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Veterinary Complex
Tripureshwor, Kathmandu, Nepal
Tel/ Fax: 4257496
P. O. Box No.: 11462
E-mail: nveta@wlink.com.np or vetnewsnepal@yahoo.com.np
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Editorial

The editorial board is highly delighted to publish this issue of Nepalese Veterinary Journal. The board has circulated to all members for contribution of scientific papers for publication. Accordingly twenty one scientific papers, review papers, clinical case reports are being incorporated in this publication. These papers are mainly based on livestock commodities eg. Cattle, bufflaoes, sheep, goats, pigs and poultry, livestock products eg meat and milk, pets and wild animals. There are many emerging and re-emerging diseases in the country on which the Veterinarians have to study with proper laboratory diagnosis. This issue of NVJ highlights mainly on livestock and poultry deseases, zoonotic deseases, fish deseases, Dairy Science and pet animals.

The papers submitted by recently graduated authors were quite similar to the thesis write up. The standard format and guidelines were not being followed which made the editorial task very difficult to bring to the standard format. So the authors are requested to follow the standard guide lines for future publication of papers in Nepalese Veterinary Journal.

The country had faced the natural calamities of earthquake, floods and landslides last year (2015). With so much of devastation of nature and man made infrastructures not sparing the precious animals of different livestock species which rural people are mostly depended on for livelihood. The authors expects papers based on loss and affects on livestock species due to the earthquake and relief works being carried out for different livestock species for future publication of NVJ.

The editorial board thanks all the peer reviewers by indicating the areas to be improved and thanks all the authors who have responded timely by correcting all the comments pointed by peer reviewer. Every efforts have been made to make the articles without any errors by checking again and again and there might be still some errors which might have been overlooked.

The conclusions, recommendation and technologies of the published papers will definitely be helpful to the Veterinarians, technicians and livestock farmers and other stake holders. With so many rules and regulations of the government of Nepal to be followed to meet the need of WTO, OIE and SPS agreement, Veterinarians had to be updated with recent advancement, technologies and animal disease diagnosis. The published articles will definitely be helpful in updating recent knowledge in the field of Veterinary Science and Animal Husbandry.

Thanks to all the members of editorial board for giving their valuable time and effort for editing the papers. Ms Pramina of NVA has really worked hard for compilation and moderation of editing of all the papers. Thanks goes to Dr Sulochana Shrestha (Jr) for necessary help in computer editing and Mr Krishna for logistic support.

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

Editor-in-Chief

Contents

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Study of Paratuberculosis of Cattle

U. M. Singh[1], P. Shrestha[2], N. Paudel[2], R. Bastola[3] and S. Sapkota[4]

1. National Animal Science Research Institute

2. Animal Health Research Division

3. NCRP, Chitwan

4. Animal Breeding Division

ABSTRACT

A total of 88 serum samples comprising of Achhami cattle (36), Lulu cattle (10), NCRP cattle (23), and Surkhet cattle (19) were collected and stored in -80^{0}C deep freezer at AHRD, Khumaltar until laboratory examination. The serum samples were rendered for ELISA (ID Vet France for presence of antibodies against paratuberculosis. A total of 8 samples (22.2%) of Achhami cattle, 2(8.69%) samples of NCRP, Chitwan and 5(26.31%) samples of cattle of Surkhet were found positive for antibodies against paratuberculosis by ELISA. Achhami catle were found positive for paratuberculosis antibodies and Lulu cattle were found tobe negative. There is a need for study on this disease in more number of animals covering wider areas.

INTRODUCTION

Paratuberculosis (Johne's disease) is a chronic granulomatous infectious disease of domestic ruminants which has been reported in wild ruminants including rabbits and camelids. This disease was reported for the first time by John and Frothingham 1985. It is caused by *Mycobactterium avium* sub sp. *paratuberculosis* an acid fast organism. The animals are infected at early age but disease appears later after one year of age. The disease is characterized by progressive weight loss and diarrhoea and emaciation. Diarrhoea occurs in later stages. It is characterized by chronic granulomatous enterocolitis, Lymphangitis, Lymphadenitis (Chiodini *et al.,* 1984). Diarrhoea occurs in later stages. It is characterized by chronic granulomatous enterocolitis, Lymphangitis, Lymphadenitis (Chiodini *et al.,* 1984). Economic loss in the herd is mainly due to decrease in production, feed efficiency, culling of infected animals cost of diagnosis and control measures. And mostly Intradermal skin test has been carried out in past years.hence in this study attempt has been made to carry out additional diagnostic tests e.g. Indirect ELISA (ID Vet France).

Absorbed ELISA was conducted in 115 serum samples from buffaloes and 98 serum samples from cattle of different districts of Western Nepal which revealed 39 samples (33.9%) and 27(27.5%) samples positive to antibodies against paratuberculosis. In the same study 11 (34%) out of 32 goats were found to be positive for paratuberculosis (Joshi and Joshi, 1999). Similarly rectal scrapings were collected from 28 buffaloes and 26 cows and 4 scrapping smears and 3 cultures were positive for acid fast organisms (Jha, 2003/2004). During the years 2006-2008 studies on pratuberculosis were conducted by using different diagnostic tests conducted at Animal Health Research Division, NARC, Khumaltar and over all prevalence of 7.10% (3.5-9.09%) were detected in DTH, 4.55% (4.25-4.85%) in faecal smear, 0.70% in faecal culture and 5.34% (4.54-5.82%) in AGID in cattle of Kathmandu valley and Chitawan district (Singh *et al.*, 2008).

There is very scanty information regarding Johne's disease in Nepalese context This study was conducted to help in carrying out future studies on bovine paratuberculosis, to find out the occurrence of paratuberculosis and develop future strategies on this disease.

MATERIALS AND METHODS

Collection of Samples

Annual reports of Central Animal Disease Investigation and Research Laboratory from 1982/83 to 1987/88 were reviewed for the incidence of Johne's disease in cattle. The cattle which were more than one year of age were selected randomly from Acchami cattle maintained at RARS, Depayal, Lulu cattle maintained at Animal Breeding Division Khumaltar, cattle maintained at NCRP, Chitawan and cattle of Surkhet district. A total of 88 serum samples comprising of Achhami cattle (36), Lulu cattle (10), NCRP cattle (23), and cattle (19) from Surkhet were collected and stored in -80^0C deep freezer at AHRD, Khumaltar until laboratory examination.

Laboratory examination

The serum samples were rendered for ELISA for presence of antibodies against paratuberculosis. The serum samples were tested as per manufacture direction (ID Screen Paratuberculosis Indirect Screening test ID Vet, France).

Results and Discussion

A total of 8 samples (22.2%) of Achhami cattle, 2(8.69%) samples of NCRP, Chitwan and 5(26.31%) of samples of cattle of Surkhet were found positive for antibodies

against paratuberculosis by ELISA (Table-1).

Table-1: Result of Serum samples tested by ELISA for Paratuberculosis

Sample	Total no of serum samples	No.positive	positive %
Achhami cattle	36	8	22.22
Lulu cattle	10	0	0
NCRP cattle	23	2	8.69
Cattle of Surkhet	19	5	26.31
Total	88	15	16.30

Achhami cattle have never been tested for paratuberculosis. The positive animals should be identified and removed from the farm to make farm Paratuberculosis free. Only 2(8.69%) samples were detected positive out of 23 cattle of NCRP, Chitwan. As the animals have been tested in previous years and the positive and suspected animals had been removed from the farm, this may be the possible reason for lower prevalence. Smilarly 59 (26.31%) samples were found positive from Surkhet District and these animals have never been tested before. Joshi and Joshi (1999) detected 27.51% prevalence out of 27 no of cattle of sample by ELISA which is higher than the findings of this study with overall prevalence of 16.30%. In other studies conducted by Singh *et al.,(*2008) an overall prevalence of 7.10% were detected in DTH(Johnin Test), 4.55% in faecal smear, 0.75% in faecal culture and 5.34% in Aga gel immuno diffusion test. The sensitivity of different test varies and the least percentage of prevalence was found in faecal culture as the microbiological culture for this disease takes long time and depends on the organism load in the faecal sample

CONCLUSION

This is a preliminary study which has indicated the prevalence of Paratuberculosis in cattle of NCRP, Chitawan, cattle from Surkhet and Achhami cattles and antibodies were not detected in Lulu cattle which indicated that more number animals covering wider areas having Lulu cattle to be tested for this disease. Another possibility could be nutritional status of lulu cattle may be better compare to the Achhami cattle which might have pre disposed to this disease.

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for collection and laboratory analysis of samples.

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Study on Bovine Venereal Campylobacteriosis (BVC) in Semipastoral Cattle and Buffaloes Herd in Western Chitwan, Nepal

A. Pandit, S. K. Sah, B. N. Devkota and R. K. Bhattarai

Agriculture and Forestry University (AFU), Rampur, Chitwan

ABSTRACT

A study was conducted from April to September 2015 on semipastoral herds raised cattle and buffaloes where natural matting was predominant to find disease status of Bovine Venereal Campylobacteriosis (BVC) by isolation, identification and biotyping and to find its association with other different reproductive disorders. A total of 56 cervico vaginal mucous samples were collected by purposive sampling method, were cultured within 2-4 hrs on a media prepared by Campylobacter Agar (M994-Himedia®) and a selective supplement (Skirrow's supplement, FD008-Himedia®) in microaerobic condition. True animal-level prevalence of Bovine Venereal Campylobacteriosis was found 17.8 %, among them 70% Campylobacter fetus subspecies veneralis (Cfv) and 30% Campylobacter fetus subspecies fetus (Cff) were found, both of subspecies was independent (P<0.05) to cause disease. About 21.42% of cattle and 14.28% buffaloes were found with BVC positive. Increased calving interval (ICI) and repeat breeding condition (RBC) were associated with BVC (p<0.05). Similarly BVC was also associated (p<0.05) with low body condition scoring (BCS). Campylobacter fetus subspecies venerealis and *Campylobacter fetus subspecies fetus were found two major etiology to cause BVC in western Chitwan Nepal.*

Key words: *Campylobacter fetus*, Bovine Venereal Campylobacteriosis, BVC, Bovine Genital Compylobacteriosis, BGC,

INTRODUCTION

Campylobacter are Gram negative, microaerophilic, curved or spiral rods in the family Campylobacteriaceae. *Campylobacter jejuni* (formerly known as *C. fetus* subsp. *jejuni*) and *C. coli* are associated with enteritis in domestic animals and humans. Some strains of *C. jejuni, C. fetus* subsp. *venerealis*, and *C. fetus* subsp. *fetus* (also known as *C. fetus* subsp. *intestinalis* and *Vibrio fetus var intestinalis*) cause infertility and abortions in sheep and cattle. *C. fetus* subsp. *fetus* is occasionally isolated from humans with septicemia. Other species of *Campylobacter* including

C. lari, *C. hyointestinalis* and *C. upsaliensis* can cause disease but seem to be of minor importance in domestic animals. Uncharacterized *Campylobacter* species may be involved in proliferative ileitis of hamsters, porcine proliferative enteritis and proliferative colitis of ferrets. (CFSPH, 2003)

Bovine Genital Campylobacteriosis, as a venereal disease of cattle also known as Bovine Genital Disease characterized primarily by early embryonic death, infertility, a protracted calving season, and occasionally abortion. Distribution is probably worldwide. (Merck Veterinary Manual, 2014). Bovine Venereal Campylobacteriosis (previously known as Vibriosis) is a venereal disease of cattle caused by the organism *Campylobacter fetus* previously known as *Vibrio fetus*, now *Campylobacter fetus* subspecies *venerealis* and *Campylobacter fetus* subspecies *fetus* are major disease causative organism characterized by infertility, early embryonic death, and abortion. The disease is caused by *C. fetus* subsp. *venerealis,* a bacterium with pronounced tropism for the genital system of cattle. Transmission of the causal agent takes place mainly during natural mating, and the presence of C. fetus subsp. venerealis in the semen of bulls creates the risk of spread of the disease through artificial insemination. (OIE 2008).

Site was selected after retrospectively studied in which more animals had shown repeat breeding condition (RBC) and increased calving interval (ICI) with low fertility rate so this study may reveal status of bovine venereal campaylobacteriosis (BVC) which might be one cause among many of reproductive diseases in those herds where natural mating predominant along. Finding of this study may representative of status of BVC in similar ecological and managemental condition of other parts of Nepal.

MATERIALS AND METHODS

All together 56 vaginal mucous samples of cattle and buffaloes of semipastoral herds where natural breeding is predominant in western Chitwan were collected by purposive sampling method. Animal was restrained by using casting rope with applying a knot on hind legs. After cleaning vulval area, urine contamination was avoided by opening the vaginal cleft and placing the swab behind the external urethral opening. The sterile swab (15 cm) was introduced into the vagina up to the cervix. At this point the swab was turned and slightly pulled back and forth for few times to ensure full saturation. The swab stick with mucus was collected in an individual sterile screw test. Then sample was transported to lab within 2 hrs for inoculation against campylobacter media prepared by campylobacter agar base (Himedia-M994)

and a selective supplement Skirrow supplement (Himedia-FD008) in Veterinary Microbiology laboratory. Then those discs were incubated at 37°C and under microaerobic atmosphere of 5–10% oxygen, 5–10% carbon dioxide and preferably 5–9% hydrogen for optimal growth. Appropriate microaerobic condition was created by gas packs. Colonies of *C. fetus* were appeared on culture media after 2–3 days. To prevent overgrowth of specific colonies by contaminants, it was evaluated daily and suspicious colonies were sub-cultured. After 2–4 days of incubation, colonies measure 1–3 mm in diameter was identified. They were slightly grey-pink, round, convex, smooth and shiny, with a regular edge. Suspicious colonies was incubated for 8 hours with Bolton broth (Himedia-M1592) then observed by gram's staining. A thin, curved bacillus, 0.3–0.4 μm wide and 0.5–8.0 μm long were identified. Short forms (comma shaped), medium forms (S-shaped), and long forms (helical with several spirals) were observed simultaneously in the living state. Old cultures were coccoid bacteria. At 72 h, a representative of a dewdrop colony which was smooth, shiny and grey to pink in colour with organisms that were Gram-negative, vibroid in shape, oxidase- and catalase-positive was transferred to campylobacter media prepared by campylobacter agar base (Himedia-M994) and selective supplement Skirrow supplement (Himedia-FD008), streaked for purity and incubated under the same conditions as the sample described above. Differential Characteristics of campylobacter species was done according to BGC guideline (OIE, 2008).

Identification procedures after incubation for 2-5 days suspect *C fetus* colonies were 1-3 mm in diameters. They were slightly grey-pink, round, convex, smooth and shiny, with a regular edge. The organism was a gram negative, curved rod. Short forms (comma-shaped), medium forms (S-shaped) and long forms (spiral rod with several turns) may be observed simultaneously. Old cultures may contain *C fetus* as coccoid bacteria. Differentiation from other Campylobacter spp may be achieved by standard biotyping methods (Table 1). (OIE 2008)

Biochemical identification of Campylobacter species

Table-1: Biochemical identification of campylobacter species (OIE 2008)

	25 °C	**42 °C**	**Oxidase**	**Catalase**	**Nacl 3.5 %**	**Glycine 1%**	**H_2S (b)**	**Nalidixic Acid**
C. fetus Subsp. *Venearalis*	V	-	+	V	-	_	_	V
C. fetus Subsp. *Fetus*	+	V(a)	+	+	-	+	-	R

(a) = although *C, fetus* does not belongs to the thermophilic Campylobacters, a

considerable number of strains of this species grows at 42º C, V = Variable; R = Resistance; (+) = Positive growth; (-) = Negative Growth

Data entry, management and analysis were done using program Microsoft Office Excel 2007 and SPSS. The association between disease and various reproductive parameters were tested by using specific statistical test.

RESULTS AND DISCUSSION

out of 56 samples 10 samples were positive for bovine venereal campylobacteriosis (BVC), with prevalence of 17.8 % (Fig.- 1).

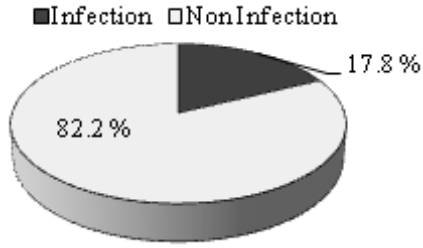

Fig.-1: True animal prevalence

Among positive, *C. fetus subsp. venerealis* (*Cfv)* and *C. fetus* subsp *fetus* (*Cff)* were found 70 % and 30 % respectively while biotyping, (Fig.- 2).

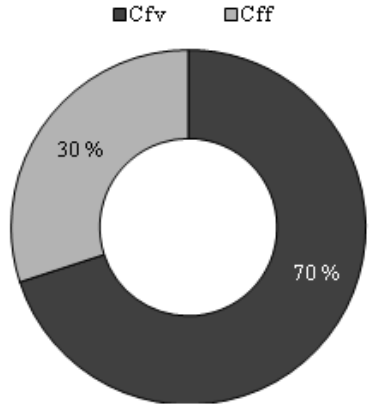

Fig.-2: Percentages of *Cfv* and *Cff* species

6 cattle have (21.42%) and 4 (14.28%) were positive. BVC infectivity of cattle was higher than buffaloes. The *Cfv and Cff* in cattle and buffaloes were 7 (12.5%) and 3 (5.35%) respectively.

True animal prevalence of BVC on this study was higher than the overall prevalence of BVC in cattle (16.4 %) in three states of northern Nigeria (Mai *et al.*, 2013). It may be due to less number of sample size in present study, though previous study was concentrated for cattle bull. In Nigeria, prevalence of 2.9-11% was reported and herd-level prevalences were 20-22% (Mai *et al.,* 2013).

Isolation rates were 2.2% for *C. fetus subsp. venerealis* and 1.5% *for C. fetus subsp fetus* from six bulls and four cows (Mshelia *et.al.,* 2014) where as in this study the prevalence of *Cfv* species were 12.5% and the prevalence of *Cff* species were 5.35%, specieswise prevalence were higher than previous study it may due be small sample size of this study. Herd and within-herd prevalence rates for *C. fetus subsppsveneralis* and *C. fetus* subsp *fetus* were 22.2% and 3.4%, respectively, while the overall active infectivity rate was 3.7% in the Lake Chad basin of Nigeria (Mshelia *et al.,* 2010) which were lower than this study, it might be due to comparatively poor herd health managemental practice than the previous study area.

Seven BVC infected animals (cattle and buffaloes) had increased calving interval (ICI) out of ten infected, rests were normal calving interval (CI). Out of forty six non-infected eight non infected animals (cattle and buffaloes) showed ICI out of forty six non-infected, rests had normal calving interval. It was analyzed by Yate's correction of Chi-Square test. Statistically found that increased calving interval (ICI) was associated with infection of BVC at 5% level of significance. Six infected animals out of ten infected had repeat breeding condition (RBC) rests had normal calving interval. Out of forty six non-infected nine animals had RBC and rests were not. It was analyzed by Yate's correction of Chi-Square test. Statistically it was found that repeat breeding condition was associated with infection of BVC at 5% level of significance.

Out of ten BVC infected five animals had BCS $\leq$ 2.75, rests had BCS >2.75. Out of forty six non-infected six animals had low BCS $\leq$ 2.75 and rests had BCS >2.75. By Yate's correction of Chi-Square test low Body Score Condition (BCS $\leq$ 2.75) was associated with BVC infection at 5% level of significance.

Adequate concentrate, nutritional supplement along with good quality of forage and fodder is needed to maintain appropriate Body condition score as in low BCS (BCS $\leq$2.75) chances of negative energy balance which is unfavorable for development of immunity power against disease **(**Hassan**,** 2015).

CONCLUSION

Bovine Venereal Campylobacteriosis or Bovine Genital Campylobacteriosis was prevalent in semipastoral herd with predominant of natural mating, *Campylobacter*

fetus subsps. *venaralis* and *Campylobacter fetus* subsps *fetus* were found major aetiological agents. There was statistical association between BVC, increased calving interval, repeat breeding condition and low Body score condition.

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Seroprevalance of *Chlamydophila abortus* in Aborted and Infertile Dairy Cattle in Chitwan District, Nepal

P. Shrestha, D.Shrestha and M. Prajapati

Animal Health Research Division, NARC

ABSTRACT

Chlamydiae are obligate intra-cellular gram-negative bacteria that cause different diseases in animals and humans. In cattle, chlamydial infections can cause abortion, polyarthritis, encephalomyelitis, keratoconjunctivitis, pneumonia, enteritis, hepatitis, vaginitis and endometritis, infertility and chronic mastitis. The aim of the study was to determine the seroprevalance of Chlamydophila abortus in aborted and infertile cattle in the Chitwan district of Nepal. A total of 92 serum samples were collected from 92 cattle with reproductive problem. Sera were tested with a commercially available ready-to-use kits ID screen® Chlamydophila abortus indirect multi-species ELISA kit (France). In this study, 7.6% (7/92) of dairy cattle with reproductive failure were positive for antibodies specific to C. abortus. This study has indicated that the agent is present in the environment and could pose a threat to public health. People living in close proximity to dairy cattle may be exposed to C. abortus. Chlamydiosis should be considered as a possible threat in humans. Future studies should include a simultaneous assessment of dairy cattle and associated human serology.

INTRODUCTION

Abortion and infertility has been a serious economic problem in domesticated ruminants in Nepal. Several bacterial, viral, parasitic, and nutritional factors cause reproductive problem including abortion and infertility in animals. *Chlamydophila abortus* may be one of the pathogens causing abortion and infertility in cattle. It causes epizootic bovine abortion in cattle. In cattle, acute infection with *Chlamydophila* species has been associated with a wide range of diseases including pneumonia, polyarthritis, polyserositis, conjunctivitis, encephalomyelitis, mastitis, and reproductive disorders including endometritis, salpingitis, vaginitis, abortion, repeat breeding, seminal vesiculitis, and epididymitis (Kaltenboeck *et al.*, 2005; Kauffold *et al.,* 2007; Yaeger and Holler 2007). Abortion has been a serious economic problem in domesticated ruminants worldwide. Several bacterial, viral, parasitic, and nutritional factors cause abortion in animals (Szymanska-Czerwinska *et al.,* 2013; Hartwig *et al.,* 1996; Kuruif 1999; Nietfeld 2001). *Chlamydophila (C) abortus* is one of these abortifacient pathogens in sheep and cattle It causes ovine enzootic abortion in sheep and epizootic

bovine abortion in cattle (Szymanska-Czerwinska *et al.,* 2013; Nietfeld 2001). *Chlamydophila abortus* is a Gram-negative, intracellular bacterium that was formerly known as *Chlamydia psittaci* serotype 1 (Da Silva *et al.,* 2006). It is considered as one of the most economically important animal pathogens of domesticated animals, which causes abortion, weak neonates and foetal loss, infertility and mastitis in sheep, goats, and cattle in many countries around the world (Szymanska-Czerwinska *et al.,* 2013; Nietfeld, 2001; Kaltenboeck *et al.,* 2005). The bacterium is also a zoonotic agent that causes abortion and other clinical symptoms in humans. The zoonotic potential of *C. abortus* is well known and poses a threat to mainly pregnant women, handling sheep and goats (Szymanska-Czerwinska *et al.,* 2013).

MATERIALS AND METHODS

Collection of Samples

Samples of dairy cattle were collected from information given by district livestock service office (DLSO) of Chitwan district. A total of 92 blood samples were collected randomly with the history of reproductive disorders such as abortion, repeat breeding and anestrus. A questionnaire format was used to record data including the name of the owner (villages) , species, breed, sex, estimated age, lactating or not, and health condition. The blood samples were collected from the jugular vein of animals; separated serum was transferred to eppendorf of tube and was transported on ice to the Microbiology laboratory of the Animal Health Research Division. All serum samples were stored at −20◦C until they were tested for antibodies at a later time.

Serological test

The commercial *IDscreen® Chlamydophila abortus* indirect multi-species ELISA kit.(IDvet, France) was used. The plates were read at 450 nm with an ELISA reader (Thermo scientific) according to the manufacturer's recommendation. The sensitivity and specificity of the test are 95 % and 100 %, respectively. To calculate the results, the optical densities (ODs) of samples were analyzed in relation to the negative and the positive controls with the following formula: The sera were considered to be ELISA-positive if they had a value of 60% or more, were suspected to be doubtful if the value was between 50% and 60% and if value was less than 50% considered negative.

$$S/P(\%) = \frac{ODsample}{ODpos} x100$$

Results and Discussion

In the present study, 92 dairy cattle with reproductive problems were examined. The specific antibodies against *C. abortus* were found in 7.6% of cattle herds examined. Seroprevalence of *C. abortus* in cattle was 7.6% in Chitwan districts. Several studies reported considerable variation in the seroprevalence of chlamydial infection in cattle. In Taiwan, Out of the 672 healthy and 63 aborted Holstein cows from 72 herds the prevalence of *C. abortus* antibodies were 51.3 % and 71.4 %, respectively (Wang *et al.,* 2001). In Poland, Niemczuk (2005) reported 19.3 % (1,333 tested) of bovine sera positive for *C. psittaci* and *C. abortus* in both complement fixation test and ELISA. The prevalence of antibodies specific to *C. abortus* in 26 herds and 192 cases of abortion in different breeds and ages of dairy cattle in Turkey was 27 % and 8.3 %, respectively (Gokce *et al.,* 2007). In Nepal, there are limited reports on abortion caused by *C. abortus*. Detection of antibodies against *C. abortus* is due to natural infection, since vaccination is not practiced in Nepal. Infected females shed vast numbers of infective *C. abortus* organisms at the time of abortion or parturition, particularly in the placenta and uterine discharges. The organisms are also shed in milk, faeces, and nasal and ocular discharges of aborting animals. *C. abortus* can also be shed in semen and transmitted to cows, possibly leading to embryonic death or infertility. Furthermore, wild animals may also serve as reservoir for this organism and play a role in the contamination of the environment and spread ofthe disease. The result of the present study indicates that *C. abortus* may causes abortion in dairy cows .It is well-known that detection of infected and carrier animals will allow appropriate control measures to be taken to reduce environmental contamination, thus limiting the spread of infection, financial losses, and the possible risk of zoonotic transmission to humans. Therefore, it is recommended that a reliable serological test should be applied regularly to flocks and herds, and seropositive animals should have proper place to eliminate.

