracin Methylene Disalycilate resulted in highly significant (P<0.001) improvements in body weight over the control diet at 14 days. Some researchers (Naga, 2012; Gao *et al.*, 2008; Zhang *et al*., 2005 and Paryad *et al.*, 2008) found that yeast culture alone (i.e. without antibiotics as growth promoter) also improved the growth performance. Gao *et al*., (2008) reported that dietary supplemental YC (Saccharomyces *cervisiae*) at 2.5 gm / kg improved the growth performance. Similarly, Naga (2012) reported that dry yeast supplementation in broiler diets at a level of 0.5% resulted in a significantly decrease in feed intake (P<0.05) and improved the body weight gain.

Immunomodulary functions

The antibody titer to NDV, serum protein, serum albumin, serum globulin and albumin to globulin ratio on 27th and 41st days of age of broiler chicks fed different levels of yeast culture (YC) have been presented in Table 4. Results showed that the inclusion of YC (*S. cerevisiae)* significantly increased the antibody titer to NDV (P<0.01), serum protein (P<0.01) and globulin level (P<0.05) on both day. . The antibody titer increased as the level of YC increased but chicks fed with YC 2, 3 and 4 gm / kg of feed had similar antibody titer to NDV(P>0.01) on both day. The serum protein level increased as the level YC increased from 0 to 4 gm / kg of feed but chicks fed with YC 3 and 4 gm /kg of feed had similar protein level (P>0.01) on both day. On both day serum albumin levels differed non significantly (P>0.05) among treatments though albumin levels were higher in YC treated groups. On both day level of globulin increased as the level of YC increased but chicks fed with YC 3 and 4 gm / kg of feed had similar globulin level (P>0.01). On both day albumin to globulin ratios were in decreasing pattern as YC level increased in treatments. It means more incensement in serum globulin concentration compared to albumin concentration as YC level increased. Increasing globulin is an indicator of increasing antibody level in blood. Thus this result supports the antidody titer result of this experiment. Antibody titer responses have been used as measures of humoral immune status of birds (Sklan *et al*., 1994). It was proposed that oligosaccharides in the yeast cell wall could bind to viruses and work as adjuvants of vaccines to increase the titers of antibody in YC-treated birds (Newman, 1994). Mannanoligosaccharide and 1,6 ß –glucan are components of the yeast cell wall that modulate immunity (Shashidhara and Devegowda, 2003). These all results indicate that YC had improved the immunity power of birds. The obtained results confirmed the previous findings of several researches (Naga, 2012; Gao *et al.,* 2008 and Paryad *et al.,* 2008) who found that yeast supplementation had the higher

(P<0.05) total plasma protein, albumin and globulin concentration. Blood plasma showed an improvement (P<0.05) in total protein, albumin, globulin, GOT and GPT when birds fed dietary yeast (Ghally *et al*., 2007).

Table 4: Effect of yeast culture supplementation with feed on immune functions of broiler chicken

Yeast culture supplementation (gm / kg of diet)							
Item	**0**	**1**	**2**	**3**	**4**	**CV %**	**Sig.**
Antibody titer to NDV							
Day 27	4.5[b]	5.75[b]	6.25[a]	6.25[a]	6.75[a]	12.38	**
Day 41	5.5[c]	6.75[b]	7.75[a]	7.75[a]	7.75[a]	9.04	**
Serum protein (g/dl)							
Day 27	2.11[b]	2.44[b]	2.84[b]	4.35[a]	4.34[a]	25.76	**
Day 41	2.50[b]	2.80[b]	4.69[a]	4.81[a]	4.97[a]	16.23	**
Serum albumin (g/dl)							
Day 27	0.68	0.72	0.68	1.08	0.99	38.44	ns
Day 41	0.75	0.81	1.03	1.09	1.14	21.11	ns
Serum globulin (g/dl)							
Day 27	1.40[b]	1.71[ab]	2.14[b]	3.26[a]	3.46[a]	36.37	*
Day 41	1.75[b]	1.99[b]	3.66[a]	3.72[a]	3.83[a]	22.54	**
Albumin : Globulin							
Day 27	0.507	0.433	0.416	0.340	0.349	58.45	ns
Day 41	0.475	0.429	0.306	0.303	0.303	36.99	ns

Means in row with different superscripts (a, b, c) differ significantly. [ns] not significantly different, * significant at 5% (P<0.05), **significant at 1% (P<0.01)

Intestinal morphology

Results of intestinal morphology summarized in Table 5 showed that chicks fed with YC 3 gm/kg of feed (T3) had the highest intestinal length and weight but these differences were non significant (P >0.05) among treatments. Intestinal mucosal micro villus height and crypt depth at duodenum (middle portion) and jejunum (initial portion) increased as the level of YC increased and highly significant differences

(P<0.01) observed among treatments. Similarly, villus height to crypt depth ratios at duodenum and jejunum were in increasing order as level of YC increased. The obtained results confirmed the previous findings of several researchers (Gao *et al.,* 2008; Spring *et al.,* 2000; Zang *et al.,* 2005; and Parks *et al*., 2001) who found that yeast supplementation improved growth of intestinal mucosal morphology. Dietary supplemental YC (*Saccharomyces cervisiae*) at 2.5 g/ kg improved mucosal micro-villi height (Gao *et al.,* 2008). YC promotes growth of intestinal microflora (Spring *et al.*, 2000) and increases growth of micro-villi (Parks *et al*., 2001). Zhang *et al*., (2005) reported greater villus height and improved performance in birds with supplementation of whole yeast or yeast cell wall. Cell wall components of yeast (ß-glucans and ɑ-mannans) may provide a protective function to mucosa by preventing pathogens from binding to villi and allowing fewer antigens to be in contact with the villi. Zhang *et al*., (2005) reported that the positive role of yeast cell wall in ileal mucosal development of broiler.

Table 5: Effect of YC supplementation with feed on intestinal morphology of broiler chicken

	Yeast culture supplementation (gm/kg of diet)						
Items	**0**	**1**	**2**	**3**	**4**	**CV%**	**LSD**
Intestinal length (in.)	70.62	74.62	73.25	75.00	73.00	5.5	—
Intestinal wt. (gm)	164.50	179.90	178.97	181.67	175.05	6.5	—
D. villus height (µm)	1012.50^{c}	1275.00^{b}	1375.0^{b}	1542.5^{a}	1520.0^{a}	5.7	117.4
D. crypt depth (µm)	780.00^{c}	905.00^{bc}	962.5^{ab}	1062.5^{a}	1050^{a}	10.7	153.6
D. villus ht: crypt dt.	1.34	1.40	1.43	1.45	1.45	9.9	—
J. villus ht. (µm)	1312.50^{d}	1425.00^{c}	1575.0^{b}	1662.5^{a}	1700^{a}	4.7	109.2
J.crypt dt. (µm)	1050.00^{c}	1075.0^{bc}	1175^{ab}	1212.5^{a}	1237.5^{a}	6.3	109.2
J. villus ht: cryp dt.	1.25	1.32	1.34	1.37	1.37	4.8	—

YC = yeast culture, in. = inch, D. = duodenum, J. = jejunum, ht.= height, dt. =depth. Figures in parenthesis are standard deviation. Means in row with different superscripts (a, b, c) differ significantly. ^{ns} not significantly different, * significant at 5% (p<0.05); **significant at 1% (p<0.01)

CONCLUSION

The results of the current study indicate that YC supplementation improves growth performance, immune functions and intestinal morphology of broiler chicken. These effects varies with the levels of YC supplementation and are the best when YC 3 g / kg of feed supplemented in broiler diet under the experimental conditions of this study though further studies with different seasons and breeds are necessary.

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Nepalese Vet. J. 32:134-140

Prevalence of Gastrointestinal Helminths in Equines of Chitwan District

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ABSTRACT

A cross-sectional study was carried out during October to December 2012 to determine the prevalence of gastrointestinal helminthes. Egg per gram for Strongyle species and Strongyloides species in equines of Chitwan district was determined using a total of 81 fecal samples selected purposively from different equine platform (Sauraha, Bharatpur Stud Farm) and their age and sex were recorded. The fecal samples were examined qualitatively by sedimentation and differential floatation technique and quantitatively by using Mac Master Technique. A questionnaire survey was conducted among 30 randomly selected equine owners regarding the management practices, equine welfare and use of antihelmintics. The study revealed 71 fecal samples (95.1%) were found positive for one or more endoparasites. The prevalence was higher in horses of older age. The Strongyloides (27.80%) was most common helminth followed by Parascaris (21.20%), Oxyuris (9.90%), Anoplocephala (9.40%), Strongylus (8.0%), Fascioloides (6.60%), Gastrodiscus (5.70%), Paranoplocephala (2.80%), Fasciola (2.40%), Triodontophorus (1.4%), Dicrocoelium (0.90%), Dictyocaulus (0.90%), unidentified (2.8%). The prevalence of Strongyle and Paranoplocephala were higher in age group between 0-5 years, while Oxyuris, Strongyloides, Dicrocoelium, Parascaris, Gastrodiscus and Anoplocephala showed higher prevalence in older age groups. The EPG count of Strongyle species ranged between 200-800. The EPG count of Strongyloides species ranged between 200-1800. Most of the farmers were unaware of helminth parasite problem and similarly equine welfare.