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Demonstration of antibodies against *Mycoplasma mycoides* subsp. *mycoides* Small Colony Type (MmmSC) among cattle of Mid and Far-Western region of Nepal

T. Uprety[1], D.R.Khanal[2] and A. Paudel[1]

1. Institute of Agriculture and Animal Science, Rampur, Chitwan.

2. Animal Health Research Division, Khumaltar.

ABSTRACT

A cross-sectional study was carried out to demonstrate antibodies against Mycoplasma mycoides subsp. *mycoides Small Colony Type (MmmSC) with an aim to study the risk factor associated with MmmSC among cattle of mid and far-western region of Nepal. A total of 160 serum samples were collected from unvaccinated cattle (against MmmSC) of Banke, Bardiya and Kailali districts. Samples were tested for MmmSC antibody using IDEXX Contagious Bovine Pleuropneumonia (CBPP) competitive ELISA (c-ELISA) test kit. Questionnaire survey was also carried out to get the information on age, sex, management status, parity and body condition of sampled cattle. Out of 160 samples, 37 tested positive for antibodies against MmmSC. Overall seroprevalence of CBPP was 23.125% (16.59%-29.66%) and true prevalence was 24% (17.38%-30.62%).The district wise prevalence was 64.86% for Banke, 27.03% for Kailali and 8.11% for Bardiya. There was significant association ($p<0.002$) between the disease and management status.*

Key Words: *Mycoplasma mycoides* subsp. *mycoides* Small Colony (MmmSC), CBPP, c-ELISA, Banke, Bardiya, Kailali

INTRODUCTION

Mycoplasma mycoides subsp. *mycoides* Small Colony (SC) type (MmmSC) is a Gram negative bacteria lacking cell wall and surrounded by plasma membrane only. MmmSC has been renamed as *Mycoplasma mycoides* subsp. *mycoides* (Mmm) and is the causative agent of Contagious Bovine Pleuropneumonia (CBPP) in cattle. CBPP is a notifiable disease and is only bacterial disease in the list A of communicable animal disease of Office International des Epizooties (OIE. 2015). CBPP is a trans-boundary disease. It has remained in endemic forms in different parts of Africa and has been reported from India, Bangladesh and Myanmar (Nicholas, 2004). The

situation in some Asian countries is unclear (OIE-Terrestrial Manual 2014). Latest report of OIE on CBPP has kept Nepal without official Status (OIE, 2015). CBPP is a disease with major impact on livestock production and potential for rapid spread. CBPP infected countries are excluded from international trade. CBPP is a disease of economic significance due to compromised food security through loss of protein and draft power, reduced output, increased production cost due to cost of disease control, disruption of livestock/product trade; inhibit sustained production in livestock, pain and suffering to animal (Paskin, 2003). CBPP causes high morbidity and mortality. It was once the most damaging infectious animal disease in China, second only to Rinderpest (Xin *et al*., 2012). According to Terrestrial Animal Health Code (2009), the most effective methods of CBPP surveillance is an enforced meat inspection program in the abattoirs followed by laboratory examinations of suspicious cattle, since cattle is not used for meat purpose and is prohibited by religion and law, serological surveillance of CBPP is found to be more suitable in Nepal. Serodiagnosis plays an important role in CBPP control strategy (Bruderer *et al*., 2002). Infected animals often persist as carrier and serodiagnosis can help in controlling the spread of disease. Mid and Far western region of Nepal have porous border with India and most of the cattle population are imported from India.

Agriculture is the back bone of Nepalese economy providing livelihood for more than 60.4% of the population. Agriculture has a share of 31.23% in total GDP (Gross Domestic Product). With a share of 25.68% in Agriculture GDP, livestock sector is an important sector of Nepalese economy. With a cattle population of 7.2 million (APSD, 2015), CBPP spread could potentially jeopardize the dairy sector. With cattle reared almost for milk purpose and annual milk deficit of 300,000 liter, there is a potential of cattle number to boom. CBPP could potentially affect the cattle population and National economy as a whole. In Annual Epidemiological Bulletin (2014), CBPP is not enlisted in the reported disease list. The total number of domestic animal infected with respiratory diseases (pneumonia, respiratory disease-unclassified and respiratory signs excluding Hemorrhagic septicemia) was 0.3 million, with death of 11.2 thousand animals. The report also showed that no animals were vaccinated against CBPP. Shrestha *et al*., (1994) had stated that CBPP is a disease of potential significance for Nepal due to its endemicity in some parts of Indian sub-continent (the possibility of its existence in the country cannot be ruled out) and stressed for its identification and study of epidemiological pattern. So this study was conducted with an aim of identifying CBPP prevalence in Banke, Bardiya and Kailali.

METHODOLOGY

A cross-sectional study along with questionnaire survey was carried out to know the age, breed, and management status of the sampled cattle. Study was conducted in samples from Banke, Bardiya and Kailali districts of Mid and Far-Western region of Nepal. These sites were selected as these districts are the areas bordering Nepal to India and cattle are usually imported from India via porous borders. At Banke, samples were collected from Chisapani, Raniyapur, Sitapur, Bageshowri, Samshergunj, Khajura and Kohalpur VDCs where as in Bardiya samples were collected from, Belawa, Kalika, Khaurapur, Motipur and Rajapur VDCs. At Kailali samples were collected from Geta VDC, Attariya Municipality, Dhangadhi Municipality, Phullwari VDC and Chaumala VDC.

Sample size was determined using EpiTool (2015). The sensitivity of the test kit was 95.4% and specificity of the test kit was 99.7%. The total cattle population in these 3 districts was 0.4 million (APSD, 2015). Sample size was calculated at a confidence interval of 95% and precision of 5%. The calculated sample size was 171; while actual sampled size was160. The targeted study population comprised of cattle of all ages. The status of CBPP in Banke, Bardiya and Kailali region is unknown with no CBPP vaccination programs.

Sampling was done during April-May of 2015. Ten ml of blood sample was collected from the jugular vein, maintaining an aseptic condition, using sterile syringe and blood was immediately transferred to sterile clot activator tubes. The samples were kept under the cool place in a slant position for four hours and centrifuged. The sera sample were transferred to serum tubes and kept at -20^0C at District Livestock Service Office of respective districts. Those samples were brought to Animal Health Research Division (AHRD) for laboratory analysis. IDEXX CBPP antibody test kit was used for screening of sera sample. The test kit is an enzyme immunoassay for the detection of antibodies directed against MmmSC in individual bovine serum samples and is a c-ELISA test kit for antibody detection directed against MmmSC based on monoclonal anti-MmmSC antibody named as Mab 117/5. "WinEpi" was used to find apparent prevalence and true prevalence. Apparent prevalence is the proportion of animals from a representative sample of the population that are positive to the diagnostic method used and true prevalence is proportion of animals that present the event of interest in the population at a given point in time.

True prevalence (T.P) was calculated as:

True Prevalence (T.P) = (A.P+Sρ-1) / (Sρ+Sε-1) where;

A. P.=Apparent prevalence (number of samples positive for MmmSC antibody divided by the total number of samples)

Sρ= Specificity of the test kit (in decimal)

Sε= Sensitivity of the test kit (in decimal)

RESULTS AND DISCUSSION

Out of 160 sera samples tested, 37 sera samples were positive for CBPP. The overall apparent seroprevalence was 23.125% (16.59%-29.66%) and true prevalence was 24% (17.38%-30.62%). The highest seroprevalence was recorded at Banke district (64.86%) followed by Kailali (27.03%) and Bardiya (8.11%). Based on the managemental status, seroprevalence was highest in stall feeding (67.57%) followed by semi-stall feeding (29.73%) and grazing system (2.7%). Based on breed, the seroprevalence was highest in exotic breeds (56.76%) followed by indigenous breeds (35.14%) and non-descript cattle (8.10%). Age wise highest seroprevalence of CBPP was found in growing cattle aged between 3 to 5 years (43.24%) followed by adults aged above 5 years (37.84%) and in calves up to 2 years of age (5.41%). In none descript cattle seroprevalence was found to be 13.51%. It was noticed that CBPP was present in comparatively higher (56.77%) in cattle with poor body condition than cattle with good body condition (43.23%). Chi square analysis (χ^2) revealed only management status was significantly associated ($p< 0.002$) with disease condition.

CBPP was present at very low percentage in calves (5.41%) when compared to other age groups. CBPP is postulated as disease of older cattle with calves mostly showing mild clinical signs and disease is manifested as arthritis and no respiratory involvement occurs (Blod *et al.*, OIE, 2015). No satisfying explanations have been put forth as why CBPP is less prevalent in calves than in older cattle and some researchers have even postulated that there is no significant difference in the manifestation of the disease in both young and older cattle (Gedlu., 2004). CBPP was more prevalent in young cattle aged 3 to 5 year (43.24%). In our finding, CBPP is more prevalent in exotic (either pure breeds or cross) breed (56.76%) than indigenous breed (35.14%). One possible reason for this variation is difference in strains and their affinity to certain breeds. Hypervirulent and velogenic strains like strain V5 were obtained from Australian, African and European outbreaks of CBPP (March *et al.*, 2000). There was a big difference in seroprevalence between Banke district and Kailali district.

Soromou *et al.,* (2014) reported a similar difference in prevalence between Banko and Dogomet region (40%) and Bissikrima (6.25%) Upper Guinea and explained that this variation in seroprevalence with region could be due to variation in cattle population densities in those regions. This high prevalence could have also resulted as large number of samples belonged to single cattle farm while from Kailali and Bardiya, samples were mostly collected from different farms. Apart from this, herd level prevalence (number of herds containing cattle positive for CBPP) is a more accurate indicator of prevalence of CBPP than individual seroprevalence (number of animals positive out of total number of sampled animals).

The main susceptibility factor of acquiring this disease is species, with cattle and buffalo are only species affected under natural condition. Breed susceptibility is another important factor. *Bos taurus* and *Bos indicus* are equally susceptible to CBPP infection (Masiga *et al*., 1996). Imported cattle of European origin are more susceptible than Zebu cattle. Exotic cattle are more susceptible than indigenous cattle. Animals recovered from disease are more resistant to further challenges. Cattle movement are responsible for transmission of CBPP from one herd region or country to another and thus type of husbandry employed plays an important role in transmission of disease. Disease is less prevalent in dry climate than in humid one (Masiga *et al*, 1996, Blod *et al*., 2007).

MmmSC may spread over a longer distance (50-200meter) if the climatic condition is favorable (Masiga *et al*., as cited in Alemayehu *et al*., 2015). MmmSC can also occur in saliva, urine (but spread of infection through urine is not fully confirmed), fetal membranes, and uterine discharges with possibility of transplacental transmission (Merck Veterinary Manual, 2010). Mortality rate in naive herd experiencing disease for first time may reach up to 60% or higher (Huebschle *et al*., as cited in Schieck *et al*., 2014).

Despite their known limitation, serological methods are still the first choice for herd diagnosis of CBPP with c-ELISA being listed as official methods in OIE manual along with Complement Fixation Test (CFT) (Schubert *et al*., 2011). The basic requirements to control CBPP are highly efficient diagnostics tests that detect MmmSC not only in symptomatic animals but also in asymptomatic carriers together with vaccination strategies (Thiaucourt *et al*., 2002 as cited in Pilo *et al*., 2007). The ideal method to control a trans-boundary disease like CBPP is the application of the stamping out policy of complete elimination of infected and exposed animals along with attendant

zoo-sanitary measures. Control of cattle movements is the most efficient way of limiting the spread of CBPP (OIE, 2015). It is believed that vaccination alone with currently available vaccines (T1/44 and T1sr vaccine) is unlikely to eradicate the disease (Nicholas, 2004). The most promising intervention scenario combines the vaccination of healthy animals with treatment of clinical cases but further work is needed to select the most appropriate antibiotics treatment regime (Xin *et al*., 2012).

CONCLUSION

Based on our finding, we can draw an inference that CBPP is prevalent in three districts of Mid and Far-Western region of Nepal. A poor health status of cattle is also another reason that could be linked with risk of acquiring disease. Management status is significantly related with the disease and cattle movement may have a role in disease spread in new herds and new areas. Detection of antibodies against CBPP, in the absence of vaccination practice in the country, is indicating the prevalence of CBPP in cattle of Banke, Bardiya and Kailali in cattle maintained in different managemental conditions and there is a potency of spread in new areas.

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A study on cattle tick and tick borne pathogens of Mid Western Nepal

T. P. Bohara[1] and S. P. Shrestha[2]
1. Central Veterinary Laboratory, Kathmandu
2. Animal Health Research Division, NARC, Lalitpur

ABSTRACT

Tick and tick-borne diseases are responsible for severe economic losses in dairy cattle enterprise due to incurring cost for parasite control along with compromised fertility, body weight and milk production. There are very limited importances on tick types and tick borne diseases in western Nepal. This study was designed to determine the tick types and tick borne pathogens in dairy cattle of Banke and Surkhet districts, western Nepal. A total of 155 tick samples and blood smears of dairy cattle were collected from May, 2013 to February 2014. Rhipicephalus (Boophilus) microplus (96.8%) was the most abundant tick followed by Haemophysalis spp.(1.9%), Ixodes spp.(0.6%) and Amblyomma spp.(0.6%. Higher abundance of R. microplus was noted in Surkhet as compared to Banke (Fisher's exact test, P<0.05). Exploration for tick borne pathogens revealed overall positivity of 6.4% (10/155). However, Anaplasma marginale (5.8%) was more in comparison to Babesia bovis (0.6%). Likewise, frequency of tick borne infections were significantly higher during summer season (Fisher's exact test p<0.05). Highest tick infestation site in cattle were dewlap (38.7%) followed by perineum and udder (23.87%), ears base (14.19 %), tail base (9.03%), abdomen (7.09%) and withers (4.51%). Higher prevalence of R. microplus indicates the possibility of blood protozoan diseases like Anaplasmosis, Babesiosis and Theilariasis. Moreover, currently prevalent economically important disease like Anaplasmosis needs to be controlled using appropriate measures in Banke and Surkhet districts of western Nepal.

***Key Words**:* Tick, Cattle, Tick borne infections, Nepal.

INTRODUCTION

Ticks are recognized as important vectors of blood protozon diseases in livestocks. Out of 867 tick species recognized globally, 10 percent of them act as the vectors

of pathogens of domesticated animals and human beings (Jongejan and Uilenberg, 2004). They possess attributes like attaching firmly, sucking blood slowly and remaining unnoticed for long period of time in their host accounting for their vector potential. Climatic factors, particularly temperature, are considered to be important determinant for tick propagation. Cattle ticks are responsible for severe economic losses in both dairy and beef cattle enterprises in the tropics (Jonsson, 2006). The major economic impacts of ticks infestation in cattle enterprise are the costs involved in parasite control along with losses in fertility, body weight and milk production. Moreover, tick-borne diseases are the problems of cattle and other livestock in Africa, Asia and Latin America (Jongejan and Uilenberg, 2004). Dairy cattle are recognized as a means of livelihood of framers in Nepal and ticks and tick borne diseases are to be explored in mid western Nepal. The objective of the present study was to study the tick types and tick borne diseases in native, crossbred and exotic cattle of Banke and Surkhet districts. Assessment of tick composition and identification of more frequent tick borne infections will assist the animal health workers and policy makers to implement relevant control strategies and programs in the study area.

MATERIALS AND METHODS

A total of 155 (N=155) indigenous, exotic and crossbred dairy cattle of the peri-urban milk production pocket area of Nepalgunj (Banke) and Birendranagar (Surkhet) of mid western Nepal were included in the study. Thin blood smears were prepared from blood collected from the ear tips, were air-dried, fixed with methanol, stained with Giemsa stain and examined under oil immersion objective of microscope. Ticks were collected manually and stored in container containing 70% ethanol with 5% glycerine and identified following the guidelines of Walker *et al.*, (2003) and MAFF (1986). Distribution of ticks and tick borne diseases by district, season and breed of animal was compared using Fisher's exact test. Fisher's exact test was calculated using IBM SPSS Statistics Version 20.

RESULTS AND DISCUSSION

The prevalence of ticks in Banke and Surkhet districts of mid Western Developmet Region is presented in Table-1 and the prevalence of Blood Protozon diseases in Banke and Surkhet is presented in Table-2.

Table-1: Identified ticks in cattle of Banke and Surkhet districts of mid western region.

Factor Variable		**No. of Cattle affected with *Rhipicephalus (Boophilus) microplus* (%) N=155**	**No. of Cattle affected with *Haemophysalis sp.* (%) N=155**	**No. of Cattle affected with I*xodes sp.* (%) N=155**	**No. of Cattle affected with *Amblyomma sp.* (%) N=155**
District	Banke	57 (36.77%)	3 (1.9%)	1 (0.64%)	1 (0.64%)
	Surkhet	93 (60%)	0	0	0
Season	Summer	86 (55.48%)	3 (1.9%)	1 (0.64%)	1 (0.64%)
	Winter	64 (41.29%)	0	0	0
Body part	Perineum and Udder	37 (23.87%)	0	0	0
	Abdomen	11 (7.09%)	1 (0.64%)	0	0
	Withers	7 (4.51%)	0	0	0
	Dewlap	60 (38.70%)	2 (1.29%)	1 (0.64%)	1 (0.64%)
	Tail base	14 (9.03%)	0	0	0
	Ear	22 (14.19%)	1 (0.64%)	0	0
Total		**150 (96.77%)**	**3 (1.9%)**	**1 (0.64%)**	**1 (0.64%)**

Table-2: Prevalence of Blood Protozon diseasesin Banke and Sukhet districts of mid western region.

Factor Variable		**No. of Cattle affected with *Anaplasma marginale* (%) N=155**	**No. of Cattle affected with *Babesia bovis* (%) N=155**	**Significance level**
District	Banke	3 (1.9%)	1 (0.64%)	Fisher's exact test, p=0.69
	Surkhet	6 (3.8%)	0	
Season	Summer	9 (5.8%)	1 (0.64%)	Fisher's exact test, p=0.011
	Winter	0	0	
Total		**9 (5.8%)**	**1 (0.64%)**	

Rhipicephalus (Boophilus) microplus (96.8%) was the most abundant tick in the study area (Table-1). It is also the most important one host tick becoming most numerous on cattle originated from South-East Asia and spread throughout the tropics (Jongejan and Uilenberg, 2004, Ghosh *et al.,* 2007). Highest proportion of *R. microplus* (60%) was recorded among cattle in Surkhet (700 m from sea level) compared to that in Banke (165 m from sea level) (Fisher's exact test, $P<0.05$) This might be due to the global warming, as reported in spread and colonization of new territories by *R.*

microplus in Africa and increase in altitude of this tick in the mountainous regions of America (Estrada-Pena and Salman, 2013).

However**,** no significant difference in prevalence of tick infestation in different season was noted. Examination of various body parts to rank the predilection sites of tick cattle revealed that dewlap (38.7%) followed by perineum and udder (23.87%), ears base (14.19 %), tail base (9.03%), abdomen (7.09%) and withers (4.51%) were the major sites for ticks (Table-1). The findings are in the agreement with Atif *et al,.* (2012) who observed perineum, udder and external genitalia (98%) as the most tick infested sites in cattle followed by dewlap, inner thighs, neck & back, tail, ears, around eyes, flanks and legs in Pakistan. Out of 155 blood samples in dairy cattles an overall rate of positivity of 5.8% (9) for *A. marginale and* 0.6% (1) for *Babesia bovis* was recorded in the present study (Table-2). The findings of Atif *et al.*, (2012) in Pakistan are also closer to the present finding in western Nepal. Global warming may influence the movement of the tick vectors and accordingly the distribution of Anaplasmosis (Jonsson and Reid, 2000). The frequency of tick borne infections were significantly higher during summer season as compared to winter (Fisher's exact test, $p<0.05$). This finding of western Nepal is in line with highest prevalence of cattle tick borne diseases in summer season in Pakistan (Atif *et al.,* 2012).

CONCLUSION

Rhipicephalus (Boophilus) microplus is the most abundant tick with higher abundance in Surkhet as compared to Banke. Tick borne pathogens revealed overall positivity of 6.4%. However, *Anaplasma marginale* was more in comparison to *Babesia bovis*. Likewise, frequency of tick borne infections was significantly higher during summer season. Highest tick infestation site in cattle were dewlap followed by perineum and udder, ears base, tail base, abdomen and withers. High proportion of vector tick R. microplus indicates the possibility of increase in the prevalence of economically important tick borne diseases like Babesiosis and Anaplasmosis escalating challenges to dairy enterprise of Western Nepal. Moreover, currently prevalent economically important disease like Anaplasmosis needs to be controlled using appropriate measures in Banke and Surkhet districts of western Nepal.

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Investigation on Diagnostic Indicators of Echinococcosis in Slaughtering Buffaloes of Chitwan District, Nepal

N. K. Yadav, I. P. Dhakal, D. K. Singh and M. P. Gupta
Institute of Agriculture and Animal Science, Rampur, Chitwan,

ABSTRACT

A study was conducted to assess the diagnostic indicators of echinococcosis in buffaloes slaughtered during December 2008 to June 2009 in Chitwan district of Nepal. One hundred and five buffaloes were randomly selected and antemortem and postmortem diagnostic indicators were evaluated. The ELISA (Enzyme Linked Immunosorbent Assay) testing of 50 cases was also performed. The mean PCV (Packed cell volume) of cystic cases was non-significant. Although the mean fibrinogen was significantly higher, the plasma protein and fibrinogen ratio was within the normal range. The mean eosinophil count in cystic cases was significantly ($p<0.05$) higher over control. Non-significant difference was observed in the other leukocytes (lymphocyte, monocyte, and neutrophil). The mean SGPT (Serum glutamate pyruvate transaminase) and SGOT (Serum glutamate oxaloacetate transaminase) values of cystic cases were significantly lower than that of control. The mean values of albumin, total plasma protein (TPP), glucose, indirect bilirubin in cystic cases were significantly higher. Non-significant difference was observed in total bilirubin, direct bilirubin, globulin creatinine and uric acid. Postmortem examination revealed the presence of hydatid cyst in the lungs only. The diameter of the cyst ranged from 3 to 13.5 cm and the weight of the cysts ranged from 0.05 to 1.5 kg. Biochemical examination of cystic fluid revealed the presence of glucose, SGOT, SGPT and ALP (alkaline phosphatase). Out of 19 cysts, 84.21% (16/19) were fertile and 15.78% (3/19) were sterile. Some of the cysts were caseated whereas some contained thick creamy pus. Cytological examination revealed the presence of protoscolices in the brood capsules that is pathognomonic to cystic echinococcosis. The histopathological examination revealed the presence of germinal layer and laminated layer that is also pathognomonic to cystic echinococcosis. The sensitivity and specificity of ELISA were found to be 84.21% and 93.55%, respectively. The prevalence of echinococcosis in slaughtered buffaloes was found to be 18.09%. The results of hospital survey showed the prevalence of 0.046% of cystic echinococcosis in human. The result of this study clearly indicates that prevalence of naturally occuring hydatid cyst infection remain relatively high in the study area. This study will support the scientific pursuit in managing echinococcosis.

INTRODUCTION

Echinococcosis was first described, in ancient times, by Hippocrates as 'cysts full of water' in a liver of a man, and by Aristotle in both human lungs and liver. The adult tapeworm is very small, usually consisting of only three proglottids and measuring 3 to 6 mm length and residing in the small intestine. Dogs are the usual definitive hosts whilst a large number of mammalian species can be intermediate hosts, including domestic ungulates and man. This disease represents a challenge of increasing concern in countries where control programs have been reduced or have not yet been implemented. Globally, the annual loss due to human hydatidosis has been estimated at US $200 million (Eckert *et al.*, 2002).

Echinococcosis is an existent problem of Nepal and was first reported when echinococal cysts were found in buffalo, goats, sheep and pigs slaughtered in Kathmandu. Shortly afterwards, preliminary study on human echinococcosis in Kathmandu indicated that there had been 47 hydatid cases of echinococcosis amongst the 30, 792 operations performed in the city's three hospitals between 1985 and 1990 of these 47 patients, 26 were male and 21 female and most had cysts in the liver (55%) or lungs (43%). There was no active screening or case finding procedure for Echinococcus infection in Nepal at that time; all 47 cases were found at a late stage or during surgery for other purposes. Ten of the cases were fatal (Joshi, 2007).

To date, and in spite of the significant progress achieved in the field of research and control, human and animals cystic echinococcosis caused by *E. granulosus* remains a considerable problem for public health and the livestock economy in developing countries. In continental control of *E. granulosus* is a very difficult and costly task that requires sustained efforts over many decades. To date, echinococcosis transmission has been successfully reduced or interrupted in some limited areas only, most notably on islands such as Iceland, Cyprus, New Zealand and Tasmania (Eckert, 2001). Echinococcosis is classified as category IV by the Infectious Diseases Control Law of Japan which requires notification of both human and canine cases of the diseases (Yasuyuki *et al.,* 2005).

The genus *Echinococcus* is of great importance because it contains a number of zoonotic species that can cause serious ill health in man. There are at least four species in the genus, but recent molecular evidence suggests that there should be a taxonomic revision to at least five species or even possibly six (Le *et al., 2002*; McManus, 2002; Thompson and McManus, 2002). There is also significant strain

variation in the species *E. granulosus*. The parasite is pathogenic and of economic significance in intermediate and aberrant intermediate hosts, where the larval parasite develops into a hydatid cyst. The genus is found throughout the world although a number of species have a limited geographical distribution.

In the intermediate host like buffalo, hydatid cysts have been found in a large variety of mammalian species and often grow slowly, sometimes taking several years to develop. Cysts most frequently affect the liver and lungs but they can also develop in other internal organs including the central nervous system. The cysts vary greatly in size and shape and may be present in large numbers in one organ. Upon postmortem examination, infiltration of the cysts can be seen in the lungs, liver, peritoneal cavity and other abdominal organs.

The definitive host canines are infected by eating the intermediate host organ that contains the hydatid cyst which contains the protoscolices which has the ability to grow into an adult worm. One small cyst may contain hundreds of protoscolices and one large cyst may contain tens of thousands of protoscolices. Following ingestion, the protoscolices develop into adult tapeworms which eventually produce eggs to complete the life cycle. Diagnosis of *E. granulosus* in the intermediate host is accomplished through necropsy examination of the animal and identifying the larval cyst in the organs, usually the liver or the lungs. Formalin fixed tissue positive on Periodic Acid Schiff (PAS) staining demonstrates a positive a cellular laminated layer with or without an internal cellular nucleated germinal membrane.

Cystic hydatid disease in humans can be a significant disease because of the mechanical and other adverse effects of the cyst. The tremendous reproductive potential of the tapeworm as well as the sheer size of the hydatid cyst can cause problems in the organs where ever they are lodged. If the cyst bursts, the resultant shock would probably be fatal. Meat is one of the most delicious and nutritious foods. In Nepal, the most commonly used meat is buff, mutton and chicken. Buffalo meat is very popular among the majority of the population and other ethnic hill groups. Due to their socio-economic conditions and culture the buffalo meat consumption is very high. The demand of buffalo meat is increasing day by day in restaurants and hotels in these days (Joshi *et al.*, 2003).

In this scenario, this study is aimed to detect the major clinicopathological findings of echinococcosis. This will be useful in revealing the prevalence of diseases in slaughtering buffalo and hence designing diagnostic tools to prevent entry of disease and other related risk factors into the food chain.

MATERIALS AND METHODS

105 buffaloes were randomly selected at four different slaughter slabs of Chitwan district. 15 ml of blood was taken before slaughter and serum was separated. The PCV (Packed cell volume), TPP (Total plsam protein) and fibrinogen were estimated by using refractometer. The blood smear was prepared and DLC (Differential leucocyte count) was done. The postmortem examination was done and lungs and livers were inspected with care. Biomertical analysis was done to determine the fertility status, size and volume of fluid in hydatid cyst. The centrifuged cystic fuid was stained with eosin 1-2% to determine the fertility. After the post-mortem examination, the tissue sample of size 1x1 cm^2 was collected in 10% buffer formalin for the histopathological examination (Chauhan and Agrawal, 2005). The histopathological slides were prepared by semiautomatic techniques and examined under high power microscope. The hydatid cyst was excised and germinal layer smear was prepared and examined under light microscope for presence of protoscolices (OIE, 2007). The biochemical paramerters (SGPT, SGOT, creatine, uric acid, albumin, glucose and bilirubin (direct and total) were analysed by using kit (Coral® India Ltd.). The ELISA (Remel® inc.) testing of 50 serum sample was also done.

A questionnaire survey was carried out from five hospitals of Kathmandu- T.U. Teaching Hospital (TUTH), Bir Hospital, Patan Hospital, Kathmandu Model Hospital, and Kanti children Hospital and one Hospital of Chitwan- College of Medical Sciences Teaching Hospital to find the prevalence of echinococcosis in human. The data were analyzed by using Excel-2007 and MSTATC. The statistical parameters like prevalence, sensitivity, specificity, predictive values (Akobeng, 2007) and relative risk were calculated by simple arithmetic procedure.

RESULTS AND DISCUSSION

Out of 105 buffaloes examined, 44 were male and 61 were females. Among female buffaloes, 19.67 % (12/61) were positive for hydatid cyst. Similarly, 15.9% (7/44) male were positive for hydatid cyst. The overall prevalence of echinococcosis was 18.09%. The statistical analysis of these factors (age, sex and breed) revealed the non-significant relation among these factors. It means that there is an equal probability of cystic infection in buffaloes of any age, sex and breed.

The mean PCV of cystic cases was non-significant. However, the mean fibrinogen was significantly higher ($p<0.05$) over control (Table-1). The mean eosinophil count in cystic cases was significantly ($p<0.05$) higher over control. However, non-significant

difference was observed in the remaining leukocytes. This finding is supported by Soulsby (2005) and Coles (1974).

Table-1: Mean±SE of hematological parameters in cystic infected and control buffalo

S.No.	Hematological parameters	Units	Control	Hydatid cyst positive
1	PCV	%	34.84±1.46	35.60±1.36
2	Fibrinogen	mg/dl	0.64±0.06[a]	0.32±0.04[b]
3	PP:Fibrinogen	Ratio	16.32±2.26	24.95±3.47
4	Lymphocyte	%	58.11±2.27	57.60±7.08
5	Neutrophil	%	17.74±2.36	26.80±6.03
6	Eosinophil	%	8.21±1.53 [a]	1.60±0.51 [b]
7	Monocyte	%	15.68±2.81	18.20±2.96

Note: Superscripts in the same row are significant ($p<0.05$).

The mean SGPT and SGOT values of cystic cases were significantly lower than that of control. The mean values of albumin, total plasma protein, glucose, indirect bilirubin in cystic cases were significantly higher than that of control. However, non-significant difference was observed in total bilirubin, direct bilirubin, globulin creatinine and uric acid (Table-2). The non-specific biochemical findings in cystic hydatid cases were reported by Radostitis *et al.* (2003).

Table-2: Mean±SE of biochemical parameters in cystic infected and control buffalo

S.No.	**Biochemical parameters**	**Units**	**Control**	**Hydatid cyst positive**
1	SGPT	U/L	13.11±1.71[b]	29.00±3.49[a]
2	SGOT	U/L	78.89±4.79 [b]	104.24±15.16 [a]
3	Direct Bilirubin	mg/dl	0.27±0.046	0.43±0.07
4	Indirect Bilirubin	mg/dl	0.67±0.17 [a]	0.24±0.06 [b]
5	Total Bilirubin	mg/dl	0.94 ±0.18	0.66±0.12
6	Albumin	mg/dl	6.16±0.41 [a]	3.93±0.67 [b]
7	Globulin	mg/dl	2.61±0.37	3.87±0.66

8	Total plasma protein	mg/dl	8.77±0.24 [a]	7.80±0.17 [b]
9	Glucose	mg/dl	102.62±11.95 [a]	59.61±7.41 [b]
10	Creatinine	mg/dl	1.42±0.13	1.74±0.09
11	Uric acid	mg/dl	5.56±0.55	6.50±0.42

Note: Superscripts in the same row are significant ($p<0.05$).

Postmortem examination revealed the presence of hydatid cyst in the lungs only. The diameter of the cyst ranged from 3 to 13.5 cm. and the weight of the cysts ranged from 0.05 to 1.5 kg. Similar biometrical values were reported by Gautam (2009) and Bajagain (2004).

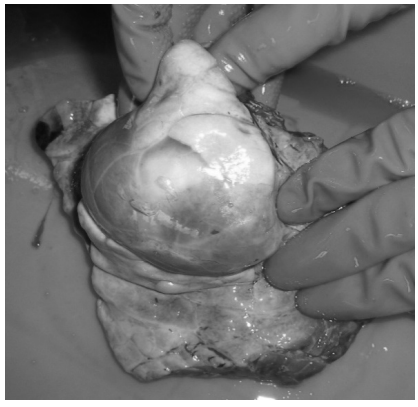

Fig: 1. Lobulated cyst

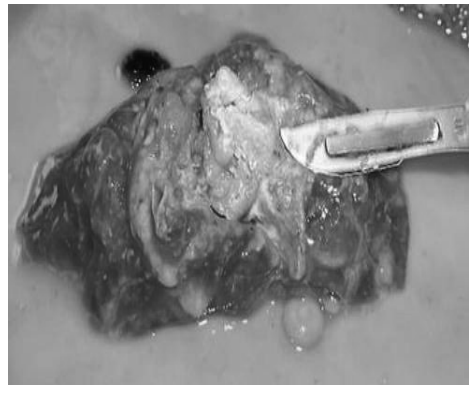

Fig: 2. Caseated cyst

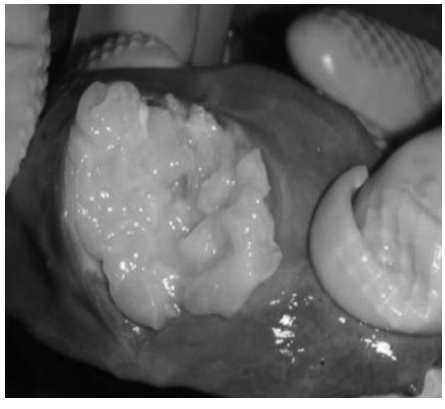

Fig: 3. Pus in the cyst

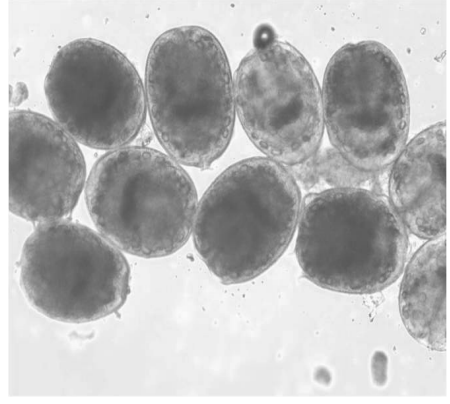

Fig: 4. Protoscolices in cytological examination

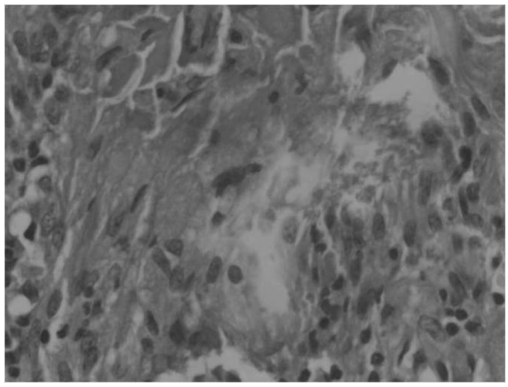

Fig: 6. Mononuclear cell infiltration

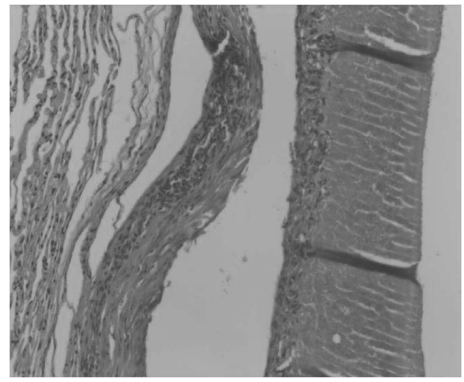

Fig: 6. mononuclear cell infiltration

Biochemical examination of cystic fluid revealed the presence of glucose, SGOT, SGPT and ALP. Out of 19 cysts, 84.21% (16/19) were fertile and 15.78% (3/19) were sterile. It could be hypothesized that high occurrence of fertile cysts in this study could be due to slaughtering of old aged buffaloes.

Some of the cysts were caseated (Fig. 2) whereas some contained thick creamy pus (Fig. 3). Cytopathological examination revealed the presence of protoscolices (Fig: 4) in the brood capsules that is pathognomonic to cystic echinococcosis.This finding is supported by Poole and Marcial-Rojas (1971). They reported the presence of brood capsules and daughter cysts suspended in the hydatid fluid. Cheesbrough (1999) reported the presence of both invaginated and exvaginated protoscolices in the cystc fluid. The histopathological examination revealed the presence of germinal layer and laminated layer (Fig. 5), which is also pathognomonic to cystic echinococcosis. Such specific histopathological findings were confirmed as cystic hydatid diseases as reported by Sun (1999). This finding is comparable to Nishant et *al*., 2005. They observed the germinal layer surrounded by a connective tissue capsule and a linear arrangement of fibroblasts around the cyst in the tissue section. They found large number of epithelioid cells and giant cells surrounded by a well developed granulation tissue infiltrated with mononuclear cells (Fig. 6) and eosinophils.

Table-3: Breakdown of ELISA test results with respect to carcass examination as gold standard

ELISA Test	Carcass examination positive	Carcass examination negative	Total
Positive	16 (TP)	2 (FP)	18
Negative	3 (FN)	29 (TN)	32
Total	19	31	50

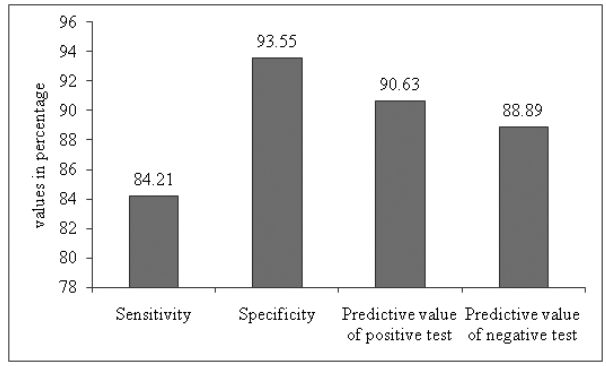

Fig.-7: Sensitivity, specificity and predictive values of ELISA (%)

The Sensitivity and specificity of the ELISA was found to be 84.21 and 93.55%, respectively (Fig.-7). The PPV of positive test indicates that this test will show false negative in 9.37% of the cases. Similarly, the PPV of negative test indicates that this test will show false positive reaction in 11.11% of the cases. Cystic patients were found at an overall rate of 0.46 per 1000 admission episodes involving 50.41% females and 49.59% males from data survey of 5 major hospitals of Kathmandu valley and one hospital of Chitwan district. Similarly, the TCC/1000 admission episodes of TUTH, Bir Hospital, Kanti Children Hospital, Patan Hospital, Kathmandu Model Hospital and CMS were reported as 0.74, 0.56, 0.10, 0.18, 1.32 and 0.07, respectively.

Hospital wise distribution of cystic patients revealed the maximum flow of cystic cases at TUTH, Kathmandu and the minimum cystic cases were operated at CMS Chitwan. This may be due to the selection of bias patients and availability of experienced surgeon at IOM.

CONCLUSION

The study revealed that echinococcosis was prevalent in both buffaloes and in human. Presence of hydatid in buffaloes, disposal of infected viscera in open places accessible to dogs and unawareness of the disease among people indicated threat for spread of infection. The MX buffaloes were found to be prone to cystic infection. Butchers/ abattoir workers and meat sellers were found responsible for the incidence of Hydatidosis

Early diagnosis of this parasite is indispensible to reduce the further infection. This study showed that some of the antemortem diagnostic indicators (fibrinogen, albumin, total plasma protein, glucose and indirect bilirubin) were significantly ($p<0.05$) different over the control. But, these significant findings were nonspecific.

Cytological examination acts as powerful tool for screening of cystic hydatid diseases. The ELISA could be used for screening test, but it has certain limitations.

Due to presence of illegal slaughtering, infected carcasses are marketed and consumed. It is recommended that the hygienic condition of slaughter house must be improved with standard guidelines of meat inspection. The meat inspection Act and Regulation should be followed strictly to avoid the hazards of such zoonotic diseases should be highlighted. Result of this study would assist in planning and implementing cost effective surveillance system, epidemiological studies, strategic control and thus support for eradication program.

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Prevalence of Brucellosis in Migratory Sheep and Goat of Taplejung and Lamjung Districts of Nepal

L. N. Das[1], S. Singh[1], U. M. Singh[2] and D. K. Singh[1]

1. Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan
2. Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur

ABSTRACT

With an objective to find out the seroprevalence of Brucella sps in migratory sheep and goat of Lamjung and Taplejung districts two tests i.e Indirect Enzyme linked immuno sorbent assay(iELISA) and Rose Bengal plate agglutination test(RBPT) were done. The questionnaire surveys were done to access the risk factors associated with the Brucellosis. Out of 385 blood samples 79 (20.51 %) samples showed positive by Indirect ELISA test and 47 (12.20 %) samples showed positive by RBPT. All the positive results showed by RBPT were also positive by Indirect ELISA test. Whereas all the positive samples by ELISA test were not positive by RBPT. The prevalence were found higher by both tests in sheep species than goat, higher in female sex than male, higher in 1-4 years age group than below 1 years & above 4 years and it was also higher in sheep and goats of Taplejung district than that of Lamjung district. Among 79 iELISA and 47 RBPT positive samples 48 (60.75 %) and 38 (80.85 %) samples were from the animals having history and symptoms like retention of placenta, abortion, orchitis, arthritis and fever respectively which were major symptoms of Brucellosis, (P<0.01). As per the questionnaires survey findings the possible risks factors which might be associated with the disease were lack of biosecurity measures, very rare or no access to Veterinary Service, lack of awareness, lack of vaccination, close contact between several flocks, lack of proper nutrition, high stocking density in the flock size and frequent contact with dogs, cattle and other animals in the flock

Key words: *Brucella sps*, iELISA, RBPT**,** caprine and ovine.

INTRODUCTION

Brucellosis is a highly contagious zoonotic disease characterized by recurrent abortions and infertility in a variety of animal species and undulant fever in man (Halle and Ajogi, 1997). Brucellosis has been reported in various parts of world affecting domestic animals and humans (Baba *et al*., 1998). Ovine and caprine brucellosis is a

significant problem for both public health and animal production in many parts of the world (Radostits *et al*., 1994). It is a major cause of economic losses in the livestock industry in terms of abortion, infertility, low conception rate and low survival rate of neonates (Enright, 1990; Halle and Ajogi, 1997; Ajogi and Akinwumi, 2001). Nutrition, genetic, management and infection are the common problems in livestock. Present livestock improvement approaches are basically centered on the introduction of exotic animals for cross breeding rather focused in animal health aspect. Abortion losses by infectious and non-infectious cause are considered as one of the major constraints in the livestock productions. This was the reason that overall production in ruminants is 12.1% (Karki and Ghimire, 2003). Among other constraints infection, abortion, less number of fetuses, stillbirth, and prolonged infertility in sheep and goat. As brucellosis has an endemic stability, it could be major cause of the significant economic losses (Joshi *et al*., 2005). Hence, it could be the great threat in future. Nepal as a member of WTO and OIE, need more attention on establishment of the disease information and epidemiological surveillance on zoonotic disease like brucellosis. The current study intends to link these gaps by engendering essential data regarding disease prevalence, its epidemiology, prevention strategies and by providing general imminent to an animal brucellosis.

MATERIALS AND METHODS

Site profile

This study was carried at Taplejung (E.D.R) and Lamjung (W.D.R) districts as a major research site of Nepal. The VDCs of Taplejung districts from where the samples were collected are Phawakhola, Baisakhe and Tringekhani. Likewise VDCs of Lamjung districts selected are Ghanpokhara and Ghalegaon.

Duration of the study

The research was conducted from June, 2013 to Janauary, 2014.

Sample size and techniques

A total of 385 blood samples were collected randomly from Migratory sheep and goat population. All samples were collected using adequate equipments and handled according to OIE requirements. The samples were carefully collected and packed, avoiding any possibility of leakage or cross contamination. Each sample was labeled by using codes describing the specific animal. Immediately collected samples were transported to laboratory and stored as recommended in the OIE manual (2009). Blood was packed in the cooler bag with ice packs and kept cool during transport

from the place of collection to laboratory. Serum was separated from clotted blood by centrifuging. Separated serum was collected in a screw caped sterilized plastic vial and was stored at -20^{o}C until testing.

Nature and source of data

Various sources and techniques were used for the collection of necessary information. Both primary and secondary sources of data were collected and analyzed.

Primary data

Farmers and veterinary technicians, veterinarians working in that area were the major sources of the primary data in the questionnaire section for the epidemiological study. With the help of the interview information such as average farm sizes, average household sizes, livestock holdings, livestock health, livestock reproduction, disease status, cases of abortion, placentitis and infertility, and farmer's view as potential solutions to these problems were recorded. Laboratory results of the blood samples of migratory sheep and goat were the sources of primary data in sero-screening of brucellosis section. Blood samples from the representative flock were collected and the sero-screening of brucellosis were performed.

Secondary data

Secondary data were collected from the publications of Department of Livestock Services (DLS), Directorate of Animal Health of DLS, Nepal Agricultural Research Council (NARC), Institute of Agriculture and Animal Sciences (IAAS), Zoonoses Control Project (ZCP), National Zoonoses and Food Hygiene Research Center (NZFHR), District Livestock Services Office (DLSOs), Central Bureau of Statistics (CBS),

Data analysis

The primary and secondary information collected from the field survey and laboratory analysis were coded, tabulated and analyzed statistically and graphically by simple descriptive statistics such as frequencies, percentage, mean and standard deviation by using Statistical Package for Social Science (SPSS.Ver.16.0), PhStat2.Ver.3.04 (Pearson Education, 2011) and Microsoft Office Excel 2007.

Chi square test compares the actual observed frequencies in the sample with the expected frequencies if there were no relationship between the variables. This test is

based on the assumption that the sample frequencies are normally distributed around the expected value, and for it to be true the expected frequencies of all cells of the table should be large (preferably ≥ 5) and no cell in the table should have a frequency of zero (Dhand *et al.,* 2005; Rangaswamy, 1995). In this study all variables have expected frequency less than 5.

RESULTS AND DISCUSSIONS

Out of 385 blood samples 79 samples showed positive Results by Indirect ELISA test showing the overall prevalence of 20.51%.

Out of 246 samples of sheep 55 (22.35 %) samples were positive by iELISA. Similarly 24 (17.26 %) samples of goat out of 139 samples were positive results by iELISA.

Table-1: ELISA test and RBPT in different species group

Species	**ELISA**		**RBPT**	
	Positive	**Total**	**Positive**	**Total**
Sheep	55 (22.35 %)	246	34 (13.82 %)	246
Goat	24 (17.26 %)	139	13 (9.35 %)	139
Total	79	3	47	385

Similarly, out of 246 samples of sheep 34 (13.82 %) samples were positive by RBPT. Likewise 13 (9.35 %) samples of goat out of 139 samples were positive by RBPT. For both the tests with the species, the obtained statistical p-Value is greater than 0.05, hence there is no significance with the either of the tests with the species group.

The study conducted by Maher *et al*, 2010 showed the prevalence of *B. melitensis* infection (tested by RBPT) in 13.85 % sheep and 17.68 % goats. The present study is further supported by a study conducted by Shrestha *et al*, 2008 showing the prevalence rate of 17.14% in goats.

Similarly the study conducted by Joshi *et al.,* 2005 showed the sero-prevalence of brucellosis in small ruminants of Nepal revealed 29.26% (12 out of 41) goats from Makwanpur, 5.12% (2 out of 39) from Chitwan and 5.4% (2 out of 37) from Kathmandu. In the same study 14.28% (2 out of 14) sheep serum from Chitwan showed the positive results for brucellosis.

Altogether 20 (16.94 %) male animals out of 118 samples of male sheep and goat showed positive for iELISA. Similarly out of 267 female 59 (22.09 %) samples were found positive in iELISA.

Table-2: ELISA test and RBPT in different sex group

Sex	ELISA		RBPT	
	Positive	**Total**	**Positive**	**Total**
Male	20 (16.94 %)	118	8 (6.77 %)	118
Female	59 (22.09 %)	267	39 (14.60 %)	267
Total	79	385	47	385

Altogether 8 (6.77 %) male animals out of 118 samples of male sheep and goat showed positive for RBPT. Similarly out of 267 female 39 samples (14.60 %) were found positive in RBPT.

In the sex group, the obtained statistical p-Value is greater than 0.05 in iELISA tests while p-Value is less than 0.05 in RBPT.

It had been reported that males are usually more resistant than female (Seifert, 1996). Different factors are probably involved in the variation of sex susceptibility including physiological and behavioral differences between males and females. There is preferential growth of the *B. sps* in the gravid uterus. On the other hand, some infected male in the testes are non-reactors or only had low antibody titers. Another factor that explains for the greater prevalence rate in the females is the behavior to lick their infected newly born calves which can lead to re-infection. (Crowford *et al.,* 1990).

The prevalence by iELISA test in the age group of below 1 years, 1-4 years and above 4 years are 14.28 %, 21.14 % and 18.18 % in the sample size of 21, 331 and 33 in the respective groups.

Table-3: ELISA test and RBPT in different age group

Age group	ELISA		RBPT	
	Positive	**Total**	**Positive**	**Total**
Below 1 yrs	3 (14.28 %)	21	1 (4.76 %)	21
1-4 yrs	70 (21.14 %)	331	42 (12.68 %)	331
Above 4 yrs	6 (18.18 %)	33	4 (12.12 %)	33
Total	79	385	47	385

The prevalence by RBPT in the age group of below 1 years, 1-4 years and above 4 years are 4.76 %, 12.68 % and 12.12 % in the sample size of 21, 331 and 33 in the respective groups. There was no significant difference on the age group ($p>0.05$).

B. melitensis infection causes disease only in adult (sexually mature) females and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response. However, susceptibility increases after sexual maturity and especially with pregnancy (Fulgencio *et al.,* 2001).

In the study conducted by Maher *et al*, 2010, the seroprevalence of brucellosis was highest in goats of 2- 3 years (35.3%) age group followed by 1-2 years (19.6%) and more than 3 years (18.4%) age group, and in sheep, it was highest in 2-3 years (27.9%) age group followed by more than 3 years (19.2%) and less in 1-2 years (14.2%) age group. The study revealed that the prevalence varied from one age group to another vastly.

Out of 201 samples collected from Taplejung districts, 47 (23.38 %) samples showed positive by iELISA. Similarly 32 (17.39 %) samples out of 184 samples collected from Lamjung districts showed positive by iELISA (Table-4).

Table- 4: ELISA test and RBPT in different districts

Districts	**ELISA**		**RBPT**	
	Positive	**Total**	**Positive**	**Total**
Taplejung	47 (23.38 %)	201	32 (15.92 %)	201
Lamjung	32 (17.39 %)	184	15 (8.15 %)	184
Total	79	385	47	385

Likewise, out of 201 samples collected from Taplejung districts, 32 (15.92 %) samples showed positive by RBPT. Similarly 15 (8.15 %) samples out of 184 samples collected from Lamjung districts showed positive by RBPT. (Table-4)

In ELISA tests there was no significant difference on the two selected districts ($p>0.05$). While in RBPT there was significant difference with those two selected districts ($p<0.05$).

Out of 79 iELISA positive samples 48 (60.75 %) samples were from the animals having history and symptoms like retention of placenta, abortion, orchitis, arthritis, fever i.e. similar to symptoms of Brucellosis and rest of the 31 positive samples were from normal animals.

Table-5: ELISA test and RBPT in different health status

Health status	ELISA		RBPT	
	Positive	**Total**	**Positive**	**Total**
Normal	31 (11.69 %)	265	9 (3.39 %)	265
Abortion	30 (46.87 %)	64	25 (39.06 %)	64
Retained Placenta	9 (30 %)	30	6 (20 %)	30
Others	9 (34.61 %)	26	7 (26.92 %)	26
Total	79	385	47	385

Out of 47 RBPT positive samples 38 (80.85 %) samples were from the animals having similar history and symptoms similar to Brucellosis as mentioned above. For both the tests there was high significant difference on the different health status of the animals ($p<0.01$).

A total of 25 farmers were selected for questionnaire for the epidemiological study in selected research site. Around 200-300 numbers sheep and goats were in each flock size in both districts which were of high stocking density. Goat covers only 1-10 % of the population. Along with the sheep and goat as major animals, most of the farmers also use to keep 1-5 number of cattle/buffalo, 1-10 number of poultry for their subsistence need as for milk, meat and egg. They also use to keep 2-8 numbers of dogs to protect sheep and goats from predators. 72 %, 16 %, 8 % and 4 % of the farmers were using river water, tap water, well water and tube well water for their flocks. Diseases in flock most frequently seen are Fascioliosis, Footrot, Chhe-Mashe (Enterotoxaemia) in Chaitra-Baisakh, Pneumonia and Ectoparasitic infestation. Reproductive cases are Abortion along with retained placenta, dystocia, pyometra and orchitis in male.