Key words: Prevalence, Helminth, *Strongyle, Strongyloides,* EPG, Equine

INTRODUCTION

Equines are the means of transport on hilly mountainous remote areas and provide livelihood to a number of rural and semi-urban population of Nepal. It is suggested that donkeys can play a great role in the frame works of food security and social equity of high food insecure countries (Karki and Manandhar, 2006). Equines have

often been found to succumb to a variety of diseases and a number of other ailments. Parasitic infestation is a major cause of illness. Documentation of parasitic infestation of equines in our country is lacking. Equines are still the major means of transport of goods in high mountains of the country

There are approximately 89 horses in Chitwan district in the dated up to December 2012. Sauraha and Bharatpur Stud farm are the two equine belts in Chitwan. Sauraha is the attraction site for tourist and horses are used for transportation and entertainment and in Bharatpur Stud Farm where horses are kept for breeding and patrolling the army.

MATERIALS AND METHODS

Site of study

The study has been carried out in different equines platforms of Chitwan district (Sauraha, Bharatpur Stud Farm) from October to December, 2012.

Field Survey

Field study was performed in each study area. A semi-structured questionnaire model

was prepared and equine owners were interacted regarding number of horses they were rearing, major health problems, feed composition, workload, their level of information about equine welfare, etc. From the collected data, knowledge dynamics of the horse owners on equine husbandry, health management, therapeutic concerns, etc were analyzed.

Sampling of animals

Fresh fecal samples were purposively taken from 81 equines of different age groups. The equines were divided into four age groups (0-5years, 5-10years, 10-15 years and 15-20 years) and 19, 22, 19, 21 samples were collected from each group respectively. The samples were kept in clean plastic bottles preserved in 10% formalin. The samples were transported to and analyzed in Parasitology laboratory of IAAS.

The sample size was determined by the given formula,

$$\textbf{Sample size (S)} = \frac{Z^2 \times P \times (1-P)}{d^2}$$

(Source: Otte, J. 1998. A field guide for the diagnosis, treatment and prevention of African animal trypanosomosis. FAO corporate document repository. Africa. pp 170)

Where,

P= Anticipated prevalence

d= Desired precision

Z= Appropriate value from the normal distribution for the desired confidence. Level of

confidence at 95%, Z= 1.960

Qualitative fecal examination

The qualitative parasitic investigation was carried out through different methods as per Urquhart (1998) and Soulsby (1982).

Quantitative Fecal Examination

EPG count of *Strongyle* spp. and *Strongyloides* spp. was done using standard Mc Master Slide Technique (Soulsby, 1982).

RESULTS

A total of 81 fecal samples were examined qualitatively and quantitatively. The test results showed that out of 81 samples, 77 were found positive for one or more endoparasites. The most common parasites were *Strongyloides* spp. (27.80%), *Parascaris* spp. (21.20%), *Oxyuris* spp. (9.90%), *Anoplocephala* spp. (9.40%), *Strongylus* spp. (8.0%), *Fascioloides* spp. (6.60%), *Gastrodiscus* spp. (5.70%), Paranoplocephala spp. (2.80%), *Fasciola spp.* (2.40%), *Triodontophorus* spp. (1.4%), *Dicrocoelium spp*. (0.90%), *Dictyocaulus* spp. (0.90%), unidentified (2.8%) (Fig. 1).

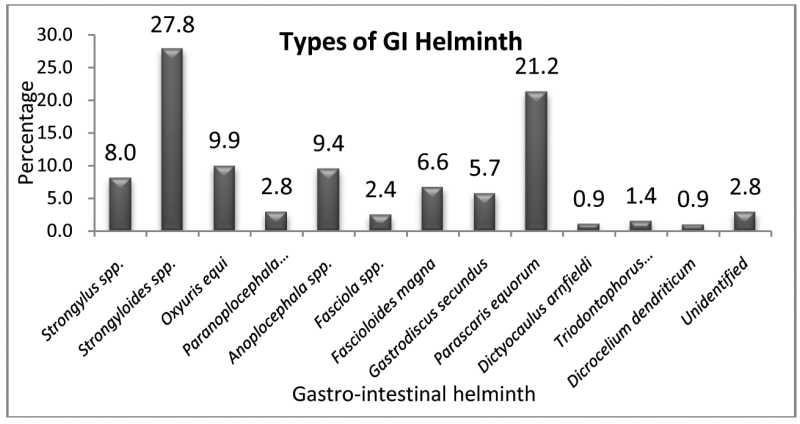

Fig. 1: A bar Diagram showing types of GI helminths in horses

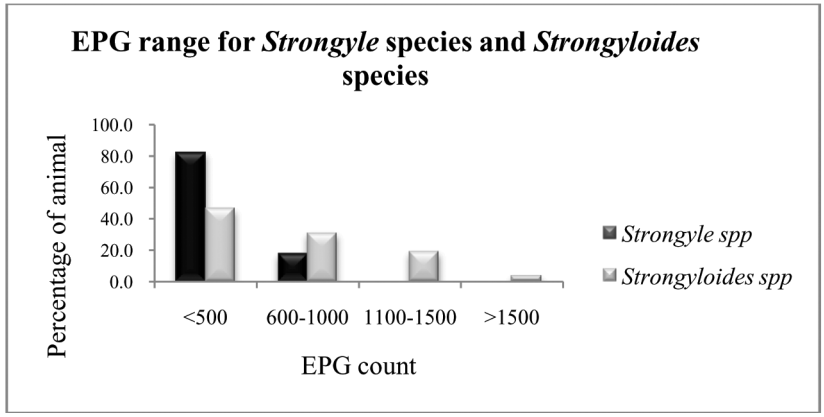

Fig. 2: A bar diagram showing EPG range of *Strongyle* species and *Strongyloides* species.

The prevalence of *Strongyle* spp. and *Paranoplocephala* spp. were higher in age group between 0-5 years, while *Oxyuris, Strongyloides, Dicrocoelium, Parascaris, Gastrodiscus and Anoplocephala* showed higher prevalence in older age groups. The EPG count of *Strongyle* species ranged between 200-800 (Fig. 2 & Fig. 3).

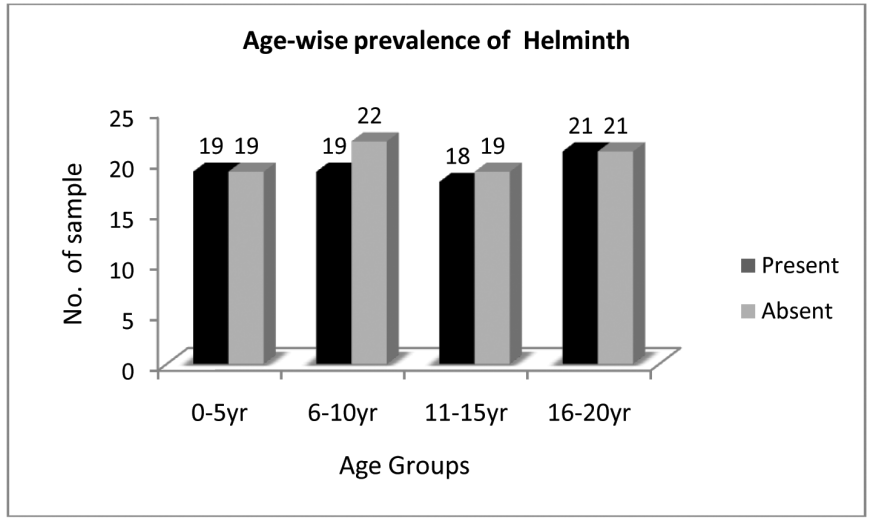

Fig. 3: A bar diagram showing age-wise prevalence of helminthes in horses

Based on the severity index (Soulsby, 1982), 17.65% of fecal samples were moderately affected and 82.35% mildly infected. Similarly, EPG count of *Strongyloides* species ranged between 200-1800 (Fig. 2) and 3.44% fecal samples were severely affected, 18.96% heavily, 31.03% moderately and 46.55% mildly infected as defined by the severity index (Soulsby, 1982).