Most of the farmers (64 % of them) used the antibiotics in the diseased animals when suggested by VAHW, VJTA, VJT and DLSO's officers. Most of the farmers used tetracyclines especially in case of footrot. But rest of them (36 % of the farmers) didnot used any antibiotics or used only locally available herbal medicines. Other information from questionnaires surveys were the outbreak of brucellosis not seen but reproductive cases/problems were frequently seen in the flocks, but the farmers were unaware about brucellosis.Therefore, they did not take any precautionary measures in abortion and reproductive cases. In addition, poor biosecurity measures and sanitation was observed. 88 % of the farmers use to rear their flocks by free

ranging system while rest 12 % of them use to rear their flock by both free ranging system and stall feeding system.

The variation of prevalence in different studies showed by different researchers could be due to different survey techniques applied by various researchers. While the whole population was taken as target population in this study, earlier studies were either confined to some selected farms or conveniently selected samples. This high prevalence may be due to unavailability of vaccination programme in the country, irregular screening of brucellosis, persistence of infection in the flock, lack of preventive and control measures and lack of awareness about Brucellosis to the villagers.

The mode of transmission of *B. melitensis* in sheep and goats is facilitated by mixing of flocks and herds belonging to different owners and by purchasing animals from unscreened sources. The sharing of male breeding stock also promotes transfer of infection between farms. Transhumance of summer grazing is a significant promoting factor in some areas as is the mingling of animals at markets or fairs. In cold climates, it can be the custom to house animals in close space and this also facilitates transmission of infection.

Dogs, cats, cattle and wild carnivores, such as foxes and wolves, may be important as mechanical disseminators of infection by carrying away infected material such as foetuses or foetal membranes (Fulgencio *et al.,* 2001). High stocking density may be the major risk factors for disseminating the brucellosis.

CONCLUSION

The present study provides the baseline status of Caprine and Ovine brucellosis in Taplejung and Lamjung Districts and the potential risk factors that would contribute to the occurrence of the disease in sheep and goats as well as possible zoonotic implications in human beings. Lack of awareness about the zoonotic nature of brucellosis, and close contact with animals, can serve as means of infection to human beings. Further studies need to be conducted on the risk of human brucellosis in this area to educate farmers on zoonotic disease and to devise measures for disease control.

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Growth Comparision of Goats Fed with Additional Supplementaion of Protein Source in Basal Diet in Western Hills of Nepal

M. R.Tiwari[1], D. Adhikari[2], D. P. Adhikari[2], R. P. Ghimire[2] and S. H. Ghimire[2]

1. Mountain Agriculture Research Institute, Guthichaur, Jumla

2. Agriculture Research Station (Goat), Bandipur, Tanahun

ABSTRACT

The study was carried out using twenty one growing female goats (50% Jamunapari, 50% Barberi and Khari; 6 each) at Agriculture Research Station (Goat), Bandipur, Tanahun for three months from 7 April to 5 July 2014. Female goats of average 9 kg were grouped into three groups having six goats in each group, as replicates, using Completely Randomized Design (CRD) having six goats of three breeds in each group (2 animals from each breed). They were drenched against internal parasites with Fenbendazole @ 5 mg/kg body before starting the experiment. Dry matter requirement (DMR) of goats was calculated based on 5 kg of body weight. Three types of ration were formulated for experimental goats. Goats of T1 group was provided seasonal fodder ad lib + compound concentrate feed @ 2% of body weight; T2 group was provided seasonal fodder ad lib + compounded concentrate feed @ 2% of body weight and T3 was provided seasonal fodder ad lib + compounded concentrate feed @ 1.5% of body weight +100 g soybean cake per head. The average dry matter feed intake per day was observed highest for T3 (554.46g) followed by T1 and T2 (504.29 g and 484.75g, respectively). Similarly, feed conversion ratio was found to be higher in T1 (10.8:1) followed by T2 and T3 (7.6:1 and 7.32:1, respectively). The total body weight gain was highest in T3 (6.81 kg) followed by T2 and T3 (5.74 and 4.2 kg, respectively) with average daily gain 75.72, 63.81 and 46.67 g for T3, T2 and T1, respectively. It can be concluded that supplementation of the oil cake is beneficial for goat fattening to get higher benefits.

***Key words**:* Crossbred goats, drenching, Feed Conversion Ratio (FCR), seasonal fodder, higher benefit

INTRODUCTION

Goats were among the first farm animals to be domesticated. As indicated by the archaeological evidence, they have been associated with man in a symbiotic relationship for up to 10,000 years (Ensminger and Parker, 1986). Goats disseminated

all over the world because their great adaptability to varying environmental conditions and the different nutritional regimes under which they were evolved and subsequently maintained. They proved useful to man throughout the ages due to their productivity, small size, and non-competiveness with him for food. Goat meat is the preferred food for all ethnic groups in Nepal. Since the demand for goat meat has outpaced domestic production, a substantial production of goat meat is consumed in Nepal is imported. The goat population of Nepal is estimated to be 9.78 million in which about 13.7 percent are crossbred goats. The goat population is growing by 2.4% each year. Goats are used for meat, pack, manure, and milk. The total production of goat meat (chevon) was 55578Mt goat meat per annum (MoAD, 2012/13).

Barberi is a small size and their color is white creamy to golden. Spotted animals also come across. Their meat conformation is considered good. Triple kidding and early maturity are common features of these goats (http://www.ansi.okstate.edu/breeds/goats/barbari). Jamnapari is white with patches of tan on the neck and head. Their heads tend to have a highly convex nose, which gives them a parrot-like appearance. They have long flat drooping ears which are around 25 cm long. They also have unusually long legs (Shelton and Maurice, 1978). Khari goats are widespread and more abundant (50%) than other indigenous breeds and are present in the mid-hills. Khari goats are relatively small bodied with body weight ranging between 20-40 kg. They are more prolific among the four indigenous breeds and can adopt in different agro-climatic zones Joshi and Shrestha (2003).

Protein is usually the most expensive component of the goat diet. Protein is required both as a source of nitrogen for the ruminal bacteria and to supply amino acids for protein synthesis in the animal's body. When the levels of protein are low in the diet, digestion of carbohydrates in the rumen will slow down and intake will decrease. Inadequate levels of protein in the diet can affect growth rate, milk production, reproduction and disease resistance negatively, because insufficient amino acids are getting to the intestines to be absorbed by the body. Unlike energy, excess of protein is not stored in the body of the goat. Therefore, it is important to feed enough protein to cover the nutritional requirements of the animal. Protein nutritional requirements vary with developmental and physiological stages and level of production. Prieto *et al.,* (2011) reported that optimum protein levels for feedlot goats ranged from 16 to 20.3%. Negesse *et al*., (2001) showed that the kids fed with 8% dietary CP level had lower average daily gain (ADG) and dry matter intake (DMI) than the kids fed with 10.5, 12.8, 15.5 dietary CP levels. Hwangbo *et al*., (2009) reported that the kids

fed with 18% CP in diet had significantly higher ADG when compared with the kids fed with 14, 16 and 20% CP in diet. In Nepal, for the optimization of crude protein level for fattener no investigated so far. Therefore, the objective of this study was to investigate effects of different crude protein levels on the growth and performance of growing goats.

METHODOLOGY

Experimental animal

This experiment was carried out on eighteen growing female goats (50% Jamunapari, 50% Barberi and Khari goats; 6 each) at Agriculture Research Station (Goat), Bandipur, Tanahun from 7 April to 5 July 2014 (070/12/24 to 071/3/21). Female goats of average 7 months old with average body weight of 9 kg were grouped into three groups having six in each group, as replicates, using Completely Randomized Design (CRD) having six goats of three breeds in each group (2 animals from each breed). They were drenched with Fenbendazole @ 5 mg/kg body weight against internal parasites before assigning in experiment.

Concentrate mixture composition

Feed ingredients maize, mustard cake, soybean cake, rice bran, minerals and salt were procured from Champadevi Feed Industry, Chapagau, Lalitpur. For T2 and T3 concentrate mixture were composed by using procured feed ingredients with 16% crude protein level (Table-1) while for T1 commercial compound feed was used made by Pancharatna Feed Industry, Narayangadh, Chitwan.

Table-1: Composition of concentrate mixture

S/n	Ingredients	Part	Crude Protein, %
1	Maize	50	4.40
2	Mustard cake	31	10.12
3	Rice bran	17	1.49
4	Mineral mixture	1	0
5	Salt	1	0
Total		**100**	**16.01**

Experimental diet of the animals

The dry matter requirement of goats was calculated based on 5 kg per 100 kg body weight. Following diets were formulated to the experimental animals (Table-2).

Table-2: Experimental diets of the animals

Treatment	Experimental diet
1	Seasonal fodder *ad lib* + compound feed @ 2% of body weight
2	Seasonal fodder *ad lib* + compound feed @ 2% of body weight
3	Seasonal fodder *ad lib* + compound feed @ 1.5% of body weight +100 g soybean cake per head

Feeding regime

Compound feed and *ad lib* amount of fodder was provided to the experimental animals individually in plastic vessel. Compound feed was provided once a day in the morning whereas fodder twice a day (morning and evening). Quantity of compound feed and fodder given daily to the animals was weighed daily and refusal was weighed in next morning. Experimental animal had free access to drinking water.

Chemical analysis

The samples of feed ingredients, prepared compound feed and forest mixed fodder were sent to the Animal Nutrition Division, Khumaltar, Lalitpur for proximate analysis. Representative samples were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE) and total ash contents (TA). The DM was determined by oven drying at 100°C for 24 hrs. Crude protein of the samples was determined using the Kjeldahl method. Ether extract was determined using Soxhlet apparatus. Ash content was determined by ashing at 550°C in a muffle furnace for 16 hrs (AOAC, 1980). Crude fibre of the samples was determined using the Van Soest method (Goering, H.K. and Van Soest, 1970).

Observation recording

The trial period consisted 90 days after an adaptation period of 7 days. Total feed intake by the goats was recorded daily for all experimental period. The body weight gain of individual animal was measured fortnightly in the morning before feeding.

Data analysis

Data of feed intake and body weight gain were analyzed by **"***One Way Annova***"** test for every measurement using computer statistical package Minitab 2003, versions 13.20.

RESULTS

Chemical composition of feedstuffs

The result of chemical analysis has been given in Table-3 and crude protein content of prepared concentrate mixture was verified in laboratory that is presented in Table-4.

Table-3: Chemical composition of different feed ingredients (on dry matter basis)

Ingredient	DM	OM	TA	CP	CF	EE
Maize	87.69	97.97	2.03	8.92	2.34	4.48
Rice bran	87.85	89.5	10.5	11.52	4.83	5.1
Mustard cake	87.27	90.5	9.5	35.52	9.19	NA
Mixed forest fodder	30	88.23	11.77	10.3	NA	NA

Table-4: Chemical composition of prepared concentrate mixture (on dry matter basis)

Particular	DM	OM	TA	CP	CF
Farm feed	93.13	88.12	11.78	14.5	8.57
Concentrate mixture	93.39	87.33	12.67	16	7.13
Concentrate mixture + soybean cake	93.38	86.72	13.28	18.2	6.79

Feed intake

Average feed and fodder intake of experimental animals is presented in Table-5.

Table-5: Feed intake of experimental animals/day/animal

Feedstuffs	Mean ± SD		
	T1	T2	T3
Feed intake, g	226.87± 61.57	205.06±96.01	271.41±65.01
Fodder intake, g	976.7±339.70	977.5±352.0	1003.4±383.20
Dry matter intake/day, g	504.29	484.75	554.46
Crude protein intake/day, g	146.31	146.26	193.26
Total dry matter intake (DMI), kg	45.39	43.62	49.9
Total crude protein intake, kg	13.17	13.16	17.39
Feed conversion ratio (FCR)	10.8:1	7.6:1	7.32:1

Table-5 showed that highest compound feed intake was found in T3 (271g) followed by T1 and T2 (226.87 and 205.06 g, respectively) which was highly significant ($P<0.001$) among diet groups. Similarly, highest fodder intake was recorded for T3 (1003.4 g) followed by T2 and T3 (977.5 and 976.7g, respectively) which was no significant among diet groups which resulted highest dry matter intake for T3 (49.9

kg) followed by T1 and T2 (45.39 and 43.62 kg, respectively). The FCR was noted higher for T1 (10.8:1) followed by T3 and T2 (7.32:1 and 7.6:1, respectively)

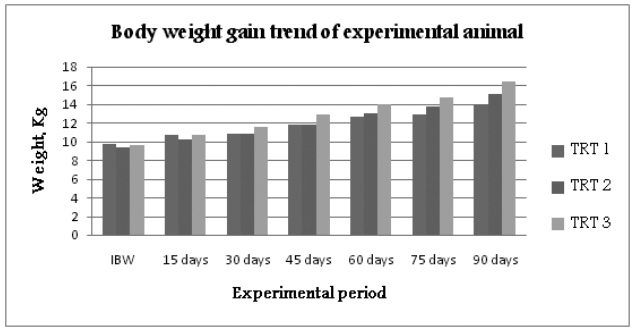
Growth performance

Average growth performance of experimental animals is presented in Table-6.

Table-6: Growth performance of goats

Parameter	**Mean ± SD**		
	T1	**T2**	**T3**
Initial body weight, kg	9.74 ± 2.65	9.37 ± 3.14	9.58 ± 1.90
Initial metabolic weight, kg	5.51	5.35	5.44
Final body weight, kg	13.94 ± 3.55	15.11 ± 4.99	16.4 ± 3.94
Final metabolic weight, kg	7.21	7.66	8.15
Total weight gain, kg	4.2 ± 1.96	5.74 ± 2.21	6.81 ± 2.25
Average daily gain, g	46.67 ± 21.88	63.81 ± 24.60	75.72 ± 25.04

In the beginning of the experiment highest initial weight was in T1 (9.74 kg) followed by T3 and T2 (9.58 and 9.37 kg, respectively) while by the end of experiment (after 90 days) total weight gain was obtained higher from T3 (6.81 kg) followed by T2 (5.74 kg) and T1 (4.2 kg). Both initial and total weight gain was no significant (P>0.05) among diet groups. The average daily gain was observed highest in T3 (75.72 g) followed by T2 (63.81 g) and T3 (46.67 g).



DISCUSSION

This experiment revealed that higher level of crude protein in goat diet increase the feed intake of T3 groups (271.41g) than that of T1 (226.87 g) and T2 (205.06 g). Similarly, fodder intake also was increased in higher level of crude protein diet group (T3) which was 1003.4 g than that of T2 and T1 (977 g). Likewise, total body weight gain was also higher in increased crude protein level diet (6.81 kg for T3) than that of T2 (5.74 kg) and T1 (4.2 kg). Moreover, experiment showed decreasing trend of FCR

with increasing level of crude protein in diet. It might be due to bypass of protein from rumen to lower guts

Sharifi *et al*., (2013) suggested that increasing CP levels from 14 to 16% in diet improved feed intake, average body gain and feed efficiency but had no effect on apparent digestibility of nutrients and body. Therefore, 16% dietary CP could be recommended for Iranian Sannen kids; however, higher ratios of CP should be tested. Kabir *et al.,* (20014) reported that feeding of goats with high protein (HP) diet significantly ($p<0.01$) increased crude protein intake (73.14 vs. 59.91 compared with low protein (LP) diet. However, no significant ($p>0.05$) difference was observed between LP and HP diet for the values of DM intake and live weight gain although there was a tendency to increase live weight gain in goats given the HP diet.

Shahjalal *et al.,* (1997) reported that growth rate of grazing Black Bengal goats can improved under conditions of increased protein supplementation. Feeding of sheep with HP diet significantly ($p<0.01$, $p<0.05$) increased CP intake (78.54 vs. 55.39 g/d) and DM intake (509.0 vs 425.9 g/d) compared with those received the LP diet. This protein intake resulted in increased ($p<0.01$) live weight gain in sheep received the HP diet. This can be related with the Lu and Potchoiba (1990) who suggested that DM intake in goats increased linearly as dietary CP level increased. The results suggest that the effect of supplementing high protein on intake and live weight was higher in sheep than goats. Chaokaur *et al*., (2012) conducted an experiment with objective to quantify protein requirements for maintenance and gain of Anglo-Nubian crossbred growing goat fed under tropical condition in Thailand and found that crude protein requirement for maintenance and gain 100g average daily gain were to be 157 and 272 g/day, respectively

CONCLUSION

Experiment proved that increased crude protein level had increased body weight gain and reduced feed conversion ratio. Therefore, further experiment should be carried out to optimize the crude protein level for higher body weight gain and reduced feed conversion ratio.

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Microbial Contamination in Meat Samples of Kathmandu Valley

S. Thapa

Himalayan College of Agriculture Sciences and Technology (HICAST), Kalanki

ABSTRACT

A total of 27 goat meat (chevon) samples were collected from nine different meat shops of Kathmandu Valley from June 2008 to August 2008 to assess the microbial quality. Laboratory examinations were performed at Microbiology laboratory, HICAST, Kathmandu. The Total Viable Count (TVC) and Coliform count were found to be 5.43±0.07 and 2.82±0.03 log cfu/g respectively. Two samples from the two different slaughter slabs were found to be positive for salmonella. Coliforms and salmonella were identified as the major contaminants. Epidemiological questionnaire survey revealed only few meat shops maintained with proper sanitization while others had limited standards for the producdtion of quality meat.

Key Words: Microbial quality, Meat shop, Total Viable Count, Coliform count and Salmonella.

INTRODUCTION

Meat and meat preparation have occupied a unique position in human diet and one of the most nutritious foods used for human consumption. Consumption of meat by human beings can be traced back to prehistoric time. The sumerians of about 3000 BC believed to have been the first livestock breeder and dairymen. Ancient civilizations were found to consume meat as important part of their diet. They also developed some technique to preserve meat and meat products for longer periods. But the role of microorganisms in spoiling of meat andtransmission of diseases was established after many centuries i.e. in 17th century (Joshi and Shah, 2003).

The total population of animals contributing to the national meat production was comprised of 4.2 million buffaloes, 7.4 millions goats, 0.81 million sheep, 0.9 million pigs, 23.22 million poultry and 0.39 millions ducks in the country. It was estimated that the total meat consumption in the country during 2007 was about 227105 MT (CBS, 2007). Meat and other meat products contribute significantly to higher incidence of food borne disease and zoonotic diseases (Prasai, 2000). Meat serves as an excellent medium for the growth of microorganisms. The microorganisms responsible for lowering the sanitary quality of meat are mainly derived from

external environments. The pathogenic bacteria like *Escherichia coli*, *Salmonella sps., Clostridium sps., Staphylococcus sps., Camphulobacter sps.*, etc. spoil meat but also cause food poisoning and other illnesses to consumers. Measures should be adopted in such a way that these organisms should not be present in meat and meat products. (Prince and Schweigert, 1970).

In Nepal lack of appropriate slaughtering facilities and unsatisfactory slaughtering techniques are causing unnecessary losses in meat which is aggravated by its slaughtering places which are frequently polluted with street dust, garbage, human excreta, animal blood, intestinal contents, and dirty effluents (Joshi, 1991). The condition of animal handling, meat producdtion and marketing in Nepal is primitive. Pre-slaughter handling is not appropriate and antemortem and postmortem inspections are not being practiced. The slaughtering condition is far from satisfactory. Meat market is filthy and totally unhygienic. Adulteration of meat is common practice. Thus the consumers are deprived of clean and wholesome meat (Subba, 2002).

Nepal being a member of WTO, should dream and plan for extending its business to international market. For the purpose we should be assured about the quality of our products. The study of this kind would help to access the quality of our products and at the same time emphasize the implementation of "Slaughter House and Meat Inspection Act 2055". This study was mainly focused on the assessment of microbial load rather than the isolation of organisms. The microbial load in the samples was compared with international standards.

MATERIALS AND METHODS

Sampling and Questionnaire Survey

This study was carried out from July to August 2008 in Kathmandu Valley. The laboratory work was performed in Microbiology Laboratory, HICAST, Kathmandu. ISO standard for microbiological methodology was followed. A random sampling was carried out from meat shops of Kathmandu Valley. A total of twenty-seven samples were collected form nine different meat shops.Three samples from each meat shops were collected at different intervals of time and the questionnaire survey was carried out to find out the sanitary condition of the meat, their premises and hygienic meat establishment. 50 grams of fresh breast muscle sample from each shop were collected aseptically in a sterile container, which were kept in ice box and transported to microbiology laboratory, HICAST Kathmandu. The samples were processed as soon as possible.

Laboratory examination

The entire media (Hi MEDIA) e.g Nutrient agar, Selenite broth, Bismuth Sulphate Agar were prepared. Initially 25 grams of meat sample was grinded and homogenated with 225 ml of buffered peptone water in a conical flask in order to get 10 fold dilution. Then 9 ml of Peptone Buffered Solution (PBS) was poured to each six sterile test tubes with the help of pipette. Thereafter,1 ml of homogenized sample was poured into the first test tube. Then after mixing the sample, 1 ml of the mixture from the first test tube was transferred to the second test tube and the similar procedure is continued serially up to the sixth tube. Finally, 1 ml solution from the last test tube was discarded. One test tube was kept as control with peptone buffered solution only.

For the estimation of total viable count1 ml of inoculum of each dilution was placed asceptically in a labeled petridish and then 15-20 ml of molten agar was cooled to 45°C and poured into each petridish and then uniformly mixed. The first three dilutions were chosen for coliform count and remaining last three dilutions were chosen for Total Viable Count (TVC). Plate Count Agar and Violet Red Bile Agar (VRBA) were used for TVC and coliform count respectively. After solidification of the medium, the petridishes were incubated at 37° C for 24 hours. A petridish was kept as control without inoculum. After overnight incubation the colony counting was carried out by using microbial digital colony counter.

For the isolation of salmonella the homogenized sample was incubated in buffered peptone water at 37° C for 24 hours for pre-enrichment. 1 ml of the pre-enriched sample was poured into 9 ml of Selenite brothe and incubated at 37° C for 24 hrs for selective enrichment. First the required volume of slective media, i.e. Bismuth Sulphite Agar (BSA) , was prepared just prior to inoculation. Then, 1 ml of selective enriched sample was poured into petridishes. One plate was kept as control. After sloidification of the media, the petridishes were incubated at 37° C for 24 hrs.

RESULTS AND DISCUSSION

All 27 samples had TVC and coliform count higher than the standard set by ISO. Out of 27 samples, 2(7.40%) were found positive for Salmonella.The average coliform count of nine mentioned meat shops mainly A,B,C, D,E,F,G,H and I was found to be 2.78,2.85, 2.83, 2.82, 2.78, 2.86, 2.87, 2.80, 2.83 (log cfu/g) respectively (Fig.-1) These findings are slightly higher than the standard set by ISO (2.69 Log cfu/g). The standard deviation of coliform was found to be 0.03.

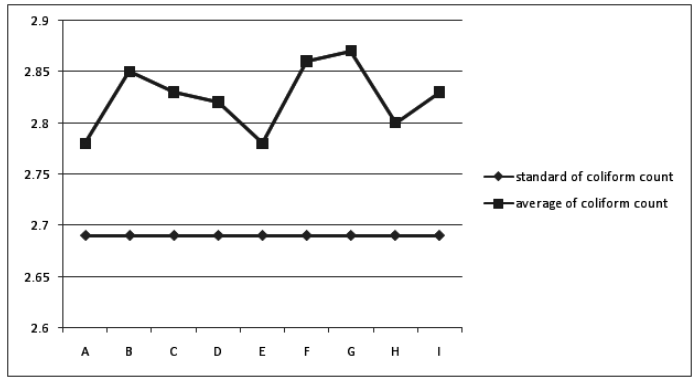

Fig.1: Total Coliform count (TCC) in meat shops

The avearage TVC of nine mentioned slaughter slabs (A, B,C,D,E,F,G,H AND I) was found 5.45, 5.35, 5.48, 5.43, 5.35, 5.45, 5.33, 5.53 and 5.48 (log cfu/g) respectively (Fig.-2). The standard deviation of TVC IS 0.07.

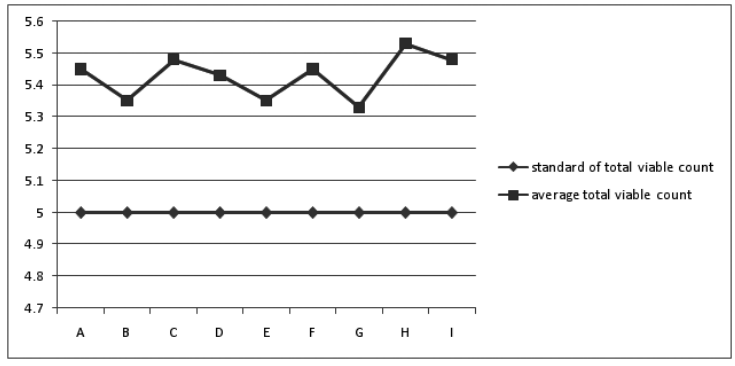

Fig. 2: Total viable count (TVC) in slaughter slabs.

Questionnare survey in nine meat shops had their own slaughtering places.Tap water was generally used for washing and cutting the meat. All the meat shops do not wash the cutting knife during the time of cutting and serving meat. Most of the meat was sold fresh and by the end of the day the remnants were stored in the refrigerator. The average total viable count of goat carcass in this study was 5.43 log cfu/g which was close to the standard prescribed by the ISO. The findings were much higher than the findings shown by the study conducted by Shankhi (2006), 3 log cfu/g. However, this was close to the standards of Bureau of Indian Standards, i.e 6 log cfu. It was also lower as compared to the results of Smith and Palumbo (1973) i.e 6.7 log cfu/g.

In this sutdy, out of 27 samples, 2 (7.4%) were found positive for salmonella. Thus the quality of the meat did not meet the standard set by ISO in which no salmonella

had to be present in 25 g of the sample. This finding was comparatively lower (11.3%) than the findings by Joshi & Shah (2003). The result is slightly higher (8%) than that of the findings of Shrestha. The coliform count of the sample was 2.82 log cfu/g which was near to the standard of ISO. This was also similar by Smith and Palumbo (1973) ie.. 3 log cfu/g.

According to this questionnaire based survey and by personal observation, none of them had maintained their basic requirements in the hygienic practices. The lab results also showed that the samples from the shops which were using untreated water had comparatively higher coliform count and total viable count. The carcass should be washed not only after final inspection but between the various stages involved in eviscerational, as it has been shown that by doing so, the numbers of coliform and salmonella on carcass were reduced because there is insufficient time for attachement to occur (Gracey and Collin, 2003).

The standard deviatioin of TVC is 0.069422 and the standard deviation of coliform was 0.03283. The magnitude of variance and the standard deviation for the TVC as well as coliform count was small which indicated that the TVC and coliform count for all the meat shops were similar and the data was in high degree of uniformity.

CONCLUSION

The epidemiological questionnaire survey results indicated that the sanitary condition of meat shops were unhygiene and had unscientific methods of handling in present study. It is suggested that antemortem as well as post mortem inspection of the animals should be done accordingly. Treated water should be used for all the procedures during slaughter.Proper disposal of sewage and wastage of the plant should be done. There should be effective implementation of the "Slaughter house and Meat Inspection Act 2055".For quality control, this type of study should be performed at regular intervals. The bacteriological standard of meat in Npeal should be developed depending on the slaughtering technique areas.

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Investigation of Microorganism in the Milk Samples of Chitwan District.

N. Malla[1], I. P. Dhakal[2], H. B. Basnet[2], R. Dhakal[3]

1. Mount Everest Kennel Club, Kathmandu
2. Agriculture and Forestry University, Rampur
3. Agricultural Technology Centre

ABSTRACT

A total of 52 apparently healthy animals of Chitwan were selected. The Age of animal ranged from 4 to 10 years from first parity to 8th parity. Teats were cleaned by cotton swab soaked in 70% ethyl alcohol. The first strip of milk was discarded and 10 ml milk sample was collected in sterile bottle. Milk samples were submitted for bacteriological culture within 30 minutes to one hour of collection. The cultures from positive samples were subjected to biochemical tests. Based on the biochemical tests 36.24% of culture were identified as Klebsiella sps. followed by Staphylococcus sps. 32.21%, E. coli1 6.78%, Streptococcus sps. 9.40% and Pseudomonas sps. 5.37%. The Klebsiella spp., Staphylococcus sps., E. coli, Streptococcus sps. and Pseudomonas sps. were the major source for problem of udder health as well as human health. Prevention of Coliform mastitis is difficult because Escherichia coli, Klebsiella, and Enterobacter were ubiquitous even in the well-managed dairies. Improving pre-milking hygiene and frequent evaluation of milking machine function may reduce bacterial load and teat end changes.

Key words: Chitwan, Culture, *Klebsiella, Staphylococcus, Streptococcus, Pseudomonas*

INTRODUCTION

Milk is synthesized in cells lining the alveoli, the small sacs at the very end of the ducts deep within the udder (Blowery & Edmondson, 2010). Udder being exposed to external environment is very prone to infections. Interaction of several management and environmental factors leads to exposure of the animals to several microorganisms. The microorganism gain entrance through the teat canal to milk secreting tissues of udder where they cause infection. This also reduces natural resistance of animal to disease (Sudhan & Sharma, 2010). Poor management, improper milking procedure, faulty milking equipments, inadequate housing, breeding for ever increasing milk

yield etc are the possible reasons behind the continuous presence of several diseases in the dairy industry.

There are two groups of bacteria responsible for most of the infections of the mammary gland. *Staphylococcus aureus, Streptococcus agalactiae and Mycoplasma bovis* are contagious and major pathogens. *Streptococcus uberis, Streptococcus dysgalactiae* are most prevalent environmental pathogens while *Streptococcus equinus* is a less prevalent one. Environmental coliforms includes gram negative bacteria *E. coli, Klebsiella* spp*., Citrobactor, Enterobactor* spp*., Enterococococcus fecalis, E. faecium* and other gram negative bacteria are *Serratia, Pseudomonas and Proteus.* Among these organisms *Str. agalactiae* causes greater production loss, whereas *S. aureus* causes the higher infection rate, greater resistance to treatment and longer duration of infection. Cogulase-Negative *Staphylococci* (CNS), *Corynebacterium bovis* is known as minor pathogens. (Rodostits *et al.*, 2006) *S. aureus, E. coli* may occasionally loss the life of animal, *Corynebacterium pyogenes* may completely loss the quarters, *Str. agalactiae* causes greater production loss, whereas *S. aureus* causes the higher infection rate, greater resistance to treatment and longer duration of infection.. In Punjab of Pakistan two hundred thirty four bacterial isolates of nine genera i.e. *Staphylococci, Escherichia, Streptococci, Pseudomonas, Salmonellae, Bacillus, Klebsiella, Enterococci* and *Corynebacterium* species were identified from the milk samples. The highest prevalence was of Staphylococci followed by *E. coli, Pseudomonas, Bacillus, Streptococci, Salmonellae, Corynebacterium, Klebsiella* and *Enterococci*. (Ali *et al*., 2011). In the study in Karnataka state of India the predominant bacterial isolates recovered were *Staphylococcus aureus* and *E. coli* followed by *Staphylococcus epidermidis*, *Streptococcus* spp., *Klebsiella* spp. and *Bacillus* spp. (Harini & Sumathi, 2011)

Among the several diseases like Staphylococcal food poisonings, Streptococcal infection, Tuberculosis, Brucellosis and Q-fever may be transmitted to man through infection of infected milk (Radostits *et al*., 2006). Studies have shown diseases such as Tuberculosis and Brucellosis and, the presence of atypical mycobacteria in milk may have great influence on morbidities and severity of infections such as HIV/ AIDS syndrome, which is now a pandemic in some developing countries (Mdegela *et al*., 2004). Consumer preference for raw milk may also predispose consumers to these diseases. The presence of harmful organisms in milk renders it unsuitable for human consumption. Thus the need of identification and isolation of possible microorganisms to control is driven by the consumers demand for wholesome, nutritious and safe milk produced by healthy animals. This analysis will provide a basis for the preventive programs for the possible public health hazard from infected milk.

MATERIALS AND METHODS

Western part of Chitwan was selected for research purpose. All together 52 apparently healthy animals were selected. Purposive random sampling was done for sampling. Teats were vigorously wiped using cotton swab soaked with 70% ethyl alcohol. The first strip of milk was discarded and 10 ml milk was collected in sterile bottle. Milk samples were first collected from two near teats (LF and LH) and then from two far teats (RF and RH). Milk samples were submitted for culture within 30 minutes to one hour of collection. The media used for culture were nutrient agar and Mac conkey agar. Culture positive sample were subjected to Gram's staining and further subjected to several biochemical test; Catalase, Oxidase, Motility Indole Lysine (MIL), Triple Sugar Iron (TSI), Simmons Citrate, Methyl Red (MR) and Voges-Proskauer (VP) tests (Quinn *et al*., 1994). Bacteria were identified on the basis of morphology and biochemical test.

RESULTS

Sample population

Udder appearances of the sampled animals were 94% symmetrical and 6% asymmetrical. Consistency and color of milk was found normal. The average milk yield of the animals was found 9.2 liter.

Culture and Biochemical Tests

All together 149 samples of nutrient agar and 87 samples of Mac Conkey agar were culturally positive. All the positive samples were subjected to Gram's staining. Several biochemical tests were performed in total 62 Gram positive samples and 87 gram negative samples. Based on biochemical test 36.24% was *Klebsiella sps*. followed by *Staphylococcus* sps. 32.21%, *E. coli* 6.78%, *Streptococcus sps*. 9.40% and *Pseudomonas sps*. 5.37%. Coliforms were found to be predominant microorganism in the milk sample.

DISCUSSION

As reported by several researchers from 2002 to 2010 more than 200 infectious causes of udder infection are known to date and in large animals the commonest pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other Streptococcus and Coliforms in Asia (Sharma *et al*., 2012). Similar organisms were also identified in our finding as well as the research carried out by Dhakal and Kapur (1989), Jha *et al.,* (1993) in the study conducted during 1991/92 in Ilam and Dhankuta, by Dhakal and Tiwari (1993) at the IAAS livestock farm of Rampur, Joshi and Joshi, (1997) in the

western hills of Nepal, Sah and Dhakal (1999) in western Chitwan, Adhikari (2009) in Chitwan district and Khanal and Pandit (2013) in Lamjung district of Nepal.

In this study Coliforms were predominant which was similar to Khakural (1996), Sah and Dhakal (1999) and Adhikari (2009). Whereas this result was different from the result of Dhakal and Kapur (1989), Jha *et al.,* (1993), Dhakal and Tiwari (1993), Joshi and Joshi, (1997) showed *Staphylococcus sps*., as predominant organism and Khanal and Pandit (2013) showed *Streptococcus sps.,* as predominant organism.

Lactating and dry cows that were housed on dirt lots (yards) or bedded (manure) packs were particularly vulnerable to Coliform infections. Periods of hot, humid weather, extensive periods of heavy precipitation, movement of cows into confined facilities and movement of cows to new facilities are often followed by periods of increased incidence of new Coliform case. Increased Coliforms may be due to the poor housing management, fecal contamination, poor bedding, unhygienic milking, contaminated milking equipments etc. Increased Coliform infection may be due to warm, rainy weather as the research was carried out in the summer and rainy season between June and August.

CONCLUSION

The *Klebsiella* spp., *Staphylococcus* spp., *E. coli*, *Streptococcus* spp. and *Pseudomonas* spp. were the major source for problem of udder health as well as human health. Prevention of Coliform mastitis is difficult because *Escherichia coli*, *Klebsiella*, and *Enterobacter we*re ubiquitous even in the well-managed dairies. Improving pre-milking hygiene and frequent evaluation of milking machine function may reduce bacterial load and teat end changes.

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Prevalence, Characterization and Antimicrobial Resistance Pattern of Thermophilic Campylobacter in Broiler Meat of Chitwan, Nepal

R. K. Bhattarai, H. B. Basnet, I. P. Dhakal, S. Thapaliya

AFU, Rampur, Chitwan

ABSTRACT

A total of 400 random samples of retail broiler meat from different location of Chitwan, Nepal, were collected to study the prevalence, characterization and antimicrobial sensitivity pattern of thermophilic Campylobacter (Campylobacter jejuni, C. coli and C. lari) during November 2008 to June 2009. The majority (179/400) of retail broiler meat samples with a 44.7% were contaminated with different Campylobacter spp. Among Campylobacter spp. the highest proportion were C. jejuni (80.4%) followed by C. coli (14.5%) and C. lari (4.9%). Among these, 13 (7.3%) broiler meat were infected with multiple Campylobacter spp. This study revealed that retail broiler meat is often contaminated with thermophilic Campylobacter spp. However, there were no significant ($p<0.05$) differences in the prevalence of Campylobacter in different sites of Chitwan. A total of 290 samples were analyzed in winter (November to February) and 110 samples in summer (May to June). There was significant ($p<0.05$) seasonal difference in the isolation of Campylobacter spp. In the summer season there was 1.31 times (odd ratio) more likely to yield Campylobacter spp. as compared to the winter season. Antimicrobial sensitivity pattern of most common antimicrobial used in the commercial broilers was evaluated against the Campylobacter spp. The results of this study revealed that C. jejuni, C. coli and C. lari were completely resistant to Cephalothin. Ampicillin showed the highest resistance (88.9%) followed by Colistin (68.9%), Ciprofloxacin (59.2%), Enrofloxacin (59.1%), Tetracycline (47.6%), Nalidixic acid (33.2%), Streptomycin (25.4%), Erythromycin (12.1%), Gentamicin (10.2%) and Chloramphenicol (6.6%). The Campylobacter spp. showed significant difference ($p<0.05$) in antimicrobial resistance against Erythromycin and Enrofloxacin. Mean disc diffusion diameter among C. jejuni, C. coli and C. lari revealed that C. coli is highly significant ($p<0.01$) for Enrofloxacin and significant ($p<0.05$) for Ampicillin. Among the Campylobacters, 77.4% were found to be resistant to more than two antimicrobials. Multi drug resistances were found more in C. coli as compared to C. jejuni and C. lari.

INTRODUCTION

Thermophilic Campylobacter is the number one causative agent of food zoonosis often attributed to consumption contaminated broiler meat (OIE, 2007). To date, no practical or effective control measures have been available. Campylobacters are a commensals organism found in fowls. They are the most common host for Campylobacter. The *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis* are commonly referred to as thermophilic Campylobacter, with an optimum growing temperature of 42.0^{0}C. In Nepal, there has not been any documented work on the occurrence of Campylobacter in broiler. Similarly, the most important concern worldwide for Campylobacter food borne infection has increased because of the frequent isolation of antimicrobial resistant species in human and food animals seems to be primarily driven by the indiscriminate use of antimicrobials in food animals. In order to gather information on the presence of Campylobacter in retail broiler meat, this study was carried out in Chitwan, during the November 2008 to June 2009. This study addressed the lack of data pertaining to the prevalence and antimicrobial resistance pattern of *Campylobacter spp*. found in retail broiler meat shops (local butcher shops) at their condition.

MATERIALS AND METHODS

Sample Collection

A total of 400 retail broiler meat samples were collected was collected from different places of Bharatpur, Narayangarh, Ramnagar, Sharadanagar, Rampur and Mangalpur of Chitwan, Nepal during the period of November 2008 to June 2009. Broiler meat samples (100 g) consisting of equal parts of meat derived from neck (with skin), breast and carcass parts collected in UV rays sterilized zipped, 5x4 inches, transparent plastic bags with the help of sterilized forceps using 70.0% alcohol. The collected retail broiler meat samples were transported to the Veterinary Microbiology Laboratory of Rampur Campus, IAAS, Rampur, maintaining cold chain with the help of icebox and were stored at refrigerator (2-8.0^{0}C) if necessary.

Enrichment and Primary culture

The methodology of isolation and identification of the organism was done as described by OIE terrestrial manual, 2008, Chapter 2.9.3 (OIE, 2007). Subsamples of 25 gm meat from individual sample were kept in 400 ml capacity plastic bag with zipper lock containing 225 ml 0.1% buffer peptone water (pH 7.2) (M614, HiMedia lab, Mumbai, India) then homogenized manually. One volume of homogenized fluid taken from plastic bag is added to nine volume of Bolton broth (CM0983, Oxoid ltd., Basingstoke, Hampshire, England) for enrichment. The inoculated broths were

incubated in microaerophilic atmosphere obtained by burning candle in candle jar system (BD1777SE, Don whitely scientific ltd, England) for microaerophilic culture at 42.0^{0}C for 48 hrs.

Following incubation, one loopful of broth culture was streaked on plates of modified CCDA (mCCDA) using the four quadrant streak method and incubated in microaerophilic atmosphere. If identified colonies were grayish, flat and moistened with tendency to spread and have a metal sheen. These typical colonies were subcultured onto tryptose blood agar (TBA) plates (supplemented with five per cent defibrinated sheep blood) under similar conditions as above. Pale yellow colonies with mucoid appearance were stained by modified Gram's staining for presumption of Campylobacter. The Campylobacters are Gram's negative and curved bacilli.

Conformation and stereotyping

The presumptive Campylobacter colony resulting from respective plates were identified based on Gram's staining reaction, cellular morphology, cultural characteristics and biochemical reactions. The thermophilic Campylobacter differentiated based on Nalidixic acid and Cephalothin sensitivity, hippurate hydrolysis and indoxyl acetate. Generally, *C. jejuni* can be differentiated from other *Campylobacter sps*. based on the hydrolysis of hippurate as this is the only hippurate positive species.

Antimicrobial sensitivity test.

In vitro antimicrobial sensitivity pattern of identified *Campylobacter spp*. were done by using disc diffusion method performed according to Clinical and Laboratory Standards Institute (CLSI, 2007). Suspected *Campylobacter sps*. colonies from the mCCDA/TBA plates were emulsified in 5 ml BHI broth and incubated with caps loosened for 48 hrs at 42±1.0^{0}C in anaerobic jar with lighting candle, having turbidity of the inoculums to the equivalent of a one McFarland turbidity standard, into BHI broth (M210, HiMedia lab, India).

Culture suspension on BHI broth (100 µl) after 48 hrs was charged and dispersed over the surface of a MHA with five per cent defibrinated sheep blood agar plate. Using sterile tweezers, antimicrobial discs were placed widely spaced aseptically on the surface MHA plate and incubated in a microaerophilic atmosphere at 37.0^{0}C for 48 hrs. The type and concentrations of antimicrobials in the discs (concentration in µg) used were: Ampicillin (A 10), Chloramphenicol (C 10), Ciprofloxacin (Cf 10), Erythromycin (E 15), Nalidixic acid (Na 30), Tetracycline (T 30), Cephalothin (Ce 30), Gentamicin (G 10) Streptomycin (S 10), Colistin (Cl 10) and Enrofloxacin (Ex 30), Cotrimoxazole (Co 25) (HiMedia Laboratory Limited, India). The diameter of these zones of inhibition was recorded to the nearest millimeters and classified as

sensitive, intermediate and resistant based on the criteria of Huysmans and Turnidge, 1997 for Ampicillin, Chloramphenicol, Ciprofloxacin, Erythromycin, Nalidixic acid, Tetracycline, and other antimicrobial as performance standards for antimicrobial disc sensitivity tests as CLSI.

Statistical analysis

All the data available from laboratory findings were subjected to statistical analysis. Prevalence of Campylobacter, significant differences in resistance rates between *C. jejuni, C. coli* and *C. lari* was analyzed by using chi-square test using commercial software (PH stat version 2.5) with significance level defined at the $p<0.05$. Data entry, management and analysis was done using program Microsoft Office Excel 2003. Significant difference in zone of inhibition of *Campylobacter spp*. were analyzed by using Tukey Kramer procedure for multiple sample (Steel and Toerrie, 1980) using commercial software PHStat version 2.5 with significance level defined at the $p<0.05$. Odd ratio and 95% CI were calculated on seasonal prevalence to determine the strength of association using software Win Episcope Version 2.0 (EPIDECON).

RESULTS AND DISCUSSIONS

Prevalence of Campylobacter spp.

Out of 400 broiler meat samples examined, 179 meat samples (44.8%) showed positive for *Campylobacter sps*. Prevalence rate of 43.3%, 44.6% and 49.6% were found in the site A, B and C respectively. Among these, 13 (7.3%) broiler meat samples were infected with multiple *Campylobacter sps*. The highest prevalence was observed in broiler meat of site C (49.6%), than site B (44.6%) and A (43.3%) (Table 1). The prevalence of *Campylobacter spp*. among various locations were non significant ($p>0.05$).

Table- 1: Prevalence of *Campylobacter sps*. among various locations in Chitwan (n=400)

Site	No of Samples	Positive	p-value
A	201	87 (43.3)[a]	p>0.05
B	130	58 (44.6)[a]	
C	69	34 (49.6)[a]	
Total	400	179 (44.8)	

Figures in parenthesis indicate percentage and with the same letter in the same column are non significant ($p>0.05$)

This is the first report of isolation of *Campylobacter spp*. from broiler meat in Nepal. The mean incidence of Campylobacter positive samples in this study was lower than that reported by Zhao *et al*., (2001) in USA, Taremi *et al*., (2006) in Iran and Meldrum *et al*., (2005) in UK, who isolated Campylobacter from 67.9% (106/156), 70.7% (130/184), 63.0% and 73.1% (538/736), respectively from raw chicken meat samples at the retail levels. Relatively high incidence of contamination, despite the general cleanliness and standard level of sanitation that generally applied in developed countries during the preparation and processing of chicken meat, indicated that contamination of chicken meat with Campylobacter is very likely to be unavoidable since the poultry are the main reservoirs for this organism. It is probable that the extra handling and manipulation required for the preparation of chicken portions in modern slaughter house of developed countries may increase the likelihood of cross contamination resulting in higher prevalence of Campylobacter.

In contrast to these higher contamination incidences, lower percentage of Campylobacter recovery (<40.0%) were determined in chicken products from Belgium (Uyttendaele *et al*., 1999), the Netherland (Dufrenne *et al*., 2001), Mexico (Castillo-Ayala *et al*., 1993).

The present finding is in close agreement with those reported worldwide in different studies (Whyte *et al*., 2003) in which *C. jejuni* was the most prevalent species identified (>75.0%) from chicken meat products, while *C. coli* was less frequently determined (<25.0%). Other studies (Whyte *et al*., 2003) reported prevalence of Campylobacter on raw chicken carcasses with a recovery rate of *C. jejuni*, ranging from 8-98.0%. Saenz *et al*., (2000) and Chattopadhyay *et al*., (2001) reported that *C. jejuni* was the most prevalent from chicken and foods containing chicken product.

In contrast, *C. coli* were isolated more frequently than *C. jejuni* from broiler chicken in Italy (Pezzotti *et al*., 2003), as well as from commercial frozen chicken livers in Chile (Fernandez and Pison, 1996). In other studies, however, Madden *et al*., (1998) Castillo-Ayala *et al*., (1993) found that 50.0% of *Campylobacter spp*. from chicken meat in Mexico was *C. coli*.

More than one species of Campylobacter was identified in 13 broiler meat samples. This study indicated that multiple *Campylobacte*r *spp*. are present in retail broiler meat which has also been observed in other studies (Zhao, 2001). Kramer *et al*., 2000 have also suggested that contamination with multiple *Campylobacter spp*. occurs in 5-10.0% of human cases of acute enteritis.

There were total of 290 samples analyzed in winter (November to February) and 110 samples in summer (May to June).

Table-2: Seasonal variation in *Campylobacter spp*. (n=400)

Season	Sample	Positive (%)	χ^2 (p-value)	OR (95% CI)
Winter	290	121 (41.7)[a]	0.048	1.31 (0.83-2.05)
Summer	110	58 (52.7)[b]		

Figures with the same superscript in same column are non significant (p>0.05) and different superscript are significant (p<0.05)

There was significant (p<0.05) seasonal difference in the isolation of Campylobacter in winter (41.7%) and summer (52.7%) in this study. In the summer season there were 1.31 times (odd ratio) more likely to yield Campylobacter as compared to the winter season (Table-2). Campylobacter, a major zoonotic pathogen, displayed a distinct seasonality pattern as obtained in various other studies (Abulreesh *et al.*, 2006).

Antimicrobial resistance

Table-3: Antimicrobial resistance pattern of *Campylobacter spp*. from broiler meat

Antimicrobials	C. jejuni	C. coli	C. lari	p-value
Ampicillin	123 (85.4)[a]	24 (93.4)[a]	6 (66.7)	>0.05
Chloramphenicol	5 (3.7)[a]	2 (8.7)[a]	1 (11.1)	>0.05
Ciprofloxacin	95 (65.8)[a]	18 (70.6)[a]	4 (44.4)	>0.05
Erythromycin	9 (6.3)[a]	7 (24.4)[b]	1 (11.1)	<0.05
Nalidixic acid	40 (27.5)[a]	11 (40.8)[a]	2 (22.2)	>0.05
Tetracycline	66 (45.3)[a]	15 (59.4)[a]	3 (33.3)	>0.05
Cephalothin	144 (100)[a]	26 (100)[a]	9 (100)	>0.05
Gentamicin	16 (10.4)[a]	3 (11.7)[a]	1 (11.1)	>0.05
Streptomycin	31 (21.6)[a]	8 (30.4)[a]	2 (22.2)	>0.05
Colistin	89 (61.3)[a]	21 (79.6)[a]	6 (66.7)	>0.05
Enrofloxacin	63 (43.5)[a]	23 (90.4)[b]	4 (44.4)	<0.05
Cotrimoxazole	12 (8.6)[a]	4 (16.7)[a]	6 (66.7)	>0.05

Note: Figures in parenthesis indicate percentage, figure with the same superscript in the same row are non significant (p>0.05) and figure with the different superscript in the same row are significant (p<0.01)

The *Campylobacter sps*. showed significant (p<0.05) difference in resistance with Erythromycin and Enrofloxacin and non significant (p<0.05) with other antimicrobials with respect to species (Table-3). The results of antimicrobial resistance for *C. jejuni*,

C. coli and *C. lari* have been summarized (Fig.-1).

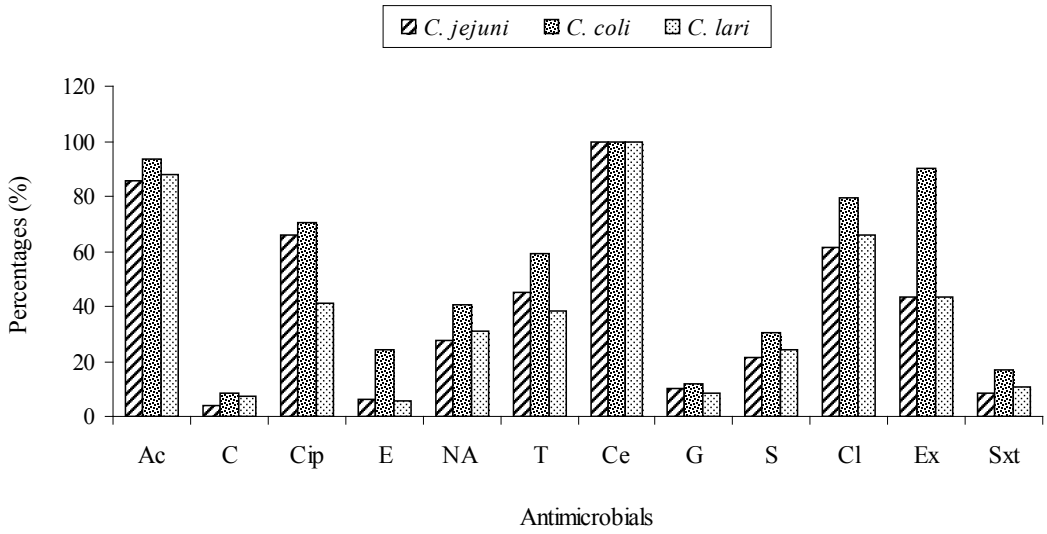

Fig.-1: Antimicrobial resistance pattern of *C. jejuni*, *C. coli* and *C. lari*

Asteric (*) above the bar indicate the significant difference ($p<0.05$). Ac=Ampicillin, C=Chloramphenicol, Cip=Ciprofloxacin, E=Erythromycin, NA=Nalidixic acid, T=Tetracycline, Ce=Cephalothin, G=Gentamicin, S=Streptomycin, C=Colistin, E=Enrofloxacin, Co=cotrimoxazole

Relatively high resistance in *C. jejuni* from chicken meat were reported from Belgium (Looveren *et al*., 2001), USA (Ge *et al*., 2003), Turkey (Bostan *et al*., 2009), Belgium (Habib *et al*., 2009), Canada (Guevremont *et al*., 2006), Lebnan (Talhouk *et al*.,1998) and Italy (Pezzotti *et al*., 2003), moderate rate were reported from Switzerland and France (Avrain *et al*., 2003), whereas limited occurrence of antimicrobial resistance among *C. jejuni* was reported from Sweden (Lindmark *et al*., 2004). A possible explanation for these differences might be that occurrences of antimicrobial resistance are reflecting the different national and regional policies in relation to the use of antimicrobial agents for food animals.