DISCUSSION

The present prevalence rate of 95.10% is almost similar to the result 93.26% obtained by Pandit *et al*. (2008) in Jammu and Kasmir. However, the observed result vary widely than that described by Sapkota (2009) who reported 45% prevalence of GI parasites in mules of Brick Kiln of Lalitpur district. This suggests that the deworming is not common practice in equine husbandry in Lalitpur district.

In other hand, the EPG count obtained, 500-800 for *Strongyle* spp. was similar to the EPG count obtained by Paudel (2007) in Shree Sainik Stud Farm, Chitwan but higher than the EPG count obtained by Katoch *et al.,* (2006) in Spiti horses of Himchal Pradesh. The EPG count for *Strongyloides* species ranged from 200-1800. The high EPG in study area suggested that either there is no regular deworming of animals or anthelmintics are used in improper dosing.

The prevalence of *Strongyle* spp. was 8% which is less than 48.78% (Paudel, 2007), 28.3% (Eslami *et al.*, 2000). The prevalence of *Strongyloides* spp. was 27.8% which is higher than 1.67% (Katoch *et al.*, 2006), 10.29% (Shrestha, 2010). This lower percentage of *Strongyle* spp. might be due to the ivermectin medication via oral route prior to the sample collection in the study site and high percent of *Strongyloides* spp. might be due to drug resistancy.

The prevalence of *Parascaris equorum* was 21.2% which was 50% in a study carried out by (Karki and Manandhar, 2006) which might due to the smaller sample size used in their study. This might be due to the high resistance of the eggs of *Parascaris equorum* to desiccation. Soulsby (1982) has stated that *Parascaris equorum* eggs are very resistant to adverse conditions, like drying or freezing and the larvae rarely hatch and infection usually takes place through ingestion of the eggs.

The observed *Oxyuris equi* with prevalence rate of 9.9% was in harmony with the work of Pandit *et al.,* (2008) who had reported 9.4% prevalence of *Oxyuris* in equines in Kashmir valley of Jammu and Kashmir. These findings however were higher than 3% prevalence reported by Karki and Manandhar, (2006) in Udaypur district. The low prevalence in this study might be the effect of relative higher temperature in the present study area which desiccates the highly susceptible *Oxyuris equi* eggs (Shrestha, 2010).

The prevalence of *Anoplocephala* species was 9.4% which was lower than 28.5% in a study carried by Rehbein *et al.,* (2012) in gastrointestinal parasites in abattoir horses in Germany. This low prevalence might be due to seasonality of Oribatid mites vectors (Soulsby, 1982).

Gastrodiscus (5.7%) was almost six times lower in prevalence as compared to Sapkota (2009). This lower prevalence might be due to difference in ecological conditions for the development of intermediate host snails and the parasite. The highest prevalence was seen in horses of older age similar to the findings by Sapkota (2009) which might be related to decreased immunity in seniles. Similarly, among all the studies conducted up-to now, the prevalence of GI helminths was higher in older horses than younger ones and EPG count was also found greater in older horses. This might be related to decrease immunity in older animals too.

The field survey revealed that the most common health problem was colic and one of the reason causing colic is the overload of endoparasites (Soulsby, 1982). The deworming interval of mules was also not uniform which might be one of the reasons behind the high prevalence rate. Although, deworming was practiced in some areas, the high prevalence might be due to re-infection and repetition of infective cycles.

CONCLUSION

There have been only a few studies regarding parasitic prevalence of horses in Nepal. Preliminary data too is also not available from DLSO. Very few efforts are being made by the Government to prevent the endoparasitic infestation in equines and improving equine welfare. Horses are being used for mankind with very less consideration about their proper management, health care and feeding. This might have lead to drastic decline in the population of equines and we can't deny the situation where the source of tourist attraction in Nepal will decrease. Few non-governmental organizations are working in the sector of equine health but it hasn't been in reach to work on district like Chitwan.

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Nepalese Vet. J. 32:141-147

Sero-detection of Leptospiral Infection in Canine Population of Kathmandu Valley

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ABSTRACT

The status of leptospiral infection in canine population of the Kathmandu valley was determined using a rapid diagnostic test kit by detecting IgM antibodies in 150 sera samples of the pet dogs brought to the Veterinary Hospitals and Veterinary Clinics with the history of fever and jaundice and samples of community dogs. Screening of the samples by the rapid diagnostic test kit (SD Bio Line, Korea) showed 2.7% (4/150) sera samples positive for leptospiral antibodies. The present result suggested that leptospiral pathogens continue to circulate in canine population of Kathmandu valley.

Key words: Leptospiral antibodies, IgM, canine population

INTRODUCTION

Leptospirosis is a zoonotic disease with epidemic potential, especially after a heavy rainfall, caused by bacteria of genus Leptospira. *Leptospira interrogans* is pathogenic to humans and animals with more than 200 serologic variants or serovars (WHO, 2003) known to exist in the world. The infection is however more common in the tropical and sub-tropical countries, since favorable conditions for its transmission exist and most of the countries in this region are developing ones (Bharti *et al*., 2003; WHO, 2003). In the developed world, leptospirosis is considered as an emerging infectious disease (Levett, 2001). Recreational activities like water-sports are also believed to be an important factor for the emergence of this infection. The earliest recognized accounts of leptospirosis were descriptions of severe illness with jaundice and renal involvement in man and these were clinically distinct from other icteric and nephritic illnesses, but the cause was not known then.

Canine leptospirosis was first described in 1899. Before 1960, *L.interrogans* serovars *icterohaemorrhagiae* and *canicola* were believed responsible for most clinical cases

of canine leptospirosis. The disease then, mainly described as acute or sub acute hepatic and renal failure, was often thought characterized by acute hemorrhagic diathesis (Sykes, *et al*., 2011). Canine leptospirosis has received increased attention as a cause of febrile illness and hepatic renal disease (Bolin, 1996). The canine disease presents as an acute infection of the kidney and liver and sometimes as a septicemia. Chronic kidney disease is a common sequel of infection and abortions may occur in pregnant dams (McDonough, 2001).

Leptospirosis occurs worldwide wherever there is a risk of direct or indirect contact with the urine of infected animals. Theoretically, any mammal is capable of being infected by any serovar of *Leptospira interrogans*. However, only a few serovars are enzootic in any particular region. Optimal conditions for survival are a warm and wet environment, with neutral or slightly alkaline water; however, leptospires are known to survive acidity of pH 5 to 6.2 for limited periods. They can survive in cold water, provided that it does not freeze (Thiermann, 1984). Because of the importance of water as a means of spreading infection, new cases are most likely to occur in wet seasons and low lying areas, especially when contamination and susceptibility are high (Nervig *et al.*, 1978).

Leptospirosis is believed to be endemic as ideal conditions exist for transmission of this infection in Nepal (Brown *et al*., 1981), however, as with other developing countries; the infection is largely under reported. Though Leptospira in dogs has been reported in south-east Asia including India, Bangladesh and Pakistan, there is lack of baseline information in Nepal. Therefore, this study was aimed to detect the seroprevalence of Leptospira in dogs of Kathmandu.

Due to lack of sufficient laboratory infrastructure this study was aimed at IgM detection in serum of febrile dogs. Therefore, the principal limitation of this study lies in the fact that detection of serovars could not be done.

MATERIALS AND METHODS

Sample Size and Site Selection

Altogether 150 blood samples were collected from dogs brought with febrile illness and jaundice at Central Veterinary Hospital (CVH), Advanced Pet Hospital, Mobile Veterinary Hospital, Dog Sanctuary of Animal Nepal and Kathmandu Veterinary Clinic. Pet dogs brought with the history of febrile illness were regularly vaccinated against leptospirosis while community dogs did not receive any such vaccination.

Blood collection and serum preparation

Blood sample was collected from the cephalic vein with a 21 G needle and a 3 ml syringe. The serum sample was separated and stored in a cryo-vial at -20°C until tested.

IgM detection

The samples were tested for *Leptospira* IgM by rapid test kit method (SD Bioline, Korea).