This high antimicrobial resistance rate could be due to the widespread use of antimicrobials in chicken particularly in feed as well as due to being use indiscriminately. Also there is a evidence to indicate that Tetracycline survives longer in the environment than do other antimicrobials which may be critical in maintaining the level of Tetracycline resistance at a high level (Frost, 1991). The low antimicrobial resistance among *C. jejuni* from chicken was probably the result of restrictive use of antimicrobials in chicken production (Berndtson *et al*., 1996).

Mean disc diffusion zone diameter among different *Campylobacter spp*.

Mean disc diffusion zone among different *Campylobacter spp*. were non significant (p>0.05) for Ciprofloxacin, Erythromycin, nalidixic acid, Tetracycline, Cephalothin, Gentamicin, Streptomycin, Colistin and cotrimoxazole and highly significant (p<0.01) in *C. coli* for Enrofloxacin and significant (p<0.05) for Ampicillin (Table 4).

Table- 4: Mean disc diffusion zone diameter for Campylobacter spp.

Antimicrobials	Mean disc diffusion zone diameter (mm)			p-value
	C. jejuni	*C. coli*	*C. lari*	
Ampicillin	7.2[a]	6.4[b]	7.0	<0.05
Chloramphenicol	25.1[b]	22.7[a]	23.6	<0.05
Ciprofloxacin	19.0[a]	19.2[a]	21.7	>0.05
Erythromycin	19.6[a]	18.3[a]	18.9	>0.05
Nalidixic acid	17.5[a]	16.9[a]	17.9	>0.05
Tetracycline	23.0[a]	19.0[a]	22.9	>0.05
Cephalothin	-	-	-	-
Gentamicin	16.2[a]	15.9[a]	16.1	>0.05
Streptomycin	14.0[a]	13.5[a]	13.9	>0.05
Colistin	9.6[a]	9.3[a]	9.4	>0.05
Enrofloxacin	18.1[a]	15.1[b]	18.1	<0.01
Cotrimoxazole	15.2[a]	14.5[a]	14.9	>0.01
Total sample	144	26	9	

Figures with the different letters in the same row are significant (p<0.01) and same letter in the same row are non significant (p>0.05)

Single and multi drug resistance *Campylobacter spp*.

Multi drug resistances (resistance with four agents) were found more in *C. coli* as compared to *C. jejuni*. Resistance to multiple antimicrobial agents were predominantly prevalent in *C. coli* followed by *C. jejuni* and *C. lari* (Table-5). Among the Campylobacters 77.4% were found to be resistant to more than two antimicrobials (Table-5). The higher percentage of Campylobacter (26.8%) were resistance to three types of antimicrobials followed by four (23.5%), five (20.7%) and six (14.0%). Similar to the findings of Humphrey *et al*., (1995) this study found of multiple Campylobacter from same sample could be both susceptible and resistant to antimicrobial.

In contrary to our findings, no multiple resistance (defined as resistance to four or more

different classes of antimicrobials) has been reported in a number of EU countries (Bywater *et al*., 2004), Sweden (Ronner *et al*., 2004) and Australia (Jeanette *et al*., 2007).

Table-5: Multi drug resistance observed among Campylobacter spp. (n=179)

Organisms	one agent	two agents	three agents	four agents	five agents	six agents
C. jejuni	4 (2.8%)	23 (16%)	42 (29.2%)	35 (24.3%)	27 (18.8%)	13 (9.0%)
C. coli	2 (7.7%)	1 (3.8%)	1 (3.8%)	8 (30.8%)	9 (34.6%)	5 (19.2%)
C. lari	2 (22.2%)	1 (11.1%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	1 (11.1%)

CONCLUSION

The *C. jejuni*, *C. coli* and *C. lari* are the prevalent species in broiler meat. The present study showed that the *Campylobacter sps*. commonly contaminates retail broiler meat with marked seasonal fluctuation. Antimicrobial resistance was commonly observed on *Campylobacter sps*. from broiler meat in Chitwan District. Cephalothin showed the complete resistance to all *Campylobacter sps*. whereas Ampicillin, Colistin, Ciprofloxacin, Enrofloxacin, Tetracycline, Nalidixic acid show highest resistance. Similarly, Streptomycin, Erythromycin, Gentamicin and Chloramphenicol showed highest sensitivity to Campylobacter. Erythromycin, Enrofloxacin, Ampicillin and Chloramphenicol showed significant in antimicrobial resistance for *Campylobacter sps*.

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Detection of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv) Antibody in Pig Population of Nepal

M. P. Acharya, S. P. Shrestha, D. R. Khanal, M. Prajapati, P. Shrestha and N. Paudyal.

Animal Health Research Division, NARC

ABSTRACT

Porcine Reproductive and Respiratory Syndrome (PRRS) was first recognized in the USA in 1987 as a new disease of swine. Later on in 1991 PRRS virus (PRRSV) isolated and identified in Netherland. Currently two major genotypes of PRRSV exist, the type 1(European) and the type 2 (North American). It is caused by a RNA virus classified as a member of the genus Arterivirus, causing reproductive failure of sows and severe respiratory problem in piglets and growing pigs. After 1990s, the disease is spreading worldwide in major pig raising countries including Asian countries. In 2006, high pathogenic PRRS (HP-PRRS) was recognized in China (An,et al 2010). Since, then the disease has spread to Philippines, Viet Nam, Combodia, Laos PDR, Thailand, and Myanmar. From December 2014, Active surveillance was conducted by Animal Health Research Division in four districts of Nepal. Out of 150 serum samples 35 samples (23.3 %) were found positive in antibody detection test (immunochromatographic test). District wise 50 %, 16.6%, &.5% and 6.6% samples were found positive from Chitwan, Makawanpur, Kathmandu and Sunsari districts respectively. To date, no vaccination is in practice in Nepal against PRRS which confirm that our pig population has been exposed to PRRSV. The positive serum and few blood samples have been kept for genotyping and further molecular analysis of virus. It is very crucial to identify the genotype of PRRSV prevalent in pig population of Nepal before introducing PRRS vaccine otherwise vaccine may not be effective.

Key words: *PRRS; ELISA; Antibody.*

INTRODUCTION

Pig rearing is an important enterprise and the main source of income especially for poor landless. In recent years, pork meat has become popular due to change in food habits and urban life style. Likewise, the rise in the price of meat of other animals particularly goat meat, the demand for pork has grown-up and pig farming is becoming more popular in commercialized scale in peri-urban areas. Industrial and intensive farming in the swine sector is developing rapidly.

Total population of pig is around 1.1 million, annual growth rate is 1.6 percentage,51 percent of the total population are in eastern region, 18, 15, 11, and 5 percent of the total population lies in the mid region, mid-western region, western region and far-western region respectively (Agri. Statistics MoAD, 2012).

To get proper return from the pig farming it is very crucial to manage common pig diseases. Recently, a devastating pig disease has emerged in the country named Porcine Reproductive and Respiratory Syndrome (PRRS) also known as blue ear disease is a widespread disease affecting domestic pigs. The symptoms include reproductive failure in adult deadly pneumonia in piglets and increased susceptibility to secondary bacterial infections. It is caused by virus classified as a member of the genus Arterivirus. PRRS was first recognized in North United Sates of America in 1987 and causative virus was identified in the Netherland in 1991. The disease has been reported from most of countries including Asian countries. It has become most important disease in intensively raised pigs throughout the world.

In Nepal, the disease was serologically reported in pigs of Kathmandu valley (Sharma, 2011). Recently, outbreak of the disease was reported from Swine and Avian Research Program (SARP), Khumaltar (Annual report, AHRD 070/071). PRRS is a new emerging disease in Nepal .Outbreak at government owned pig farm at Khumaltar showed heavy losses. (Annual report, AHRD 070/071)

PRRSv belongs to the viral family Arteriviridae and is an enveloped RNA virus with a diameter of 48-83 nm (Benfield et al., 1992). Significant genetic and antigenic variation in PRRSV suggests that the virus consist of two distinct genotypes. Type 1 European genotype and type 11 North American genotype (Wensvoort, *et al*., 1995). Variation within the genotype is also persist. Cross protection is very limited. Recombination between PRRSv strains may occur to lead PRRSv evolution. This recombination within the type may be easier than between the types. (Van Vugt, *et al* 2001). This study was conducted to find the status of PRRS in different pig raising districts of Nepal.

MATERIALS AND METHODS

Study design

Four districts were selected for investigation.An investigation was performed jointly with veterinarians and technicians from DLSO's of respective districts. Commercial farms and households were visited of those four districts for investigation.

Pig farms in four districts (Chitwan, Makawanpur, Kathmandu and Sunsari) were

visited and were tested on the spot using rapid test kit. (Colloidal Gold immune-chromatographic cards made by SchenzhenLvshiyuan biotechnology Co. Ltd, China).Those pigs which were found positive in rapid test again blood were collected for Enzyme linked immune-sorbent assay (ELISA) test and genotyping of virus. The serum samples were analyzed for the presence of PRRS antibody by ELISAfollowing manufactures protocol. (Shenzhen Lvshiyuan Biotechnology Co. Ltd. Shenzhen, 518120, China). Among the samples which have shown strong positive reaction in rapid test were sent to High Security Animal Disease laboratory, (HSADL) Bhopal, India for genotyping

RESULTS AND DISCUSSION

Out of 150 serum samples tested 50, 40, 30 and 30 from Chitwan, Kathmandu, Sunsari and Makawanpur respectively, overall, 23.3% (35/150) were found positive for PRRS antibody in immuno-chromatograhic test. Districtwise highest prevalence was found in Chitwan (50%) followed by Kathmansdu, (16.6%), Makawanpur (6.6%) and Sunsari (5%) respectively. In ELISA test out of 92 pulled sera samples only three samples showed positive reaction.

To date there is no vaccination practice against PRRS in Nepal. However, in all four districts there was pig population with sero positive reaction. It clearly indicates that, those pigs have exposed to virus in their life time. This is very preliminary investigation carried out by Animal Health Research Division (AHRD).

It is already mentioned that PRRS is a major constraint among the prevalent diseases in pigs worldwide. However, in Nepal systematic investigation on this disease is not done yet. There are several reports relating to abortion and piglet mortalities and studies conducted till date couldn't identify the specific cause of the problem. Production is adversely affected by this disease which causes reproductive failure in adult and heavy mortality with pneumonia in piglets associated with abortion, still birth and death due to pneumonia. Current control measures include the use of vaccines, the management of incoming replacement gilts and implementation of bio-security protocols validated to reduce the risk of PRRSv spread within and between herds. Methods of eliminating virus from endemically infected herds include whole herd depopulation/repopulation, test and removal and herd closure.

CONCLUSION

In our context, it is very important to know the genotype of PRRS virus before introducing any vaccine. Otherwise, vaccine may not be effective. This is very new

diseases in our country, therefore, need to make trained about different aspect of the disease field veterinarians and technicians including advanced molecular techniques for diagnosis, virus isolation, identification and genotyping of the virus for laboratory technologist/technicians. Now the investigation has been expanded to other districts of the country where the pig population is dense. Effective strategies for PRRS disease management will increases production from pig farming which ultimately assist in poverty alleviation and improvement of food security.

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Study on Immune Response of New Castle Disease Vaccines in Layer Chicken

U. M. Singh[1], M. P. Acharya[2], B. R. Joshi[1], and M. Prajapati[2]

1 National Animal Science Research Institute, khumaltar, Lalitpur

2 Animal Health Research Division, khumaltar, Lalitpur

ABSTRACT

*A totlal of 250 day old chickens of Newhampshire breed were procured and raised at shed of Swine and Avian research programme. Khumaltar.The birds were grouped in five different groups in different experimental rooms and immunized with RD F1 at second day of age, at 4th week by ND(*Newcastle disease) *LaSota and F1 and R_2B at eight weeks of age. Treatment group consisted of I – RD F1+RD F1, II – RD F1+LaSota, III – RD F1+RD F1+R_2B, IV – RD F1+LaSota+R_2B, V - No vaccination (control) with 50 chickens in each group. Blood samples were collected from the birds every 15 days post vaccinations, serum separated, transferred to serum collection vial, labeled and stored at -20°C at Animal health Research Division, Khumaltar until laboratory examination was carried out on the serum samples. ND antigen and Antisera (CVL, UK) were used for Haemagglutination Inhibition (HI) test. ND F1 vaccine at day old chicken and ND F1 vaccine repeated at fourth weeks of age produced less than log 2^3 showing no immunity development in experimental chickens. ND LaSota vaccine given at fourth weeks of age produced more immunity (log 2^{5-7}) in vaccinated birds two weeks post vaccination than ND F1 vaccination. ND LaSota vaccine produced in Nepal produced immunity for longer duration. ND R_2B vaccination at 8th weeks of age has produced protective immunity 14 days, 28 days post vaccination and decrease in no of birds with protective immunity after 42 days and 56 days post vaccination in immunized chickens however there was decrease in antibody titre after 70 days post vaccination. ND R_2B vaccination at 8th weeks of age conferred immunity only for 70 days and booster vaccination is required prior to this period.*

Key Words: ND (Newcastle disease), Vaccine, HI Titre

INTRODUCTION

There is growing number of broiler farming in the country. Newcastle disease is one of the most economic important diseases of poultry. Outbreak of New Castle Disease in commercial poultry farms in Chitwan valley was reported which affected estmated

population of 0.4-0.5 millions of commercial chickens and morbidity and mortality was estimated to be more than 50 and 5% respectively. The virus was identified as very virulent New Castle Disease (VVND). Bohora *et al.,* 1996).

Newcastle disease F1, LaSota and R_2B vaccines are being used in the poultry. This study has helped in identification of exact schedule for the RD F1, RD LaSota and R_2B vaccines.

Newcastle disease F1 and LaSota vaccines are produced in country by the Biological Production Division, DLS, Tripureswor, Kathmandu. The vaccine is recommended to be given at 1-7 days and repeated after 4 weeks of age for F1 vaccine and LaSota is recommended at 19-21 days of age and repeated after 3-4 weeks of age, similarly for R_2B at 8-12 weeks of age (Anon, (064/065 BC/2007/2008 AD). This study was proposed to clarify the vaccination schedule against New Castle Disease in poultry flocks of Nepal.

MATERIALS AND METHODS

A total of 250 day old chickens of New Hampshire breed were procured and raised at shed of Swine and Avian Research Programme, Khumaltar. The birds were grouped in five different groups in different experimental rooms and immunized with RD F1 at second day of age, at 4th week by LaSota and F1 and R_2B at eight weeks of age as per Table-1.

Table-1: Experimental Groups of Chicken

Treatment Group	Vaccination	Number of Birds
I	RDF1 + RDF1	50
II	RDF1 + LaSota	50
III	RDF1 + RDF1 + R_2B	50
IV	RDF1 + LaSota + R_2B	50
V	No vaccination (Control)	50

Blood samples were collected from the birds every 14 days post vaccinations, serum separated, transferred to serum collection vial, labeled and stored at -20°C at Animal health Research Division, Khumaltar until laboratory examination. ND (HI) antigen and antiserum were procured from CVL, UK and Charles River USA.

Haemagglutination Inhibition Test

Haemagglutination (HA) and Haemagglutination Inhibition (HI) tests were conducted as described protocol (OIE, 2012). Briefly, to determine HA unit, 25µl of serial two fold dilutions of antigen with equal volume of phosphate buffered saline

(PBS, 7.2 pH) and 25µl of 1 % chicken RBC was added to each well in V shaped microtiter plates and incubated at room temperature for 40 minutes. Likewise for HI 25µl of serial two fold dilution of serum were performed with equal volume of PBS (7.2 pH) and 25µl of 4 HA unit antigen was added to each well in V shaped microtiter plate and incubated at room temperature for 30 minutes. Then, 25µl of 1% chicken RBC was added to each well and again incubated for 40 minutes. The HA titer was expressed as reciprocal of highest antigen/virus dilution that completely showed haemagglutination activities whereas HI titer was expressed as the reciprocal of highest dilution of serum that completely inhibited haemagglutination of 4 HA units of the virus antigen/virus. The value of serology in diagnosis is clearly related to the expected immune status of the affected birds. HI titres was regarded as being positive if there was inhibition at a serum dilution of 1:16 ($\log 2^4$) when expressed as the reciprocal) or more against 4 HAU of antigen. Some laboratories prefer to use 8 HAU in HI tests. While this is permissible, it affects the interpretation of results so that a positive titre is 1:8 ($\log 2^3$) or more. HI titres may be used to assess the immune status of a flock. At present, the HI test is most widely used for detecting antibodies to NDV in birds (OIE Manual, 2009).

RESULTS

Laboratory examination

Table-2: Result (HI Titre) of HI test of post vaccination serum samples from experimental chicken

Age of the birds (days)	Group				
	F1	F1+F1	F1+Lasota (Nepal)+R_2B (Nepal)	F1+LaSota (India)+R_2B (Nepal)	Control
	6 chickens	6 chickens	6 chickens	6 chickens	4 chickens
28	< log 2^2	<log 2^2	< log 2^2	< log 2^2	< log 2^2
42	< log 2^2	log 2^7(1)	log 2^7(1)	log 2^{5-7}(3)	< log 2^2
56	log 2^{5-6}(3)	log2^5(4)	log2^{4-6}(6)	log2^{4-5}(6)	log 2^2
70	No titre	No titre	log2^{6-8}(6)	log2^{6-8}(6)	No titre
84	No titre	log 2^4(1)	log2^{3-9}(6)	log2^{3-5}(6)	No titre
98	No titre	log 2^{3-5}(4)	log 2^{4-6}(5)	log 2^{3-4}(2)	<log 2
112	No titre	No titre	log 2^6(1)	log 2^{4-5}(2)	No titre
126	No titre	No titre	log 2^6(1)	log 2^4(1)	No titre
140	No titre	No titre	log 2^5(1)	No titre	No titre

Note-HI titre less than log 2^3 titre are considered as not protective immunity

-No. in parentheses indicates no of chicken showing protective immunity against ND virus

ND F1 vaccine at day old chicken and ND F1 vaccine repeated at fourth weeks of age produced less than log 2^3 showing no immunity development in experimental chickens. ND LaSota vaccine given at fourth weeks of age produced more immunity (log 2^{5-7}) in vaccinated birds two weeks post vaccination than ND F1 vaccination. ND LaSota vaccine produced in Nepal produced immunity for longer duration. ND R_2B vaccination at 8^{th} weeks of age has produced protective immunity 14 days, 28 days, 42 days and there was a decreasing no of birds with protective immunity after 56 days and 70 days post vaccination in immunized chickens however there was decrease in antibody titre after 70 days post vaccination. ND R_2B vaccination at 8^{th} weeks of age conferred immunity only for 70 days (Table-2).

DISCUSSION

New Castle Disease vaccines (F1, Lasota and R2B) are used in poultry flocks. There are different brands from different countries are being imported. F1 vaccine given at day old and booster vaccine at 4th week of age gave protective immunity in less number of birds compared to La Sota group. So it is better to give booster vaccination with ND La Sota at forth weeks of age. R2B vaacination at eight weeks of age conferred immunity for 70 days post vaccination so it is neccessary to give booster vaccinnation before the end of that period. ND La Sota vaccine produced in Nepal produced immunity for longer duration.

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A Study of Mycoplasmosis in the Broilers and Layers of Chitwan and Kaski Districts of Nepal

K. Pandey[1], U. M. Singh[2], B. R. Joshi[2]

1. Institute of Agriculture and Animal Science, Rampur, Chitwan

2 National Animal Science Research Institute, NARC, Khumaltar

ABSTRACT

Mycoplasmosis is one of the economically important diseases in poultry worldwide causing poor weight gain, low egg production, low feed conversion efficiency, and high mortality.The study was undertaken to assess sero-prevalence of Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) in commercial layers and commercial broilers in two locations namely Chitwan and Pokhara from January to June 2014. Two stage sampling was done to select the research sites and birds were selected purposively. Fifty sera samples were collected from each research site for each category of birds totaling 200 samples and all necessary information about sampled bird were taken. The collected samples were tested by Rapid Serum Agglutination (RSA) test by using MG and MS antigens (Lillidale diagnostics, U.K.) followed by ELISA for MG(X-OvoFlockscreen, Mycoplasma gallisepticum (Mg) Antibody ELISA kit, X-Ovo limited, U.K.) to detect the presence of antibodies against MG and MS. The overall prevalence for MG and MS on RSA was 26.5% (n=53) and 42.25% (n=85) respectively. The prevalence of MG was higher in birds of Chitwan which was not significant compared to that of Pokhara, and the prevalence of MS in birds of Chitwan was significantly higher to that of Pokhara ($p<0.01$). The prevalence of MG was found to be 6.0% (n=11) under ELISA. On RSA, the prevalence of MG and MS for commercial broilers (100) and commercial layers (100) were 24% & 36% and 27% &32%; respectively. The sero-prevalence of MG and MS infection is widespread which poses a high risk to commercial poultry production. The birds are to be checked periodically to determine the status of Mycoplasma infection and sero-positive ones should be culled to take effective control measures.

Key words: Mycoplasmosis, Sero-prevalence, RSA, ELISA, poultry, Chitwan, Pokhara

INTRODUCTION

Avian mycoplasmosis is an economically important poultry disease worldwide, caused by several pathogenic mycoplasmas of which *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) are the most important (OIE, 2008). *Mycoplasma gallisepticum* is responsible for chronic respiratory diseases (CRD) of chickens (Ley and Yoder 1997) which is characterized by respiratory rales, coughing, nasal discharges, conjunctivitis and infraorbital sinusitis and Mycoplasma synoviae (MS) causes mainly the joint infections CRD takes a long course to develop and infection remain longer. CRD caused by *Mycoplasma gallisepticum* infection is also known as "Air sac disease" because of severe air sacculitis as a result of complication or aggravation by respiratory viruses like infectious bronchitis virus or New castle disease virus and usually by *Escherichia coli* (Ley and Yoder, 1997). MG is transmitted vertically through infected eggs and horizontally by inhalation of contaminated dust, airborne droplets and feathers. The most characteristic signs of Mycoplasmosis in adult flocks are tracheal rales, swelling of head and face, nasal discharge, and coughing. The birds usually have reduced body weight with reduction of feed consumption. In laying birds, the egg production decline down severely and remain in lowest point. *MG* can be diagnosed based on morphological and cultural characteristics, biochemical and serological properties. *MG* serology methods, such as serum plate agglutination (SPA),enzyme linked immunosorbent assay (ELISA). This study was carried out to find the status of mycoplasmosis in broiler and layer chickens.

MATERIALS AND METHODS

Study area and selection of birds

Under cross sectional study method, two stage sampling method was done during the selection of the birds. On first stage the districts- Chitwan and Kaski were selected and on second stage the places- Mangalpur V.D.C from Chitwan and Pokhara sub-metropolitan city from Kaski were selected. The birds were selected randomly. A total of 200 blood samples -100 from commercial broilers and 100 from commercial layers; 50 each samples from Kaski district and 50 samples from Chitwan districts were collected from January 2014 to June 2014.

Sample Collection and Laboratory Examination

The blood was collected from the wing vein aseptically in 3 ml syringe by using 23 gauze needle. The syringe was left for over-night in slant position. Serum was harvested in ependorf tube. It was then stored in freezer at -20^{0}C till laboratory test was carried out. The serological analysis was done in the laboratory of Animal Health Research Division (AHRD), NASRI, NARC, Khumaltar, Kathmandu, Nepal.

Rapid Serum Agglutination Test (RSA) and ELISA

The RSA test was performed by using Lilli Test MG & MS Rapid Serum Agglutination (RSA) Test Antigen (Lillidale diagnostics, U.K). 25 µl serum was placed in the plate using Micropipette and immediately equal volume of serum sample was added. The antigen and the sample were mixed properly by using glass rod or stick. After 2 minute the observation was done for the formation of granules for the positive cases.Agglutination was assigned score from (+) to (+++). The sera samples having agglutination score (++) or greater were recorded as positive and used for calculation of prevalence.

The X- OVO Flock screen ™ *Mycoplasma gallisepticum* (Mg) Antibody ELISA kit (X-Ovo limited, U.K.) was used as a screening test to confirm whether a flock has been exposed to *Mycoplasma* and therefore whether there are likely to be carrier birds in the flock. Significantly elevated Immunoglobulin G (Ig G) levels are detectable by ten days of post infection. The protocol according to the manufacturer's instruction was followed.

Briefly, samples were diluted five-hundredfold (1: 500) with the diluent i.e. 1µl of serum sample and 500µl of sample diluent was dispensed in a well of a plate previously coated with MS antigen. Plates were incubated for about 30 minutes at room temperature and were washed with 300µl wash buffer for four times. The enzyme conjugant was added and then the plates were again incubated for 30 minutes and washes as before. Again, the substrate reagent was added and the plates are incubated for 15 minutes at room temperature. Finally, the stop solution was added and the plates were read under wavelength of 550 nm. Results were then expressed as serum-to-positive ratios (S/P) ratios relative to standard positive control. Serum samples, greater than 1220 mg- titre (>0.275 S/P) were considered as positive.

Statistical Analysis

The information regarding bird was taken during blood collection. Descriptive study was displayed through percentage, frequency and averages. Association of prevalence and risk factors were studied through chi square test. The statistical analysis was completed using Excel 8.1 version and SPSS 16.0 version. The p value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

The overall prevalence of *Mycoplasma gallisepticum*and *Mycoplasma synoviae* can be shown from the Table-1.

Table-1: Sero-prevalence of MG and MS in poultry of Chitwan and Pokhara

Test	Result	Chitwan	Pokhara	P value (χ2test)
Rapid serum agglutination Test for *Mycoplasma gallisepticum*	Positive	30 (30%)	23 (23%)	2.516 (0.113)
	Negative	70	77	
Rapid serum agglutination Test for *Mycoplasma synoviae*	Positive	55 (54.5%)	30(30%)	24.610 (0.000**)
	Negative	45	70	
ELISA (MG)	Positive	**6 (6.5%)**	**5 (5.5%)**	**0.293 (0.864)**
	Negative	87	88	
	Suspect	7 (13.5%)	7 (12.5%)	

Broilers

The result of RSA test (MG), RSA test (MS) and ELISA (MG) of samples of broilers are presented in Table-2

Table-2: Prevalence of MG/MS in commercial broilers

Test	Result	Location			χ2test		
		Chitwan	Pokhara	Total	Value	Df	Asymp. sig. (2 sided)
RSA Test (MG)	Positive	14	10	24	0.877	1	0.349 (NS)
	Negative	36	40	76			
RSA Test (MS)	Positive	26	10	36	11.11	1	0.001**
	Negative	24	40	64			
ELISA (MG)	Positive	1	1	2	0.086	2	0.086 (NS)
	Suspect	3	1	4			
	Negative	46	48	90			

*significant, NS= Non significant, Df= Degree of freedom

Regarding MG in broiler farms, the prevalence was 33.33% in Iran (Feizi *et al*, 2013). In this study, the prevalence of MG in broiler parent was 19% which is almost half the prevalence mentioned by Fiezi *et al., (*2013) (33.3%). Fiezi *et al.,* (2013) also reported higher MG infection in female parent than in male (10.14%) broiler parents. A study of MG conducted in Iran by Seifi and Shirzad (2012) stated highest prevalence of 21.4% in 2003 in ELISA test. The flock size has not significantly affected the infection prevalence. The sero-prevalence of MG in serum plate agglutination test was 58.90% in a total of 382 sera samples of broiler parents in Bangladesh, (Sarkar *et al.,* 2005) which was found to be far greater than our findings. This is also contrast to the finding of Feberwee *et al.*, (2008) reported that only 6% in broiler parents were infected with MG in Holland.

Layers

The result of RSA test (MG), RSA test (MS) and ELISA (MG) of samples of layers are presented in Table-3

Table-3: Prevalence of MG/MS in layers

Test	Result	Location			χ2test		
		Chitwan	Pokhara	Total	Value	Df	Asymp. sig. (2 sided)
RSA Test (MG)	Positive	18	9	27	4.11	1	0.043*
	Negative	32	41	73			
RSA Test (MS)	Positive	21	11	32	4.59	1	0.032*
	Negative	29	39	68			
ELISA (MG)	Positive	3	1	4	1.45	2	0.484 (NS)
	Suspect	9	7	16			
	Negative	38	42	80			

Regarding the result in layer birds, this is in line to the finding by Osman *et al.,* (2009) for commercial layers while it is in contrast to the findings in young birds .The prevalence of MG in commercial chickens in this study is also in contrast to the findings of Sarkar *et al.,* (2005) and Hossain *et al.* (2007). They described the overall seroprevalence of MG infection in different flocks of commercial layer chickens in Patuakhali as 56.9%, in Feni as 58.9%, and 55.13% respectively in Rajshahi district of Bangladesh. Similarly, Feberwee*et al.*, (2008) in Holland reported much higher (73%) positivity in commercial layers which is almost thrice to the present finding. In another study, Hossain *et al.,* (2007) found 45.1% prevalence of MG in Rajshahi and surrounding districts of Bangladesh.

CONCLUSION

Mycoplasmosisis an important infectious disease of poultry and poultry farms are at greater risk of outbreak of diseases. In this study the overall prevalence was also high which was found higher in the Chitwan than in Pokhara. The infection seems to be increasing in the places where the poultry industries are raising. The reason might be due to the higher population density of birds in poultry farms and warmer climate at Chitwan causing heat stress. Poor ventilation and insanitary practices might also

have played a role in it. The lacking biosecurity and mishandling of the equipment's by the personals working may have played an important role. The breeder farms also were found affected by the mycoplasma infection in this study, which might be due to the maintenance of breeder stock for a long period of time and replacement of breeder stock with the progeny of the same flock.

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Evaluation of Thermostability of NF I-2 Vaccine for Newcastle Disease Prevention in Village Chicken

M. P. Acharya[1]. S. K. Adhikari, [2] U. M. Singh[3], S. P. Gautam[4] and S. Shrestha[5]

1. Animal Health Research Division, NARC, Nepal
2. District livestock service Office, Morang,
3. Himalayan College of Science and Technology, Kalanki
4. Central Biological Production Laboratory, Tripureshwor
5. Hester Biosciences Nepal P. Ltd, Nepal

ABSTRACT

A study was conducted at Animal Health Research Division, Khumaltar, to evaluate thermostability of ND I-2 vaccine for Newcastle Disease prevention in village chicken. The vaccine was prepared at Biological Production Division, Tripureswor. Titration of vaccine was done in embryonated eggs of 10 days, HA test was done as per OIE guidelines and EID_{50} was calculated. ($10^{6.64}$ /0.1 ml) Three treatments of Vaccine were made and kept in different temperature. Treatment A: at 4°c in Refrigeration for 15 days, Treatment B: at Room temperature for 15 days and Treatment C: at 37°c temperature in an incubator for 7 days. 120 local chicks of day old age were reared and randomly divided into 4 groups (30 birds in each) named as Treatment Group A, B, C and Control. After 4 weeks birds were given ND, I-2 vaccine by intra ocular route @ 0.1ml/bird except control group. Before vaccination blood were collected for base line antibody monitoring against ND. Blood samples were collected again 14 days and 28 days post vaccination for antibody analysis. All chickens of 4 treatment groups were challenged by field virus of ND @ 0.1ml ($EID_{50}10^6$ /0.1 ml) by intraocular route and birds were observed daily and PM was performed. Death records of Treatments A, B, C and control group were found 3.34%, 13.33%, 23.34% and 90% respectively. Average antibody titre against Newcastle disease in pre-vaccinated blood were 2^0 $2^{2,}$ $2^{0,}$ 2^2, in 14 days post vaccinated blood were $2^{6,}$ $2^{6,}$, $2^{3.4}$, $2^{1.5,}$ and in 28 days post vaccinated blood were $2^{6.2,}$ $2^{5.2,}$, 2^4 , $2^{2.4}$ in treatment A, B,C and no titre in control group respectively. Antibody titre and Mortality % showed that ND I-2 vaccine used for treatment A and B were found effective in prevention of New Castle Disease in village chicken. Evaluation of Longevity of immunity and Field trials are needed for verification and field application.

Key words: *ND I-2 vaccine, Themostability, Antibody titre, EID_{50}*

INTRODUCTION

Newcastle disease (ND) is an acute viral infection of great economic significance to the poultry industry worldwide. ND is a highly contagious viral disease that affects all age groups of birds (Alexander *et al.,* 1997). The ND caused by an avian paramyxovirus serotype 1 (APMV-1), family Paramyxoviridae. (Lamb *et al*., 2000). Depending upon the pathotype involved and susceptibility of the flock, the virus causes mortality ranging from 0 to 100 percent.

The ND is considered as one of the most economically important viral diseases of poultry in Nepal. It is an endemic and sometimes epizootic disease in chickens and it causes a great loss in backyard chickens where vaccination is not in practice till date and one of the main sources of animal protein (Spradbrow, 1993).

Prevention of Backyard chickens from virulent Newcastle disease is an essential first step to improve their productivity. ND is a major constraint to backyard poultry production throughout developing countries, frequently causing mortality rates of 75 to 100% in unvaccinated flocks (Spradbrow, 1992). ND can be controlled by vaccination. There are several types of ND vaccines suitable for use in commercial chickens but which are not suitable for backyard chickens due to various region like availability in multi dose, difficulty in maintaining cold chain and may not suitable for existing strain of virus.. The ND, I-2 vaccine has been developed for local or regional use in controlling ND in back yard chickens. Vaccines and vaccination programs vary widely, depending on several factors such as level of biosecurity, local strain, maternal immunity, along with storage of vaccine. Not much research has been done on the control of ND in backyard chickens in Nepal. Therefore an attempt to evaluate the thermostability of ND I-2 vaccine was conducted in this study.

MATERIALS AND METHODS

Preparation of thermostable ND I-2 vaccine

The master seed of the avirulent thermotolerant NDV (strain I-2) was imported from Department of Veterinary Pathology of the University of Queensland, Australia and vaccine was prepared at Biological Product Division, Tripureswor.

Titration of vaccine

The titer of ND vaccine was measured by preparing tenfold serial dilutions of the vaccine suspension, inoculating these into embryonated eggs of 10 days following OIE manual. Results of vaccine titration were determined mathematically by the method of Reed and Muench (1938) which was as follows.

$$\text{Index} = \frac{(\%\text{ infected eggs at dilution immediately above 50\%}) - 50\%}{(\%\text{infected eggs at dilution above 50\%}) - (\%\text{ infected eggs at dilution below 50\%})}$$

Experimental designs

Storage of Vaccines for Thermostability

For evaluation of thermo stability of ND I-2 vaccine, prepared vaccines were divided into 4 groups and kept at different temperature.
Treatment A: at 4°C in Refrigeration for 15 days,
Treatment B: at Room temperature (25^0-30^0 C) for 15 days
Treatment C: at 37°C temperature in an incubator for 7 days, there after vaccine were kept in Refrigeration.

Experimental trial of ND, I-2 vaccine

Embryonated eggs from local chickens were hatched in Suwal Hatchery, Bhaktapur. 120 day old a chicks were reared and randomly divided into 4 treatment groups (30 birds in each) named as Treatment Group A, B, C and Control. Chicks were provided feed and water as per requirements according to respective ages. IBD vaccine was given on 15 days. Before vaccination, on 27^{th} day blood were collected for base line antibody monitoring against ND. Vaccine was first diluted in 0.5 ml PBS then added 4.5 ml PBS to make 10 fold dilution and 4 weeks old birds were given ND I-2 vaccine by intra ocular route @ 0.1ml/bird as described in protocol.
Treatment A: Vaccine kept at 4°C in Refrigeration for 15 days
Treatment B: Vaccine kept in Room Temperature for 15 days
Treatment C: Vaccine kept at 37 °C in an incubator for 7 days
Control group: No vaccine but kept as control.

Blood were collected on 42 (14 DPV) and 56 days (28 DPV). All chickens of 4 treatment groups were challenged by field isolate of velogenic NDV@ 0.1ml by intraocular route on 56 days of age. Clinical signs of birds were observed daily and PM was performed. Tissue samples were collected for histopathological examination.

Table-1: Protocols of experimental trial

Days	**Activities**
Day 1	Rearing of 120 chicks, providing feed, water etc.
Day 7	Birds were randomly divided into 4 groups (30 birds in each group) and named as Treatment group A, B, C and control
Day 15	Vaccinated with IBD vaccine
Day 27	Blood were collected for base line antibody monitoring
Day 28	Given ND I-2 vaccine by intra ocular route @ 0.1ml/bird for each group
Day 42 and 56	Blood were collected for antibody monitoring
Day 56	Challenged by field virus of ND @ 0.1ml by Intraocular route. Clinical sign, PM reports and death records were noted thereafter for analysis.

Serology

All the serum samples were analyzed using Haemagglutination (HA) and Haemagglutination Inhibition (HI) test described in OIE manual 2012.

Results

Immunization with different vaccines in experimental birds

Table-2: Comparative antibody titre using HI test in sera of experimental birds of different treatment groups before and after vaccination.

Treatment groups	Days of Vaccination	Antibody titer using HI test									Protective level of specific immunity of NDV
		1:0	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Treatment A (Refrigeratio)	1 DPrV	-	-	5	-	-	-	-	-	-	0%
	14 DPV	-	-	-	1	4	5	7	13		96.67%
	28 DPV	-	-	-	-	-	2	13	8	7	100%
Treatment B (Room temp^{r})	1 DPrV	4	8	18	-	-	-	-	-	-	0%
	14 DPV	-	-	-	-	-	5	17	4	4	100%
	28 DPV	3	-	-	2	-	12	10	3	-	83.34%
Treatment C (Incubator)	1 DPrV	15	6	1	2	3	3	-	-	-	20%
	14 DPV	5	-	2	3	8	10	2	-	-	66.67%
	28 DPV	3	2	4	6	6	6	3	-	-	50%
Control	1 DPrV	9	12	9	-	-	-	-	-	-	0%
	14 DPV	12	-	14	2	2	-	-	-	-	6.67%
	28 DPV			19	8	3	-	-	-	-	10%

When we analyze antibody titers of sera of experimental birds as shown in Table-2, it was observed that all treatment groups had no specific immunity against NDV on 1 DPV. Treatment groups A and B had protective level of specific immunity of NDV on 14 DPV & 28 DPV but immunity level of group B was decreased from 100 % on 14 DPV to & 83.34 % on 28 DPV

Challenge of virus in experimental birds

All experimental chickens of 4 treatment groups were challenged by field isolate of velogenic NDV at the dose rate of 10^6 EID_{50}/0.1ml/birds by intraocular route (I/O) on 56 days of age after collecting blood for determining post antibody titers.

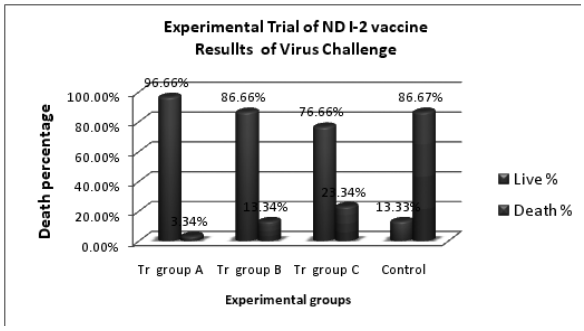

Fig. 1: Comparision of live and death in experimental birds after challenge

It was observed that 96.66 % birds of treatment group A and 86.66 % birds of treatment group B were live. It clearly indicates that ND I-2 vaccine are effective in treatment group A and B. Similarly it was found that vaccines were not effective in treatment group C and control because live percentage of birds were below the protective levels (≥ 80 % live) (Fig.-1).

DISCUSSION

A specific objective of this study was to evaluate the thermostability of ND I-2 vaccine for Newcastle Disease prevention in village chicken. Commercial vaccines are not thermolabile. Cold chains maintaining is not possible in rural areas in our context. Village flocks are small, scattered and multi-aged. In this scenario such thermostable vaccine has great value to prevent Newcastle disease.

All the experimental birds were in unprotected state at 1 DPV but at 14 DPV the protective level of specific immunity to NDV were 96.67 %, 100% , 66.67%, 6.67 % and at 28 DPV were 100 %, 83.34 % , 50 %, 10% respectively for treatment group

A, B, C and control. The result showed that group A was found effective in birds giving 100% protection whereas group B (vaccine kept in room temperature ranging between 25^0c -33^0c in an average) was found effective in birds giving 86.67% of protective level of immunity to NDV and there was increasing trend in HI antibody titer up to 14DPV for group A and B, which is in agreement with the statement of Illango, (2008) where the author reported that eye drop application of thermostable ND I-2 vaccine in housed chickens attained 100% protection to Newcastle disease with Mean ND antibody HI $\log_2$ titer of 6.19± 1.3 compared with 89% protection by unhoused chickens with Mean ND antibody HI $\log_2$ titer of 5.40. ± 2.5. Tu *et al*. (1998) reported that freeze-dried I-2 vaccine lost about 1 $\log_{10}$ of infectivity when stored for 6 days at 26-32°C and vaccine reconstituted after storage for 24 days at 30-35°C still produced substantial protection in vaccinated chickens. It should be possible to reach village chickens in any part of Vietnam with this vaccine, without refrigerated transport.

In this study at 1DPV in all treatment and control group there was no specific immunity against ND. Similarly mean HI titre of sera on 14 DPV of group A was highest (78.13) and control was lowest (3.46) value but group A, B, C have protective level of immunity. The HI titer ≥ 3 ($\log_2$) were considered protective based on the findings of *et al* (1991) who reported that birds with HI titres ≥ 3 ($\log_2$) were protective against challenge with a virulent strain of ND virus. The protective antibody titres >3 ($\log_2$) recorded in 143 (8.91%) out of 1605 village chickens screened for HI antibody before vaccination suggested previous exposure of the birds to field strains of ND virus (velogenic).

During study of virus challenge, it was observed that 96.66 % birds of treatment group A and 86.66 % birds of treatment group B were live after challnege with field isolate of velogenic NDV which indicates that ND I-2 vaccine are effective in treatment group A and B where as group C and control have higher mortality than protective levels (≥ 80 % live). It was also observed that death of birds were started on 6^{th} day in treatment group B & C and maximum number of death were found from 9^{th} to 12^{th} day in groups B, C, and control. Likewise in control group, death was observed from 9^{th} to 13^{th} day and maximum numbers of death was recorded on 12^{th} day. In a study Barlleng Leonard Mogoje (2006) reported that more than 85 % of vaccinated chickens, irrespective of the method of application, survived the challenges. Mortalities started to occur on the 48 hours after challenge while, on the 4^{th} day all the unvaccinated SPF chickens died as well as the unvaccinated

free-range chickens In a similar study by Wambura, Kapaga and Heyera (2000), the I-2 strain thermostable ND vaccine used to vaccinate free range chickens, got protection of 100 % by means of the eye drop route and 80 % in both drinking water and feed application.

Post mortem findings of dead birds after virus challenge showed severe hemorrhage and necrotic plaques in proventriculus, hemorrhage in intestine, trachea and caecal tonsil in all dead birds. Proventriculus, caecal tonsil and gut haemorrhage scored more than 90 % in all groups that showed infection with ND virus. The results were very similar to the Barlleng Leonard Mogoje (2006).

CONCLUSION

The mean antibody titre of experimental birds at 14 DPV, 28 DPV and live percentage after challenge indicate that ND I-2 vaccine kept both in refrigeration and room temperature was effective in controlling NDV. Therefore, it can be concluded that this vaccine has a positive impact in controlling N D on chicken flocks. it is affordable to all farmers to use, it don't require strict cold chain facilities and easy to administer by farmers and can be used irrespective of age. The control of ND will contribute to improve village poultry production as well as commercial poultry. If village chicken production can be optimized, there will be major benefits in terms of poverty alleviation and human nutrition

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Nepalese Vet. J. 33:104-109

A review on effect of microbiota in the gastrointestinal tract of chickens

Tanka P Prasai

Department of Livestock Services

ABSTRACT

Microflora of the chickens gut are responsible for proper nutrition, detoxification of toxins, growth performance and protection against pathogenic organisms. Various physiological roles are served by the microbial communities of the gastrointestinal tract and changes to gut microbiota can affect animal health and productivity. Use of antibiotic growth promotants have been banned in Europe and this trend has been extending to other nations via consumers influenced and increased awareness promoting more natural organic farming system.Beneficial bacteria in the guts can decrease populations of harmful bacteria and are associated with strong immune system. Microbial identification has traditionally involved culturing, with use of selective and non-selective media. The majority of microbiota (up to 90% in some environments) are not able to be cultured. Recent advances in DNA sequencing techniques have revolutionised the ability to document microbial diversity of whole microbial populations, including of the gastrointestinal tract by 16S ribosomal sequencing.These advances in technique open the door to a greater understanding of the gut microbiome in response to a range of treatments, including prebiotics.

INTRODUCTION

The gastrointestinal tract (GIT) is the major digestive and absorbing body part and it plays avital role in birds' growth and production. A varied microbiota is found throughout the GIT and is most extensive in the caecum. Nutrition, detoxification of toxins and toxic metabolites, growth performance, protection against pathogenic bacteria are the major effects of the GIT microflora of the chickens. The GIT microbiota are responsible for influencing health and productivity of host animals and birds(Amit-Romach *et al.,* 2004).Microbiota present in the GIT have been considered as another organ of the body (Possemiers *et al.,* 2011). Many physiological roles can be served by the microbiota of GIT and changes to GIT microbiota can affect animal health, and vice versa (Gabriel *et al.,* 2006)%u.

Antibiotic growth promotants

Antibiotics were in the past provided in feed as antibiotic growth promotants (AGPs), particularly in meat bird production, with the intent of suppressing pathogenic bacteria in the gut. This practice was prohibited from 1 January 2006 in the European Union (Huyghebaert *et al.,* 2011) and this trend is extending to other developed countries via consumer influenced decrease of AGP usage in favour to more natural farming practices such as free-range or organic (Gabriel *et al.,* 2006), primarily in response to concerns related to the enhancement of antibiotic resistant microorganisms and AGP residence in product. Animal antibiotics registered for use as growth promoters/feed efficiency for layers chicken in Australia include arsenicals (3-nitro-arsonic acid) and Flavophospholipol or Bambermycin (Sarmah *et al.,* 2006), and virginiamycin, with the condition of a withholding period of 21 days. Antibiotics were commonly used for control of the pathogenic GIT coccidia, which can produce extensive damage to the mucosal layer (Kimura *et al.,* 1976). There is therefore need for alternatives to antibiotic treatment.

Microbiota in the GIT of Poultry

There is great dissimilarity in the anatomy and physiology of monogastric and multi-gastric animals, and it is no surprise that poultry have a different GIT microbiota compared to simple stomach animals (Gabriel *et al.,* 2006). Microbial activity in the small intestine is low relative to that of the crop and the caeca of the GIT (Fuller, 1984; Gong *et al.,* 2002). Gram positive facultative anaerobes dominate in the crop through to the distal end of the ileum. Strict anaerobes are the dominant form present in the caeca but other facultative microbes are also present in minor numbers (Lu *et al.,* 2003). However, GIT species composition can vary between birds, reflecting inoculation history, diet, animal health and associated environment (Zhu *et al.,* 2002). Thermal stress and breeding density are associated with a decrease in the level of beneficial microbes (Knarreborg *et al.,* 2002; Lu *et al.,* 2003), and an increase in the pathogenic microbial population in the GIT (Apajalahti *et al.,* 2004; Suzuki *et al.,* 1989).

Procedures for the maintenance of a favourable GIT have received relatively little scientific attention, although there are a range of pro and pre-biotic feed supplements on the market. Prebiotics are natural or chemical substances whereas probiotics are beneficial microbial cultures, with both used as additives in chicken rations. Various antimicrobial metabolites can be produced by beneficial bacteria with interaction

with the intestinal mucosa in the gut. These metabolites have bacteriostatic and bactericidal properties and prevent the growth of pathogenic microorganisms. Microbes like *Campylobacter sps., Yersinia, Salmonella, Clostridium perfringens; Listeria, Escherichia coli,* etc. are examples of pathogenic bacteria associated with the GIT of birds.

Another common commercial practice of consequence to the GIT microbiome is the hatching of eggs in a sterile environment with well-maintained biosecurity measures, with the GIT of the newborn chicken isolated from parental inoculum sources. The microflora that first colonise the GIT obstruct growth of other microbes in a process termed 'competitive exclusion' (Gabriel *et al.,* 2006). Apajalahti *et al.* (2004) noted that in day old chicks, the intestinal contents of ileum and caeca contain 10^8 and 10^9 microbes per g FW, while by the three day old chicks, the microbial population increased to 10^9 and 10^{11} microbes per g fresh weight (FW), a level maintained until 30 days of age. Hatching units can be treated with caecal microbiota of well-matured healthy birds or selective microbiota (pro-biotics) should be considered at hatching to diminish the chances of colonisation by Salmonella species. (Stavric and D'aoust, 1993) concluded that better results in terms of bird health and productivity are achieved by application of numerous bacterial species than with a few species of microorganisms

Beneficial GIT microbiota

Rolfe (1991) described beneficial bacteria as those that can decrease GIT populations of harmful bacteria. An induced change in the ability of the harmful bacteria to adhere to GIT, or to a decrease in growth of pathogenic bacteria. Additionally, beneficial flora are associated with a strong immune system (Rolfe, 1991; Stanley *et al.,* 2012). For instance, lactic acid produced in the bird crop by Lactobacilli is lethal to coliforms and other species of microbes, but is beneficial to Lactobacilli (Fuller, 1984). Similar effects are noted for short chain fatty acids (Van der Wielen *et al.,* 2000). Furthermore, proteaceous toxins, termed bacteriocins, generated by lactobacilli also inhibit the growth of pathogenic bacterial strains. Similarly, microbes like *Salmonella sps, coliforms* and *campylobacteria* are harmful bacteria whose growth can be prevented by a toxin, reuterin, produced by *L. reuteri* (Mulder *et al.,* 1997). Thus the lactobacilli produce an overall inhibiting effect on other (harmful) bacteria. Lactobacilli may also retard growth of other microorganisms through nutrient depletion, a process known as competitive antagonism. As a result of interaction between the intestinal

mucosa and microbiota, various metabolites like polyamines and short chain fatty acids can be produced in the gut. These metabolites can produce positive effects on anatomy and physiology of birds (Mitsuhiro and Jun-ichi, 1994).

Microbial identification has traditionally involved culturing, with use of selective and non-selective media. However, Lan *et al.* (2002), Hugenholtz and Tyson (2008) and Stanley et al. (2013) report that the majority of microbiota (up to 90% in some environments) are not able to be cultured. Recent advances in DNA sequencing techniques have revolutionised the ability to document microbial diversity of whole microbial populations, including of the GIT (Stanley *et al.,* 2014), by 16S ribosomal sequencing. More recent research has indicated the presence of more than 900 microbial species, belonging to 140 genera (Apajalahti *et al.,* 2004; Wei *et al.,* 2013). Up to 90% of species were unknown (Apajalahti *et al.,* 2004; Gong *et al.,* 2002; Lan *et al.,* 2002; Zhu *et al.,* 2002). These advances in technique open the door to a greater understanding of the GIT microbiome in response to a range of treatments, including prebiotics.

CONCLUSION

Microbiota present in the GIT of chickens is responsible for balance nutrition and detoxification of various toxins present in the digestive tract. Useful microorganisms also reduce pathogenic bacteria and maintain the health and well being of birds. Traditional culturing techniques for identification of bacterial species should be substituted by recent advanced in DNA 16S ribosomal sequencing for more precise proper diagnosis of microbial species.

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Detection of antibodies against *Toxoplasma gondii* in different livestock species in Nepal

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

Animal Health Research Division, NARC, Khumaltar, Nepal

ABSTRACT

Toxoplasmosis is a cosmopolitan protozoan zoonoses caused by Toxoplasma gondii*, which infects humans and most warm-blooded animals and is responsible for major economic losses in all classes of livestock through abortions, still birth and neonatal losses, especially in sheep. In Nepal, there is very little documented information on the epidemiology of toxoplasmosis in livestock and its public health implications. A study was carried out to determine the prevalence of Toxoplasma gondii in different livestock species of Nepal using ID Screen Toxoplasmosis Indirect Multi-species ELISA kit (ID Vet, France). A total of 1347 pre-monsoon serum samples were collected in which mountain, hill and terai region contributed 523, 387 and 303 samples respectively. Similarly, a total of 1256 post monsoon serum samples were collected in which mountain, hill and terai region contributed 481, 352 and 413 samples respectively. During pre-monsoon, the total samples collected from goat, sheep, cattle, buffalo, swine, yak and chyangra (Mountain goat) were 393, 110, 387, 262, 91, 70 and 34 respectively. During post monsoon, the total samples collected from goat, sheep, cattle, buffalo, swine, yak and chyangra were 381, 102, 362, 281, 60, 35 and 35 respectively. The overall prevalance of toxoplasmosis in different livestock species in Nepal was found to be 22.6%. The overall prevalance of toxoplasmosis in different livestock species in Nepal during pre-monsoon was found to be 27.7%. Highest prevalence was found to be in Swine (53.84%) followed by Chyangra (50%), Cattle (32.56%), Goat (31.55%), Sheep (17.27%), Buffalo (13.74%) and Yak (2.85%). The overall prevalance of toxoplasmosis in different livestock species in Nepal during post-monsoon was found to be 17.03%. Highest prevalence was found to be in Chyangra (74.29%) followed by Swine (58.33%), Yak (37.14%), Goat (31.55%), Sheep (18.63%), Buffalo (7.83%) and Cattle (2.21%). Pre-monsoon prevalence of Toxoplasmosis in different livestock species in mountain, hill and terai region was found to be 29.44%, 23.77% and 41.9% respectively. Similarly, post monsoon prevalence of Toxoplasmosis in different livestock species in mountain, hill and terai region was found to be 24.74%, 11.9% and 12.8% respectively.*

Key Words: Toxoplasmosis, cattle, buffalo, sheep, yak, swine, goat, chyangra, ELISA.