RESULTS AND DISCUSSION

Out of 150 sera sample screened by rapid diagnostic test (RDT) kit, 4 samples were found positive for IgM of *Leptospira interrogans* indicating seroprevalence of 2.7% (4/150). Previous investigators reported canine *leptospira* in stray dogs of Kathmandu was 6.67% (Sharma, 2012). Chuan-Jiang Lai *et al.,* (2005) found 45.6% seropositivity of *Leptospira* in dogs in Northern Taiwan. However, Meeyam *et al.,* (2004) found 11% in Thailand and Gautam *et al.,* (2010) found 8.1% seropositivity of *Leptospira* antibodies in dogs in USA. In a survey conducted in Japan over a period of three years, 1.2% seroprevalence was observed in dogs (Ryu, 1975).

The prevalence of *Leptospira* antibodies in dogs has varied among different countries: 21.3% in India (Venkataraman and Nedunchelliyan, 1992) and 6.36% in Italy (Cerri *et al*., 2003). Climate may be an important factor affecting the prevalence of *Leptospira* in each area. The suitable climate for *Leptospira* is the tropical climate, and the prevalence of *Leptospira* has been found to be the highest in the rainy season (Ward *et al*., 2002). Prescott *et al*., (1991) found 8.2% seropositivity in dogs in Southern Ontario. Seropositivity was 57.47% among domesticated animals and 72.72% in wild animals in captivity and 33.03% in rodents in Tamil Nadu (Koteeswaran, 2006).

Among the *Leptospira* positive cases, more number of cases belonged to the age group 12-48 months (i.e. more than 1 year of age). This might be due to the fact that the samples collected were more in number in this age group. This is similar to the study done by Ghneim *et al.,* (2007). However, Ward *et al.,* (2004) found dogs between 4 and 9 years of age were more likely to acquire infection than dogs more than 1 year of age. No significant difference was observed in terms of age in the seroprevalence of leptospirosis by Dayou *et al*., (2012). In contrast, Aslantaş *et al.,* (2005) reported that adult dogs were more commonly infected than juvenile dogs.

Table 1: Gender wise prevalence of *Leptospira* among dogs

Sex of dog	Result of Rapid Test Kit		Total
	Positive	Negative	
Female	2	57	59 (3.38%)
Male	2	89	91 (1.19%)
Total	4	146	150 (2.70%)

Leptospira positivity was slightly more in females (Table 1) than in males in the present study which is similar to Harkin *et al* (2003). However Rentko *et al.,* (1992) and Zwijnenberg *et al.,* (2008) found more infected males than females. In a study conducted by Dayou *et al.,* (2012), no significant difference was observed with respect to sexes. In a retrospective study conducted by Kikuti *et al.,* (2012) from 2003 to 2010 at the Laboratory of Zoonosis Diagnosis at the Veterinary Hospital of São Paulo State University (UNESP) in Botucatu, São Paulo state, Brazil male dogs were connected with higher rates of leptospirosis antibodies.

Table 2: Vaccination status with *Leptospira* IgM positivity in dogs

Vaccination Status	Result of Rapid Test Kit		Total
	Positive	Negative	
Vaccinated	4	115	119
Not vaccinated	0	31	31
Total	4	146	150

Detection of IgM antibodies in four of the vaccinated dogs (Table 2) would not necessarily indicate vaccine response as the sera samples were collected from icteric and febrile dogs presumably having on-going infection. Since vaccination against leptospirosis was done six months before sampling, the development of IgM antibodies in the present study is attributed for on-going leptospiral infection.

CONCLUSIONS

Screening of canine sera for leptospirosis with rapid diagnostic test (RDT) kit for detecting IgM antibodies revealed 4 positive samples out of 150 samples (2.7%) in Kathmandu valley, clearly indicating for more detailed investigation in large population with need of isolation of the organisms from clinical cases.

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Prevalence of Haemoparasites in Community Dogs of Lalitpur District

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ABSTRACT

Haemoprotozoa of dogs with major clinical significance include Babesia spp., Trypanosoma spp., and Rickettsials such as Anaplasma spp. and Ehrlichia spp. An investigation on prevalence of haemoparasites in community dogs in Lalitpur was carried out from February to May 2014. Blood samples were collected purposefully from the febrile dogs brought at the Animal Shelter Home, Animal Nepal which works in the community dog population of the Lalitpur District. Out of 110 blood samples, 10% were positive for haemoparasites by using Giemsa Stained Thin Smears. Babesia spp. had the highest prevalence in 10 (9.3%) samples as compared to Ehrlichia spp. in 2 (1.8%). There were significant decreases in packed cell volume (PCV) in infected dogs as compared to the healthy dogs.

Key words: Feral dogs, Haemoparasites, Prevalence, Giemsa stain thin smears

INTRODUCTION

Rearing of pets and some wild animals has occurred since ancient time for the benefits of people in many ways such as companionship, draught, hunting, guard and commercial purpose. Dogs are the most common pet animals worldwide and perform different cultural, social, and economic functions in society. In Nepalese context, dogs are reared for pet, guard and breeding purpose. Dogs are the most successful canids, adapted to human habitation worldwide and have contributed to physical, social and emotional well-being of their owners, particularly children. Parasitic problem is one of the most commonly encountered health problems in dogs. Haemoparasitic problem is observed in dogs of all ages, but the prevalence is usually higher in puppies due to the fact that certain modes of transmission are exclusive

to newly whelp or neonates as young dogs do not have acquired immunity to fight parasites. Stray dogs mostly reside nearby public places where there is huge flow of human population such as temples, parks, gardens in the search of food and shelter. Stray dogs harbor wide range of blood parasites even without clinical manifestations. Blood parasites include *Babesia spp., Ehrlichia spp., Anaplasma Spp., Trypanosoma Spp.* and *Dirofilaria immitis*. These blood parasites are mostly diagnosed and identified by blood smear examination.

MATERIALS AND METHODS

A cross-sectional study was conducted from February 2014 to May 2014 in Kathmandu valley. A total of 110 blood samples were collected from Animal Nepal, Chobar, which works for the control of stray dog population. 3 ml of blood were collected asceptically from cephalic vein and kept into EDTA vials labeled with age and sex of dogs. The collected taken to central Veterinary Laboratory (CVL), Tripureshwor in cool box and processed for examination. Examination of parasite in blood samples was performed by examination of Blood Smear (WHO, 1991) and Haematocrit Centrifugation Technique.

Blood Smear Examination

A drop of blood was taken near one end of the clean glass slide and another slide used to prepare the blood smear. The smear was allowed to air dry and were fixed in methyl alcohol (absolute) for 5 minutes and allowed to dry. The dry smears were placed in a coplin jar containing working Giemsa stain for 30 to 40 minutes. After that the smears were taken out and washed with distilled water to remove excess stain. The slides were allowed to dry in air and were examined under oil immersion (100 x) microscope.

Haematocrit centrifugation technique

Microhaematocrit tubes were filled two-third with blood and one end of each tube was sealed. The tubes were centrifuged at 8000 rpm for 5 minutes. The tubes were then broken 1 mm below the buffy coat layer. The contents of the tube containing buffy coat and plasma were tapped out onto a glass slide. A cover slip was applied on it and observed under microscope.

Data Analysis

Data were analyzed by descriptive method using Open EPI version 2.3 and Microsoft

Excel Sheet version 2013. The data were analyzed by grouping the dog age wise as 2 months to 2 years, 2 years to 6 years and 6 years to 12 years and gender wise (male and female). Effect of sex and age on prevalence was evaluated by chi- square ($\chi 2$) test. Values of $p < 0.05$ were considered significant at 95 % level of confidence.

RESULTS

Result of blood smear examination

Overall prevalence of blood parasites in dogs

Out of 110 dogs blood samples examined, 12 (10%) were positive for presence of blood parasites (Fig 1). In the study 10 (9.3%) samples were detected positive for the *Babesia spp*. and 2 (1.8%) samples were positive for *Ehrlichia spp*. (Fig. 2)

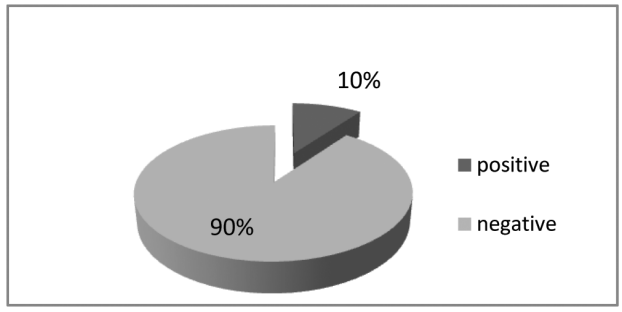

Fig. 1: Prevalence of Overall blood parasites in community dogs of Lalitpur

Haemoparasitic species isolated in positive blood samples of dogs

Out of 12 positive blood samples, *Babesia spp*. was found in 10 dogs and *Ehrlichia spp*. in 2 dogs.

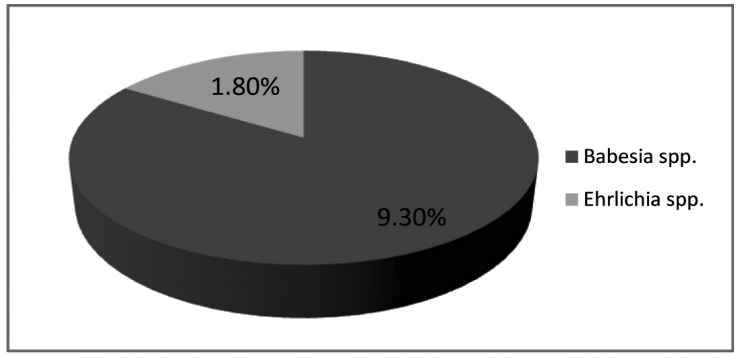

Fig. 2: Species wise prevalence of haemoparasites in Community Dogs of Lalitpur

Age wise prevalence of blood parasites in dogs

Out of 110 blood samples, 40 samples were from dogs of 2 months to 2 years of age, 50 samples were from dogs of 2 years to 6 years and 20 samples were from dogs of 6 years to12 years of age. Among 40 sample of dogs below two years, 9 (22.73%) were found positive and among 50 samples of dogs between 2 years and 6 years, 10 (21.15%) were found positive and among 20 samples of dogs between 6years and 12 years, 1 (7.60%) were found positive(Fig 3). The result showed that the prevalence of blood parasites was statistically non significant (p = 0.255) between different age groups of dogs.

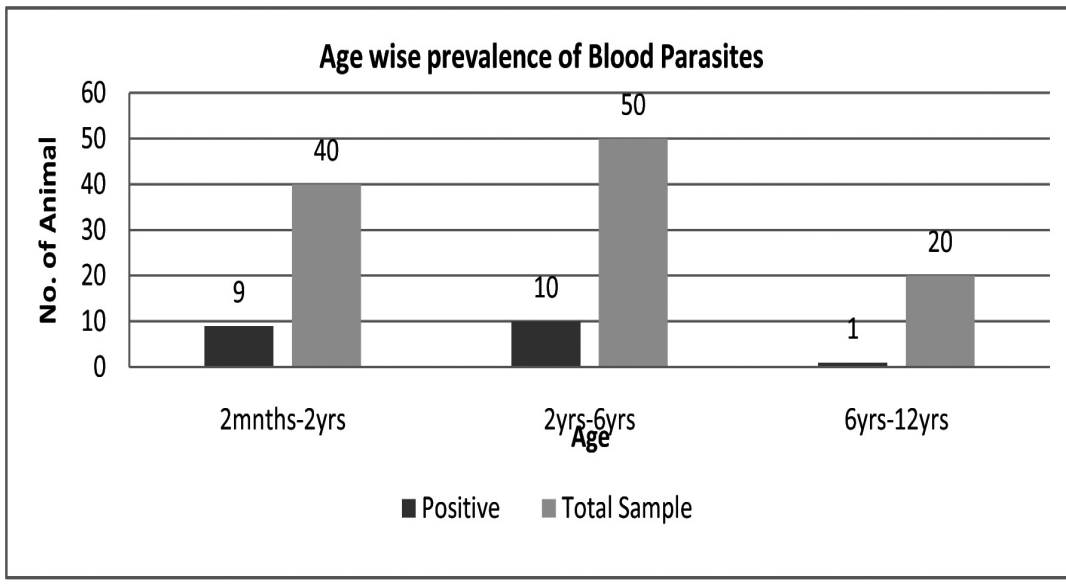

Fig. 3: Prevalence of blood parasite according to Age-groups

Prevalence of Blood Parasites according to Sex

It was observed that there was no significance difference in the occurrence of this infection amongst the different age group and sex. Though there was no significant difference in prevalence between the sex and age, the number of positives was higher among males (58.33%) than females (41.66%). And more in 2months to 6 years age group than remaining two age groups(Fig. 4).

It was found that there was significant influence ($p<0.05$) on the occurrence of the blood parasites with the decrease in PCV. Thus, we can say that blood parasites might be one of the causes of anemia.

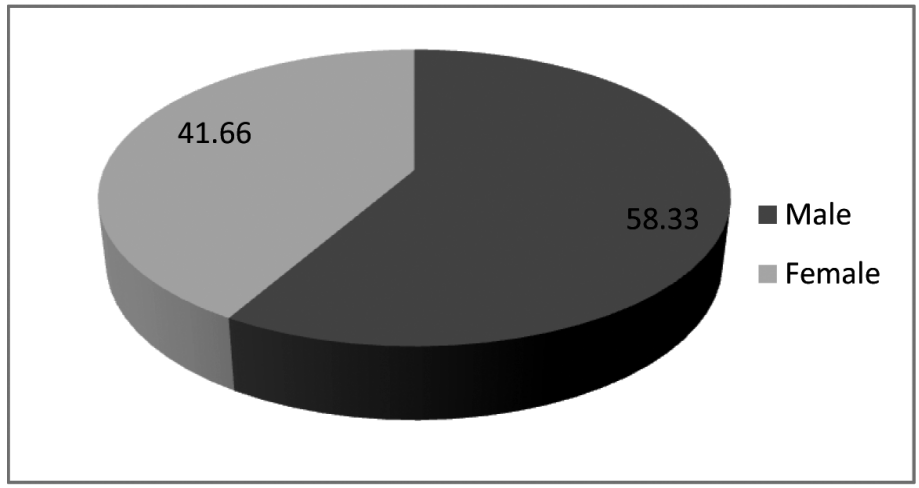

Fig. 4: Sex wise prevalence of the blood Parasites

DISCUSSION

The result of this study is close to the findings of Subedi and Gaire (2012) who found in dogs of Kathmandu valley. This similar prevalence is probably due the sample selection from the febrile dogs, geographical area and adopting the method of Geimsa Stained blood Smear technique.

But the result obtained is higher than that of Bashir *et al*., (2009) had reported that the prevalence to be 2.62% in Lahore. The higher prevalence is probably due to sample selection from febrile dogs only which would otherwise had reduced the prevalence rate.

Among 110 samples tested, 10 samples (9.09%) were found to be positive for the *Babesia spp*., 2 samples (1.08%) were found to be positive for *Ehrlichia spp*. This finding shows high prevalence of *Babesia spp*. Followed by *Ehrlichia spp.*

The presence of hemoparasites *Babesia spp.* was reported to be 10% in this research which is similar to Pam *et al*., (2013) who found the only prevalence of *Babesia canis* 43 (100.00%) in the study area, Nigeria. which might be due to the effort of hemolysis as in *Babesiosis* are known to multiple in the host blood cell. Thereby leading to heamolysis and anaemia. In severe cases, they cause slugging of blood in peripheral vessels leading to disseminated intravascular coagulation and organ failure. The high incidence of babesiasis could be attributed to abundance of tick vector *Rhipicephalus sanguneus* in this environment almost all year round. This study also suggests that

dogs in the study area are vulnerable to blood parasites and these have effect on their heamogram. Hence, owners of dogs in this area are advice to carry out control of the vectors to control parasite infection.

CONCLUSION

This study confirmed that there is still a considerably high prevalence of blood parasites in the dogs of Kathmandu Valley. High prevalence in community dogs reveals the exposure of the stray dogs to the vectors of the blood parasites. Higher prevalence of blood parasites in community dogs could be the source of contamination to pet dogs. Blood Parasites are found to be one of the causes for raise in temperature in dogs. So, it is necessary to carry out the blood smear test to those dogs having increased body temperature. Before reaching to any conclusion, it is important to establish all the risk factors of blood parasites and study in advance about these risk factors.

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Livestock, Livelihood and Climate Change Issues of Small Holder Farmers of Nawalparasi District, Nepal

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ABSTRACT

Smallholder farmers are going to feel the brunt of climate change. Their vulnerability comes from their exposure to climate change and their limited capacity to respond and adapt. Unfortunately, the kinds and actual nature of impacts will be challenging to predict because of (i) the variations in type of livelihoods activities that the smallholder farmers are engaged with, (ii) intrinsic characteristics of these systems, particularly their complexity, their location-specificity, and their integration of agricultural and nonagricultural livelihood strategies, and (iii) their vulnerability to a range of climate-related and other stressors. With focus on Nawalparasi district of Nepal, this paper shed light on the types and characteristics of smallholder farmer and the research needs to understand their sensitivity to climate and other ongoing changes.

Key words: smallholder farmers, climate change, animal feed in Nepal, Livestock diseases in Nepal.

INTRODUCTION

Some of the most important impacts of global climate change will be felt among the populations, predominantly in developing countries, referred to as "smallholder" farmers. Their vulnerability to climate change comes both from being predominantly located in the tropics, and from various socioeconomic, demographic, and policy trends limiting their capacity to adapt to change.

The total land mass in Nepal is about 14.75 million ha (LRMP, 1986). Of this total area, the agriculture land commands only cultivated area of 4.01 million ha (27.2%) and grazing land of 1.70 million ha (11.5%), the forest land includes forest areas of 5.61 million ha (38.0%) and shrubs 0.69 million ha (4.7%) . Nepal can be divided into five major physiographic regions trans-Himalaya/high Himalaya, high mountain Siwaliks/lower hills and the Terai (LRMP, 1986).Based on the topographic features, Nelson (1981) divided the country into five major physiographic regions. The Terai

region commands the major agriculture areas below 300 meters in elevation. This region is heavily transferred by all major rivers of Nepal. This region accounts for 14% of the total area, 42% of the cultivated and supports 37% of the total Population (LRMP, 1986). The Climate and vegetation of this region is humid and sub-tropical in nature with major precipitation during monsoon.

The major animals are local Terai cattle, local and crossbred buffaloes, Terai goats, local Hurrah pigs and exotic pigs, Lampuchhre sheep, local ducks and pigeon. The improved/ crossed Hurrah buffaloes and crossbred cattle of Haryana, Jersey, and Holstein Friesian breeds are increasingly raised in the vicinity of the towns and market accessible areas. Nawalparasi District covers an area of 2,162 km2 and has a population (2001) of 562,870. The Nawalparasi district lies in Terai, mid hill and Chure region. Crop residues, crop weeds, and forest supplies make up the diet of the livestock in Nepal. Agricultural lands contribute about 60 percent of the total feed requirement, mainly in the form of low quality crop residues, and forest and grazing lands contribute the remaining 40 percent. On the whole, the Nepalese livestock are underfed at least by about one-third (TLDP, 2002).

Non-climate-related stressors and trends

Non-climate stressors have been affecting smallholder and subsistence agriculture in Nepal. The complex interaction of such stressors in increasing vulnerability can be illustrated in Chure regions of Nepal. Where farmers can have lot of goats based on forest resources it may cause environmental degradation in such vulnerable and fragile dry land areas.

Community forestry in Nepal has made it possible to spread dense forest in Hilly region. But livestock rearing becomes harsh to poor people. They have limited access in such forest. Though, the members of community are better facilitated.

Other stressors are seen as contributing to an increased vulnerability to drought, which in turn feeds back in to environmental degradation, conflict and underdevelopment of livestock and agriculture market systems.

Coping and adaptation

Smallholder, subsistence systems, especially those located in marginal environments, areas of high variability of rainfall or high risks of natural hazards, are often characterized by livelihood strategies that that have been evolved (*i*) to reduce overall vulnerability to climate shocks (adaptive strategies), and (*ii*) to manage their impacts *ex-post* (coping strategies).

Many defining features of livelihoods and adaptive strategies to climate variability and postulates four major elements of adaptation:

1. Making use of biodiversity in cultivated crops and wild plants.
2. Increasing integration of livestock into farming systems (at a cost of increased labor demands).
3. Working land harder, in terms of labor input per hectare, without increasing external non-labor inputs.
4. Diversifying livelihoods.

Shifting to irrigate farming is sometimes seen as a coping strategy in the face of climate variability across the developing world. In South Asia, agricultural strategies such as increasing livestock production relative to crops, and selection of crop varieties, are responses to both drought and floods.

Impacts of climate change on smallholder and subsistence agriculture

It is important for producing maps of vulnerability to climate change for Nepal, based on existing geographical data sets of current farming systems and of indicators of socioeconomic vulnerability, and projections of length of growing period, animal feed availability and density of animals in particular areas or eco-zone of Nepal. This analysis highlights "hotspots" for vulnerability: crop and livestock interface in Terai regions of Nepal. A conceptual framework is now needed that can understand impacts of climate change on smallholder and subsistence agriculture.

Such a framework should:

1. recognize the complexity and high location-specificity of these production systems,
2. Incorporate non-climate stressors on rural livelihoods and their contribution to vulnerability.

Complexity and location specificity

These livelihood systems are typically complex; they involve a number of crop and livestock species, between which there are interactions-for example, intercropping practices or the use of draught animal power for cultivation, and potential substitutions such as alternative crops. Many smallholder livelihoods will also include use of wild resources, and nonagricultural strategies, such as use of remittances. Remittances are important in Nepalese agriculture economy too.

Non-climate stressors and vulnerability

Management of non-climate stressors, such as poor market access, by governments and development donors, would itself constitute a powerful strategy for assisting adaptation, but some stressors, particularly various forms of environmental degradation, will themselves be influenced by climate change.

Biological processes at organism and field level

1. New syntheses of the growing number of regional and global simulation studies of changes in crop yields against temperature suggest that in the tropics, even moderate temperature increases (1–2°C) are likely to have negative impacts on yields of rice, maize, and wheat (the three major cereals worldwide and among smallholder and subsistence farmers). Higher levels of warming will have serious negative impacts on yields of maize and wheat, and less so on rice.

2. Increases in temperature may increase irrigation water requirements of major crops, increasing water stress, particularly in Southeast Asia as well as in Terai region of Nepal.

3. The conclusion of the Third Assessment Report of the IPCC of likely significant negative impacts on semiarid rangelands is confirmed, although there are still very few impact studies for tropical grasslands or rangelands. There is also new knowledge on the direct negative effects of thermal stress on productivity, conception rates and health of livestock that may be relevant to exotic (*Bostaurus*) cattle kept for small-scale dairy production in the tropics.

4. There is evidence of increased risk of crop pests and diseases of crops under climate change, although knowledge of likely impacts in the tropics and on smallholder systems is much less developed.

5. A general principle that crosscuts these projections of impacts on crops and livestock species is the increased understanding of the importance of extreme events.

6. Increases in frequency of extreme events may go beyond the impacts of mean climate change in lowering long-term yields by damaging crops at particular developmental stages, making the timing of agricultural operations more difficult, and reducing incentives to cultivate.

Environmental and physical processes

One such impact is the effects of decreasing snowcap on high Himalaya and mountain of Nepal and it causes less irrigation in foot hills and Terai regions in dry season.

Non agricultural climate change impacts

Some negative impacts of climate change on tourism in developing countries have already been projected due to emergence of new disease such as Ebola, SARS and swine flu.

In general, however, the location of a large body of smallholder and subsistence farming households in the dry land tropics gives rise to especial concern over temperature-induced decline in crop yields, and increasing frequency and severity of drought. These lead to the following generalizations:

1. increased likelihood of crop failure;
2. increased diseases and mortality of livestock and/or forced sales of livestock at disadvantageous prices;
3. Livelihood impacts including sale of other assets, indebtedness, out-migration and dependency on food relief.;
4. possible feedbacks through unsustainable adaptation strategies into environmental degradation including loss of biodiversity; and
5. Eventual impacts on human development indicators such as health and education.

MATERIALS AND METHODS

Livestock are raised from the plain areas of the Terai to the rain shadow areas of the Himalayas, but in all ecological regions, a strong integration of crops with livestock, forestry and marketing have been felt in Nepal. Nawalparasi is the Terai district of the western development region. The district composes its land from hill, Chure and Terai region. Therefore, the district is the important hot spot in climate change issues.

The fifty household surveys were done by using standard format. The surveys were carried on Kolhwa, Sibamandir, Pragatinagar and Sansariya VDC of Nawalparasi districts. Based on the information provided by household (HH) the data was collected, tabulated and interpreted for livestock keeping and climate change in the district.

The key information was asked to farmers and narrated in the perspective of smallholder farmers and livelihood aspect.

RESULTS AND DISCUSSION

The family size

The average family size were of 6 (2-13). 28.9% of the people were illiterate where

as 71% were literate. High literacy would help for introduction of mitigation plan of action for climate change in local level.

There were 22.4% of farmers without land. It means they have manpower without resources. They are dependent on landholder of others and can get land on lease for their livelihood.

31.25% farmers have no rice field areas. 30.6% of farmers have no land. The average lowland holding was 13.9 Kattha in Nawalparasi district.

Land fragmentation

The land fragmentation is very common in Nawalparasi district.

81.4% of farmers worked on their land where as 18.6% people worked on other people lands for their livelihood.

Rice and maize was the major crops. Rice was cultivated by 53.06% farmers and maize is cultivated by 48.98% farmers.

Garlic, potato, onion, chilly and cauliflowers were major vegetable crops in the district. Rice straw and maize were the major feed for animals. Rice straw could be utilized as fodder by 82.2% household from their own field and similarly 69.39% of farmers utilized maize straw.10.20% did not utilize such things from their field either due to unavailability of animals in their farms or no land with them. 40.82% of farmers had fodder plants whereas 38.78% farmers did not have such plants.

The disease conditions on domesticated animals were reported by 18.37% of HH, whereas 59.18% of HH have not. The important diseases on the districts were Foot and Mouth Disease (FMD), Hemorrhagic Septicemia (HS), Black Quarter (BQ) and Peste des petites ruminants (PPR).

26.53% of HH had problems of parasitic infestation, 12.24% of HH did not report of parasitic diseases. Among parasitic infestation, ecto-parasites, liver fluke and ascariasis were important with 28.57%, 26.53% and 24.49% of HH coverage respectively.

No vaccinations were given to livestock by 30.61% of HH. The practice of vaccination against HS, BQ, FMD and PPR was carried out by 34.69%, 26.53%, 14.86% and 10.2% of HH respectively. The disease situation in the districts remained same in last five year as per 46.94% of HH. The disease trend is decreasing in the same period has been reported by 14.29% of HH. Whereas 8.16% HH reported that there have been increasing trend of disease.

Climate change parameter

The production of crops had negative impact on 24.49% of HH; there was positive impact on production 18.37% of HH. There was no change on production of crops in the period as per 4.08% of HH. The 57.14% of HH had been using chemical fertilizers where as 24.49% did not use chemical fertilizers. Drinking water sources became scare in the dry season. The source became different as per 26.63% of HH and 26.63% of HH did not have any idea about changing source of water. But 44.9% of HH believed that there was no change in water sources in their areas. Irrigation water sources became scare in the dry season. The source became different as per 38.77% of HH and 22.45% of HH did not have any idea about changing source of water. But 32.65% of HH believed that there was no change in water sources in their areas. The production of grass reported to be decreasing by 36.73% of HH, it was in increasing trend by 8.16% of HH. The grass production was about same by 6.12% of HH. The grass production was not known to 4.o8% of HH. There was excess or under rainfall has been observed by 57.14% of HH. The normal situation was observed by 36.73% of HH. There was change on animal herd by climate change as per 67.35% of HH. There is no change observed in 4.08% of HH. Climate change has effect on animal production as per 36.73% of HH. There was no change on 40.81% of HH and 2.04% are unaware of such change on animal production. The livestock keeping is going on dangerous phase in 26.53% of HH, it is on smooth phase in 59.18% of HH. There is no change observed by 2.94% of HH.

Effects of climate on flowering, seeding time

There was no change on different stages of plants as per 79.59% of HH. There were changes observed by 8.16% of HH. The change on pasture and forage quality was not known to 75.51% of HH. The global warming effect was observed by 38.77% of HH. The 44.90% observed no difference in temperature. But 4.08% of HH observed decreasing on temperature. The rainfall pattern was the same in the area as per 42.86% of HH. The rainfall was decreasing by 40.81% of HH. The rainfall was increasing as per 4.08% of HH. Hailstone pattern was same observed by 55.10% of HH. It was in decreasing trend as per 40.61% of HH. It was in increasing trend as per 6.12% of HH. Soil erosion and flood situation remained same as per 75.51% of HH. It was in increasing trend by 8.16% of HH and it was in decreasing trend by 6.12% of HH.

Top soil run off may be the result of overgrazing. It is estimated that loss of 1 mm top soil removes 10 kg nitrogen, 7 kg phosphorus and 15 kg potash from1 hectare land (Carson B,, 1992). Transportation was the major problem in the district as per 46.94% of HH and electricity is the second problem of 34.69% of HH. Government of Nepal and NGO shall work together for development and solving above mention problems as per 73.47% of HH. 2.04% of HH feels it is government duty.

Smallholder and subsistence farmers will suffer impacts of climate change that will be locally specific and hard to predict. The variety of crop and livestock species produced by any one household and their interactions, and the importance of nonmarket relations in production and marketing, will increase the complexity both of the impacts and of subsequent adaptations, relative to commercial farms with more restricted ranges of crops. Small farm sizes, low technology, low capitalization, and diverse non-climate stressors will tend to increase vulnerability, but the resilience factors—family labor, existing patterns of diversification away from agriculture, and possession of a store of indigenous knowledge-should not be underestimated.

Social-scientific study of the future impacts of climate change on poor rural people in developing countries has tended to be concerned with the increased frequency of extreme events with generalized impacts.

CONCLUSIONS

There is decreasing rain fall in the region with some extreme weather and natural calamities reported. The frequency of disease outbreak of FMD, BQ and HS has been observed. Fertility and organic matter of soil is in decreasing trends. Soil erosion is in fast pace. Seed availability of forage grass is inadequate and forage crops have not been considered as a main crop. The pace of climate change may hamper livelihood of farmers of Nawalparasi districts. In this context livestock farming is an option to them.

Agriculture sector is a basis of livelihood of Nepalese farmers, contributing to 31% of the country's GDP and employing 66% of its labor force (MoAC, 2008).

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

secondary plant residue (9%) in Nepal (Sherchand, 2001). Continuing deforestation and degrading land fertility further jeopardize the livelihood of households in the community as it increase the daily tasks of women (Bhatt *et al.,* 1994). In recent years' cultivation of exotic grass in farm land and other lease land boost the production of forage and ultimately help for improved livestock feeding system in mid hill and Terai region of Nepal.

Nawalparasi is the one of the most vulnerable district due to climate change in western development region. The fodder plants are there but the number is not sufficient for current livestock population. The fodder plants are more in 17 VDCs of hilly areas. Therefore intensity of cropping can be increased and their wastage can be utilized for animal feed. New technology of complete ration shall be introduced and animal genetics resources awareness campaign shall be done. The mitigation majors can be made in collaboration with local NGO, local bodies and District Livestock Services Office (DLSO). The Regional Directorate of Livestock Services (RDLS) shall give guidelines and support to this effort.

The Nepalese farming system is at subsistence level, and fodder and forages produced can increase the production. The increased amount of biomass can inspire the poor farmers to increase the herd composition. The commercialized dairy industry has high scope of green forage and fodders during this period of limited farmland.

Women contribute significantly to livestock raising , providing 70% of the work effort and are reported to be more knowledgeable than men about treating sick animals (Annon a, 1995 and Annon b , 2000). The production of milk and meat can be enhanced with environmental friendly livestock farming in this district with participation of women and smallholder farmers.

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Productive Performance of Cultivar (Cv.) *Mulato II Brachiaria* Hybrid with Respect to Delayed Planting in Chitwan Nepal

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ABSTRACT

Feed deficit condition is gradually emerging as a main problem of livestock production in Nepal. Among the possibilities of producing more dry matter to feed animals, use of hybrid forage species, such as cv. Mulato II Brachiaria hybrid could be one of the approaches to consider. A study on Mulato II was conducted using different planting dates at Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan, during July 2009 to January, 2010. A randomized complete block design was used. Morphological attributes such as plant height, leaf length, number of leaves per plant, and number of tillers per plant were determined for three occasions along with dry matter yield estimation. Similarly, chemical constituents of the cv. Mulato II Brachiaria hybrid were also analyzed thrice covering the entire experimental period. Findings revealed that early planting dates (27 July) had increased plant height, leaf length, number of leaves per plant, and number of tillers per plant, and thus contributed positively to increase fresh herbage mass and dry matter (DM) yield of Mulato II. The DM yield of Mulato II in second harvest and third harvest was 19 t ha $^{-1}$ and 78 t ha $^{-1}$ respectively. The DM, crude protein (CP), crude fibre (CF), ether extract (EE) and total ash content of Mulato II (averaged over different harvesting dates) were 22.22 %, 14.50 %, 23.10 %, 3.28 %, and 8.26 %, respectively, which was higher for 27 July plantation compared to the other treatments. Moreover, Mulato II was very palatable and had remained green during dry period. Results of these experiments thus indicated that there is a good potential of growing Mulato II in early summer at Chitwan conditions as an alternative and higher biomass producing grass which would be of more productive if planted earlier in the season.

Key words: Mulato II hybrid, fodder production, nutrition, delayed sowing

INTRODUCTION

Nepalese livestock, in general, are with low level of productivity. Average milk production of indigenous cattle is 880 litre/cow/year, buffalo-1,158 litre/buffalo/year (ASD, 1998). On the whole, Nepalese livestock are underfed at least by about

one-third (TLDP, 2002). Government of Nepal, Department of Livestock Services has introduced many pastures and fodder species focusing to improve the natural pastureland, farmland, as well as forestation sides. Mulato II is one of them which have been slowly introduced at the farm level. The main objectives of exploiting new forage resources are to substitute imported livestock feedstuffs with local resources to produce livestock efficiently through nutritional manipulations.

However, response of delayed planting time on productive performance of introduced species studied considering the nutritional changes. Review on agronomic studies shows cv. Mulato II grows well but scientific information with respect to the morphological attributes and their persistency particularly delayed planting, herbage mass production and nutrient contents in Nepalese context is scanty.

Major objective was to assess the productive performance and nutritive value of Mulato II on the farm conditions in relation to its response under varied transplanting scheme and specific objectives of the study were to evaluate the response of varied transplanting dates to the morphological attributes & herbage mass yield potential of Mulato II, and to determine the chemical constituents, and digestibility of major nutrients of Mulato II, grown at varied transplanting dates.

MATERIALS AND METHODS

Two experiments were conducted covering seven months period to fulfill the objectives of the research. First Experiment was conducted in IAAS Forage crops demonstration Unit. The experimental design was Randomized Complete Block Design (RCBD). Three treatments were used because Seasonality of the pasture growth is mostly governed by the monsoon. In Nepal, over 60 percent of the rain occurs from June to September (Pandey, 1997); each replicated for five times. However, the Second experiment was on digestibility trial in Goats. Statistical package MSTATC (Version 1.3) and MS Excel were used for data analysis and α level was set as (0.05).

RESULTS AND DISCUSSION

The number of tiller plant $^{-1}$ significantly differed ($P<0.05$) at 100 DAP among the planting dates. Accordingly, at 100 DAP, highest mean tiller number plant $^{-1}$ reached close to 80, whereas the tiller number plant $^{-1}$ for 6 August and 16 August planting was about 15 % and 30 % less, respectively to that of 27 July planting .

At 115 DAP, planting date had significant effect to the mean tiller number plant $^{-1}$, but the number decreased approximately 25 % for 27 July planting, compared to that of 100 DAP. On the other hand, mean number of tillers plant $^{-1}$ was increased about 10 % if planted on 6 August, but remained similar to the value of 100 DAP planting if planted on 16 August.

The overall mean of tillers plant $^{-1}$ was statistically different ($P<0.05$) among the planting dates, with the maximum number for 6 August planting, followed by 27 July planting, though, the value was 69 and 66 , statistically similar (Table 1).

Table 1: Effect of Planting dates on tiller number (TN) of cv. Mulato II *Brachiaria* hybrid for 100 days onwards of transplanting to 155 days at IAAS Rampur, 2009.

Planting date	Re-growth after first harvest			Re-growth after second harvest		
	100 DAP	115 DAP	Mean	140 DAP	155 DAP	Mean
27-Jul-09	78.28 a	59.28 b	68.78 a	58.80	73.00 a	65.90 ab
6-Aug-09	66.92 ab	73.36 a	70.14 a	66.64	72.36 a	69.50 a
16-Aug-09	56.26 b	54.76 b	55.51 b	57.24	59.32 b	58.28 b
F- Value	4.91 *	7.46 *	4.51*	1.51 ns	3.84 ns	3.30 ns
P- Value	0.04	0.01	0.04	0.27	0.06	0.09
CV %	16.54	12.71	13.13	15.06	12.91	10.93
LSD	16.2	11.58	12.41	13.37	12.85	10.29
SEM	4.14	3.45	3.23	3.75	4.18	3.15

DAP = Days after planting, CV = coefficient of variation, LSD = Least significant difference, SEM = Standard error mean, ns = non-significant, * significant at 5 % ($P<0.05$), * significant at 1 % ($P<0.01$).

The cumulative fresh herbage mass of Mulato II planted on different dates did not differ statistically ($P>0.05$). Nevertheless, the cumulative fresh herbage mass was highest (43. 7 t ha $^{-1}$) for 27 July planting, followed by that for 6 August and 16 August planting(Table 2).

Table 2: Effect of different planting dates on fresh herbage mass production of cv. Mulato II *Brachiaria* hybrid at IAAS Rampur 2009/10.

Time of Planting	First harvest (85 DAP)	Second harvest (125 DAP)	Third harvest (165 DAP)	Cumulative
27-Jul-09	41.19	1.99 b	0.53 c	43.71
6-Aug-09	34.46	2.01 b	1.65 b	38.11
16-Aug-09	31.29	2.98 a	2.02 a	36.28
Mean	35.65	2.33	1.40	39.37
F- value	1.74 ns	15.35 *	83.27 **	1.03 ns
P- value	0.24	13.85	0.00	0.40
CV %	24.02	13.85	13.57	21.61
LSD	12.47	0.47	0.28	12.41
SEM	3.82	1.14	0.08	3.80

Nutritional analysis of Mulato II

The effect of planting date to the crude protein content of Mulato II was significant ($P<0.01$) at first harvest, but was statistically similar ($P>0.05$) at third harvest (Table 3). At first harvest, mean crude protein content was 16.83 %, but that decreased to 12.15 % at third harvest (165 DAP).

Table 3: Mean CP percent of *Brachiaria* hybrid cv. Mulato II in response of planting date (averaged over different harvesting date) at IAAS Rampur, 2009

Planting date	Crude Protein %	
	First harvest	Third harvest
27-Jul-09	14.92 b	12.656
6-Aug-09	17.54 a	11.168
16-Aug-09	18.038 a	12.634
F- Value	15.80 *	0.5197 ns
P- Value	0.0017	0.05
CV	5.6	21.77
LSD	1.374	3.858
SEM	0.4214	1.1829

DAP = Days after planting, CV = coefficient of variation, LSD = Least significant difference, SEM = Standard error mean, ns = non-significant, * significant at 1 % ($P<0.01$).

The average air temperature, relative humidity, and total rainfall at the Mulato II cultivation site during the experimental period (2009-2010) are presented in Fig .1 .

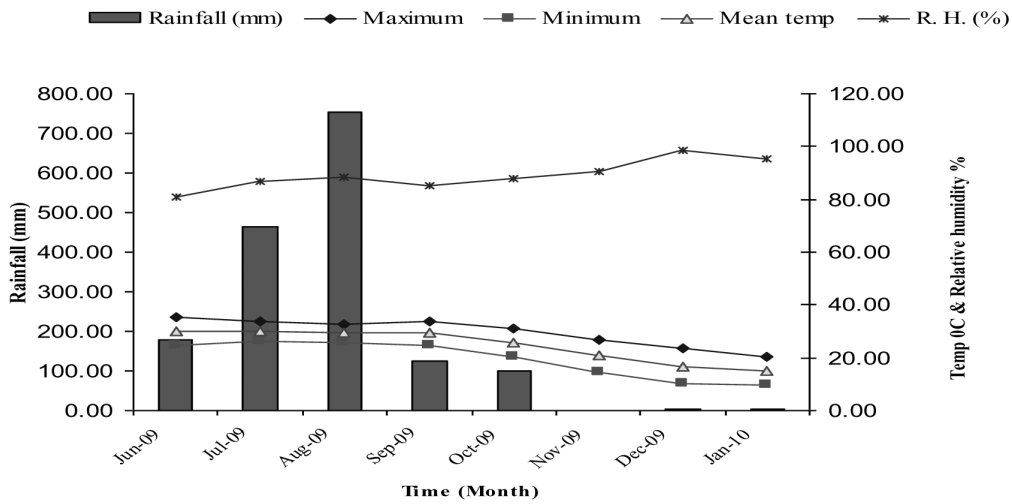

Fig.1: Average air temperature, relative humidity, and total rainfall at the Mulato II cultivation site during the experimental period (2009-2010).

CONCLUSION

Morphological attributes such number of tillers plant $^{-1}$ was significantly higher for early planting (27 July) that well contributed to the cumulative green herbage mass and corresponding DM yield of Mulato II, with no adverse, but positive effect to the major chemical constituents including CP, CF and EE; Mulato II was proved as fairly palatable and easily digestible grass with no untoward effect on goats provided it was mixed with maize grits. The digestibility coefficient of Mulato II was fairly comparable to *B. mutica* suggesting its potential livestock feeding.

Mulato II performed very well and produced higher DM yield, even during dry season, mainly due to its ability to produce green leaf but delayed planting had reduced herbage mass, DM production, with increased in dead matter production, indicating its sensitivity to the delayed in sowing.

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