INTRODUCTION

Toxoplasmosis is a cosmopolitan protozoan zoonoses caused by *Toxoplasma gondii*, which infects humans and most warm-blooded animals. The distribution of this parasite depends on regions and weather condition where the oocysts survive in environment. (Dubey, 2004 & Dubey, 2008). The occurrence and distribution of *T. gondii* have been found to be influenced by climatic conditions such as temperature, rainfall and humidity (Arko-Mensah *et al.,* 2000).

Felids are the definitive host of this protozoan parasite being the only species able to excrete sporulated oocysts into the environment while mammals and birds act as intermediate hosts. Intermediate hosts become infected by ingestion of sporulated oocysts, csyst-contaminated meats, especially from pig and sheep and also after contact with free tachyzoites or congenitally by placenta.

It is responsible for major economic losses in all classes of livestock through abortions, still birth and neonatal losses, especially in sheep (Van der *et al.,* 2000). However the infection in cattle does not usually cause clinical symptoms as they have a high natural resistance to this parasite (Dubey *et al.,* 1994).

In Nepal, there is very little documented information on the epidemiology of toxoplasmosis in livestock and its public health implications. Similarly, there is virtually no data on the seroprevalence of toxoplasmosis in livestock species. The main objective of the present study was therefore to establish the prevalence and distribution of anti-*T. gondii* antibodies in livestock species in the three different ecological zones of Nepal.

METHODOLOGY

Site selection

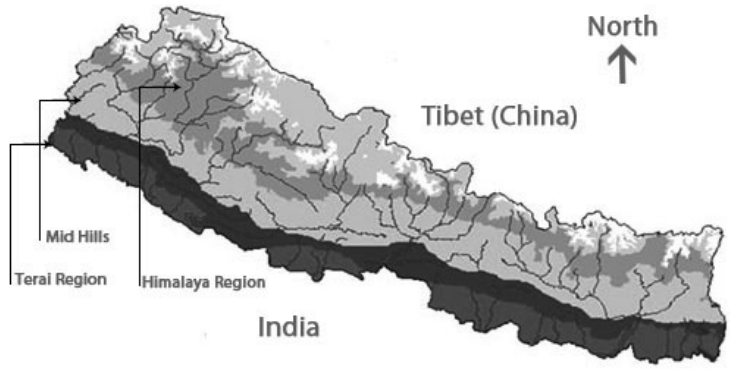

Fig.-1: Three different ecozones of Nepal

Three different ecozones were included in our study: mountain, hill and terai. For mountain region, the study sites selected were Ghatlang (Rasuwa), Mustang, Jumla and Murtidhunga(Dhankuta). Similarly, Baitadi, Sukhet, Dhankuta and Lamjung were selected for hills and for terai, Sunsari, Bara, Bardiya, and Kanchanpur were selected.

Collection of sample

The serum samples were collected from cattle, buffalo, goat, sheep, swine, chyangras and yaks during pre-monsoon and post-monsoon seasons. A total of 1347 pre-monsoon serum samples were collected in which mountain, hill and terai region contributed 523, 387 and 303 samples respectively. Similarly, a total of 1256 post monsoon serum samples were collected in which mountain, hill and terai region contributed 481, 352 and 413 samples respectively. During pre-monsoon, the total samples collected from goat, sheep, cattle, buffalo, swine, yak and chyangra were 393, 110, 387, 262, 91, 70 and 34 respectively. During post monsoon, the total samples collected from goat, sheep, cattle, buffalo, swine, yak and chyangra were 381, 102, 362, 281, 60, 35 and 35 respectively.

Laboratory analysis

ID Screen Toxoplasmosis Indirect Multi-species ELISA kit (ID Vet, France) was used for detecting antibodies against *Toxoplasma gondii* in different species of farm animals.

Results

From the total of 2603 serum samples collected, 587 serum samples were found to have contained antibodies against *Toxoplasma gondii*. Hence, the overall prevalance of toxoplasmosis in different livestock species in Nepal was found to be 22.6%.

Table-1: Prevalence of Toxoplasmosis in different livestock species

S.No.	Species	Total samples	Positive case	% positive
1	Goat	774	215	27.7
2	Sheep	212	38	17.9
3	Cattle	749	134	17.89
4	Buffalo	543	58	10.68
5	Swine	151	84	55.63
6	Yak	105	15	14.28
7	Chyangra(Mountain goat)	69	43	62.3
	Total	2603	587	22.6

Prevalence of Toxoplasmosis during Pre-monsoon

A total of 373 serum samples were found to have antibodies against *Toxoplasma gondii*. Hence, the overall prevalance of toxoplasmosis in different livestock species in Nepal during pre-monsoon was found to be 27.7%. Highest prevalence was found to be in Swine (53.84%) followed by Chyangra (50%), Cattle (32.56%), Goat (31.55%), Sheep (17.27%), Buffalo (13.74%) and Yak (2.85%) (Fig.-2).

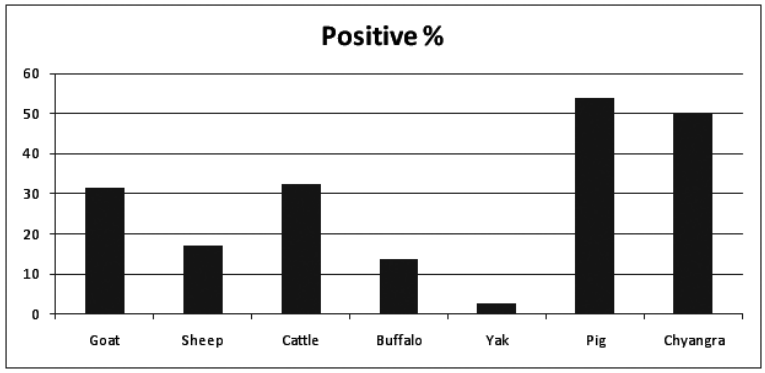

Fig.-2: Species wise prevalence of Toxoplasmosis in Nepalese Livestock

Prevalence of Toxoplasmosis during Postmonsoon

A total of 214 serum samples were found to have antibodies against *Toxoplasma gondii*. Hence, the overall prevalance of toxoplasmosis in different livestock species in Nepal during pre-monsoon was found to be 17.03%. Highest prevalence was found to be in Chyangra (74.29%) followed by Swine (58.33%), Yak (37.14%), Goat (31.55%), Sheep (18.63%), Buffalo (7.83%) and Cattle (2.21%) (Fig.-3).

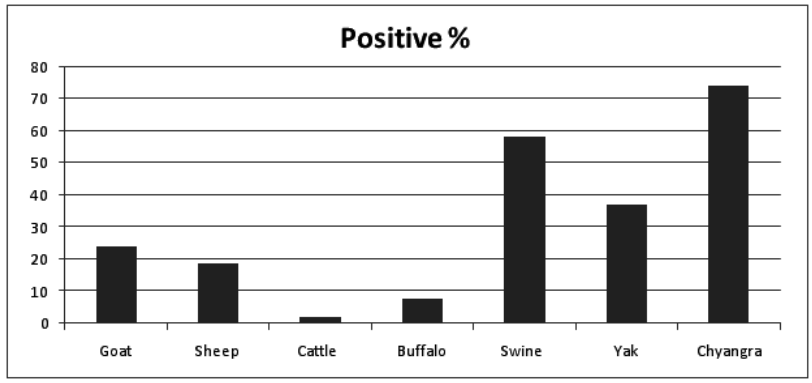

Fig.-3: Species prevalence during post monsoon season

Ecozone-wise prevalence

Pre-monsoon prevalence of Toxoplasmosis in different livestock species in mountain, hill and terai region was found to be 29.44%, 23.77% and 41.9% respectively. Similarly, post monsoon prevalence of Toxoplasmosis in different livestock species in mountain, hill and terai region was found to be 24.74%, 11.9% and 12.8% respectively (Fig.-4).

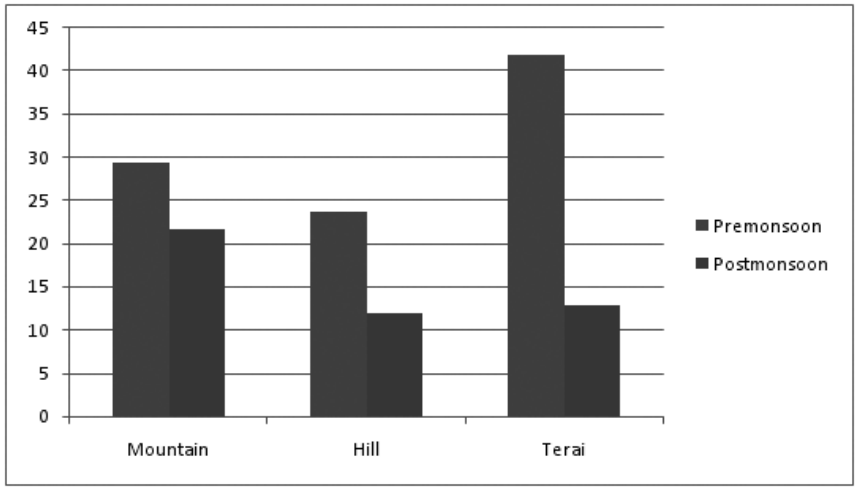

Fig.-4: Ecozone wise prevalence during premonsoon and postmonsoon season

DISCUSSION

This study indicated that exposure of livestock species to *T. gondii* infection in Nepal was common, with an overall prevalence of about 22.6%. The overall highest prevalence was found in Chyangra. This might be due to small sample size as compared to other livestock species. This study showed that the premonsoon prevalence (27.7%) was higher as compared to post-monsoon (17.03%). Similarly, this study showed significsntly higher prevalence in terai region as compared to moutain and mid hill region. This might be owing to higher favourable climatic conditions of temperature, humidity and so on. The estimated worldwide seroprevalence of toxoplasmosis in livestock has been reported as 30% in sheep, 15% in goats and 9% in cattle (Dubey, 2004). In case of Nepal among these three species, goat has the highest prevalence i.e. 27.7% followed by sheep (17.9%) and cattle (17.89%).

In this study the overall prevalence of toxoplasmosis in goat is found to be about 27.7% which is higher than estimated worldwide prevalence in goats which is about

15% (Dubey, 2004). This result is almost similar to the findings of Ghana (26.8%) (Van der *et al.*, 2000). This finding is lower as compared to that of 42.8% from West Indies (Chikweto *et al.*, 2011), 53.84% from Pakistan (Shah *et al.,* 2013), 31% from Uganda (Bisson *et al.,* 2000) and 30% from Mazandaran province, Iran (Sharif *et al.,* 2007). However, this result is higher as compared to 19.20% of Nepal (Koirala and Jha*,* 2015) and 19.25% from Iran (Hashemi-Fesharki, 1996).

Similarly, the overall prevalence of toxoplasmosis in sheep is found to be 17.9% which is lower than estimated worldwide prevalence in sheep which is about 30% (Dubey, 2004). This result is almost similar to the findings of Bangladesh (17.65%) (Samad *et al.*, 1993) and slightly lower to that of 19.88% from Southern Punjab, Pakistan (Lashari and Tasawar*,* 2010). This finding is lower as compared to that 25% of Nepal (Koirala and Jha*,* 2015), 33.2% from Ghana (Van der *et al.,* 2000), 35% from Mazandaran province, Iran (Sharif *et al.,* 2007) 44.1% from West Indies (Chikweto *et al.,* 2011) and 36.6% from Pakistan (Shah *et al.,* 2013).

The second highest prevalence was found in swine which is about 55.63% which is higher as compared to 19.15% of Nepal (Koirala and Jha*,* 2015), 23.1% from West Indies (Chikweto *et al.,* 2011), 39% from Ghana (Arko-Mensah *et al.,* 2000), 16.56% from Spain (García-Bocanegra *et al.,* 2010).

Likewise, the seroprevalence of toxoplasmosis in cattle is found to be 17.89% which is higher than estimated worldwide prevalence in cattle which is about 9% (Dubey, 2004), 12.14% of Nepal (Koirala and Jha*,* 2015), 8.4% from West Indies (Chikweto *et al.,* 2011), 1.6% from Urmia Northwest of Iran (Raeghi *et al.,* 2011) and 16.10% Bangladesh (Samad *et al.,* 1993).

Similarly, the overall prevalence of toxoplasmosis in buffalo is found to be 10.68%. This result is higher than 8.8% of Khoozestan province, Iran (Navidpour *et al.,* 1998), 1.5% of southern Vietnam (Huong *et al.,* 1997). Whereas, this finding is lower to that 15.16% of Punjab and Pakistan (Ahmad *et al.,* 2014).

The overall prevalence of toxoplasmosis in yak is found to be 14.28% and 11.8% of Qinghai, China (Liu *et al.*, 2008 & 2011) and 7.65 % of Qinghai, China (Chen *et al.*, 2010).

CONCLUSION

This study provides only the base line data on the presence of disease in different livestock species in Nepal. As this disease has public threat, further epizootiological and parasitological investigation should be given priority from the national level to determine the magnitude of infection in livestock and human.

ACKNOWLEDGEMENT

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Effect of different Fat Levels on the Quality of *Chhurpi* prepared from Cow and Buffalo milk

D. N. Sah

Directorate of Regional Livestock Services, Pokhara

ABSTRACT

A study was carried out to standardize the method of manufacturing of Chhurpi on the basis fat content of cow and buffalo milk. On the basis of market survey, three different combinations of recipes for cow milk (0.20%, 0.50%, and 0.80%) and for buffalo milk (0.20%, 0.50%, and 0.80%) were selected for preparation of Chhurpi. The Chhurpi preparation included skimming of milk, pasteurization, cooling to 82^{0}C, inoculation of citric acid using 2% and coagulation occur until clear whey obtain at 15-20 minutes. The 1/3 rd whey was drained after coagulation and filtering using a muslin cloth. The curd was cooked for 15 minutes, wrapped in muslin cloth. The curd were pressed over night and dried in a room temperature on shade for 14 days. The prepared product was selected on the basis best sensory scores by using 9-point Hedonic rank sum method. The Chhurpi having 0.8 % fat content in cow and buffalo skim milk Chhurpi was highly acceptable product in which gumminess of cow milk with significant ($p<0.05$) improvement in sensory attributes.The prepared Chhurpi was analyzed in laboratory and compare its quality with control product. Parameters used to monitor to the quality of Chhurpi were chemical (moisture%, fat%, protein%, total ash%, lactose%, acidity %) and microbial quality (yeast and moulds). The physicochemical parameters showed that there was significant ($p<0.05$) variation in moisture, fat, protein and total ash and lactose which were non-significant ($p<0.05$). The yeast and mold count in prepared product and control product of cow and buffalo milk Chhurpi did not show significant (($p>0.05$) difference in the initial and final count of yeast and molds. The product yield obtained was higher in more fat concentration i.e. 3.98% and 4.05% of cow and buffalo milk prepared Chhurpi, respectively. The higher percentage yield occurred to the cow and buffalo milk having high percent of fat. The average selling price of prepared Chhurpi was found Rs.206.56 per kg and average market price of Chhurpi was found Rs.210 per kg in the study area.

Key words: *Chhurpi,* Citric acid, Fat effect, Sensory evaluation, Composition of milk, Product, Quality.

INTRODUCTION

Chhurpi is casein based traditional dried milk product (Pradhan and Shrestha 2003). It is often called hard cheese. It is good source of Protein and mineral-Calcium. The casein contain in milk is coagulated with acid and/or previous day whey. The coagulated casein is pressed with heavy load overnight which expel the moisture to a minimum level. Then it is dried for a long time to reduce moisture to an acceptable level. It is use as a nutritious masticatory milk item (Karki, 1986). *Chhurpi* is a highly nutritious and shelf stable product with characteristic flavour and pronounced chewiness and gumminess. The production of *Chhurpi* is confined to traditional families and is manufactured in crude manner, which results in variation in its quality attributes. With advancement of the technology and increasing demand for this product, there is a need to standardize the manufacturing methods and packaging of the product (Pal *et. al.*1996).

Chhurpi by comparing traditional methods and effect of various ratio of milk fat on chemical, organoleptic and microbial quality of *Chhurpi* in consistency from soft to hard (Krofa *et al.,* 2004). *Chhurpi* is used fresh or can be used after certain period, ranging between days to months. It is used as chewing like nut. Now a day consumption of *Chhurpi* has been increases in the urban areas where people like most. Besides that it has been used in a grinding. It is very popular among trekkers and campers because of their advantages of being light weight, easy to found and pack to eat (FAO, 1990).

Product diversification from milk has been very limited in Nepal. Around 63% of milk products have not been accessed with organized milk market, more than 90% of the processed milk in the country is sold as pasteurized milk with only a very small share converted into other milk products. With the development of transportation net-work, organizing livestock farmer communities, commercializing, semi-commercializing dairy farming the present trend of milk offered is increasing at the rate of 14% while the demand is at 8% (NDDB, 2001). This increased milk production and the availability of surplus milk to be used for fulfillment of growing demand of expanding rural market development and for product diversification which promotes dairy enterprises. The main objective of this study was to analyze the nutritional quality, evaluate the yeast and mold count and assess the yield and production cost of the prepared and marketed product of cow and buffalo milk *Chhurpi.*

MATERIALS AND METHODS

Market survey

Primary data was collected from a survey. A set of questionnaire was prepared. The survey work was carried out to assess the marketing and socio-economic status; to prepare recipes and production of traditional technology of the *Chhurpi in* 3 districts of Dolkha, Ilam and Baglung. For this, almost all of the producer of *Chhurpi* in the targeted areas were listed and interviewed to obtain primary information. Secondary level information was obtained from different publication sources. The data obtained from survey were compiled and analyzed.

The materials for the preparation of *Chhurpi* were mainly fresh cow and buffalo milk skimmed and standardized skim milk of 3 recipes of were prepared based on fat percentage. The recipes were prepared for cow milk (0.20%, 0.50%, and 0.80%) and for buffalo milk (0.20%, 0.50%, and 0.80%). The reasons behind the prepared recipes having lower than 0.8% fat leads to hard body and texture in *Chhurpi* while higher fat level *Chhurpi* resulted in not drying easily, could not be stored for long time and there was problem of mould growth. Products were collected from Baglung of (buffalo milk *Chhurpi)* and Ilam of (cow milk *Chhurpi)* and control product from each site were also collected.

Preparation of *Chhurpi*

The preparation of *Chhurpi* manufacture was carried out on the basis of fat content by which different recipe was formulated. On the basis of these recipes different types of *Chhurpi* were prepared by using different levels of treatments with 2% citric acid. The tables, cloths and equipments were cleaned properly by using the hot water and ethanol. The milk was tested for fat for standardization for skim milk i.e.0.5 per cent fat and 8.7 per cent SNF. The standardized and filtered milk was heated to 90°C and cooling the milk at 82°C and then inoculation of coagulants (2% citric acid). Coagulation occured until clear whey was obtained at 15-20 minutes and then whey was drained. The coagulum obtained (green curd) was cooked in an open pan over water bath for 15 minutes. The hot cooking curd was wraped in muslin cloth and pressed in a wooden hoop at 9 kg /cm^2 pressure for over night. The mass was cut into small square shape and dried at room temperature (28°-32°C) on shade or air for 14 days. Then *Chhurpi* was packed in polythene bags.

Sensory evaluation

The sensory attributes of 3 experimental products of cow milk and one control product (purchase from Ilam ,mangalbare) and similarly 3 experimental products of buffalo milk *Chhurpi* and one control product (purchase from Baglung Cooperative) were evaluated by conducting sensory evaluation with the help of panel of 10 trained judges. The parameters for evaluation were gumminess, chewiness and overall acceptability assessed by using 9-point Hedonic scale (Rangana, 2001) after 14 days drying of the product. The scoring data obtained from different panelists were statistically analyzed. The chemical analysis of the product was made by the standard methods given.

Physico-chemical analysis of prepared product of both cow and buffalo milk and respective control product of *Chhurpi* was analyzed by Ranganna (2001). The obtained data were statistically analyzed by using SPSS (version 16). The analysis of variance (ANOVA) was calculated to see the significance difference. The means were separated by use of the least significant difference (LSD).

RESULTS AND DISCUSSION

Formulation of *Chhurpi*

The preparation of *Chhurpi* is based on the combination of different fat percent of cow and buffalo milk. The required combinations of fat of cow and buffalo milk are Recipe No.1= 0.2%, Recipe N0.2= 0.5% and Recipe No.3=0.8% respectively. All these 3 experimental product of *Chhurpi* were prepared as per the standardized flow process.

Sensory evaluation of *Chhurpi*

The combination type 3 was found to be superior to other combinations and control product (Table-1, Fig.1). The combination 3 with acceptability of sensory characteristics was selected as a best product and corresponding procedure and level of basic ingredients used *Chhurpi* preparation. The cow milk produces *Chhurpi* with moist surface, light yellow colour, too soft body and sooth texture. It was more suitable for *Chhurpi* preparation than the buffalo milk *Chhurpi,* the later being hard in body and coarse in texture, besides whitish colour.

Table-1: Mean (±S.E.) value of sensory evaluation of *Chhurpi*

Products	Gumminess		Chewiness		Overall acceptability	
	Cow	**Buffalo**	**Cow**	**Buffalo**	**Cow**	**Buffalo**
CC	5.6±0.54 (18)	5.7±0.21 (29.5)	6.1±0.45 (19.5)	6.3±0.36 (21)	6.7±0.33 (17.5)	6.6±0.30 (24.5)
ER-1	5±0.29 (31)	5.6±0.40 (28)	5±0.39 (32)	5.3±0.42 (31.5)	4.9±0.17 (35)	5.9±0.40 (31)
ER-2	5.7±0.26 (26.5)	5.8±0.32 (27)	5.5±0.40 (28.5)	5.6±0.33 (28)	5.6±0.33 (30.5)	6.5±0.26 (26)
ER-3	6.7±0.26 (15)	6.6±0.26 (15.5)	6.9±0.37 (17.5)	6.6±0.37 (18.5)	7±0.11 (15.5)	7.1±0.17 (18.5)

Note: CC- Control *Chhurpi* ER-Experimental Recipe

The number within the parenthesis denotes related to the quality of product. Since, cow milk *Chhurpi* in each column carried lower rank and was regarded better than buffalo milk *Chhurpi.* According to sensory evaluation, significant difference ($p<0.05$) was observed in the different *Chhurpi* prepared from cow and buffalo milk in respect to gumminess, chewiness and overall acceptability.

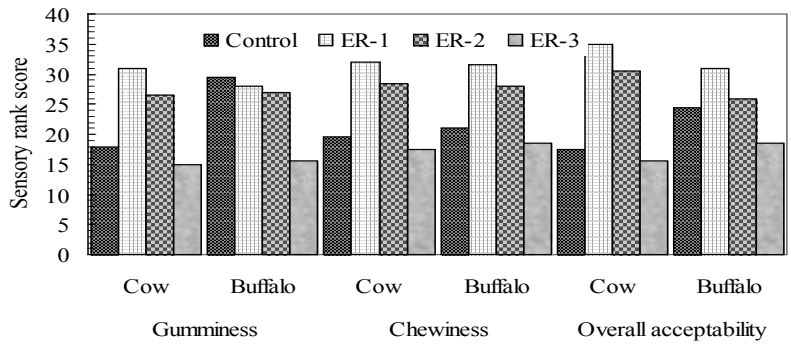

Fig. 1: Sensory analysis of three prepared and one control product of *Chhurpi*

Physico-chemical qualities

Moisture content

The moisture content of prepared and control product of cow and buffalo milk *Chhurpi* is presented in Table-2. The average with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 8.18±*0.*26, 11.57±0.44, 9.19±*0.35*

and 10.04±0.23, respectively. The water activity of that product was between 0.35 and 0.50; the humidity at this level was suitable to prevent the microbial growth. Lawire (1979) revealed that rancidity did not develop when the moisture content reduced to 1.5%. The high moisture content of *Chhurpi* has poor mouth-feel, low shelf-life. In this study, the moisture content was ranging from the lowest value 8.18% and highest value 11.57%, it increased the storage period and longer would be the shelf life of the *Chhurpi.* This result in this study showed that the moisture content of almost all the samples of *Chhurpi* remained less than 11.57%, in which 8.18% and 9.19% of cow and buffalo prepared milk *Chhurpi* than control product, which was optimum to prevent the microbial growth during three months of storage at room temperature.

Fat content

The average fat content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 5.13±0.03, 6.26±0.11, 5.08±0.10 and 5.38±0.38, respectively Table-2.

Table-2 Physico-chemical parameters of prepared and control product of *Chhurpi*

S.N.	Physico-chemical parameters	*Chhurpi* type			
		Prepared product		Control product	
		Cow milk	Buffalo milk	Cow milk	Buffalo milk
1	Moisture (%)	8.18±0.26	9.19±*0.35*	11.57±0.44	10.04±0.23
2	Fat (%)	5.13±0.03	5.08±0.10	6.26±0.11	5.38±0.38
3	Protein (%)	77.49±*0.37*	77.74±*0.23*	73.77±0.01	74.92±0.29
4	Total ash (%)	5.40±0.40	4.72±0.09	5.16±0.28	6.11±0.04
5	Lactose (%)	3.10±*0.05*	3.08±0.03	3.02±0.02	3.1±0.03
6	Acidity (%)	0.22±0.01	0.16±0.01	0.21±0.01	0.18±0.01
7	Gross energy (Kcal/kg)	5492±28.70	5530±17.32	5610±19.49	5540±55.49
8	Calcium (%)	4.61±0.01	4.16±0.01	2.22±0.15	3.10±0.20

The climatic conditions (temperature, humidity etc.) can affect the quality of *Chhurpi. C*omparatively in the hills and mountains high fat content signify the good quality *Chhurpi* prepared having lower than 0.8% fat leads to hard body and texture in *Chhurpi* while higher fat level *Chhurpi* resulted in not drying easily, could not be stored for long time and there was problem of mould growth. The fat content was 5.13% of prepared and 6.26% of control cow milk *Chhurpi* and fat content was

5.08% of prepared and 5.38% of control buffalo milk *Chhurpi*. The prepared cow and buffalo milk *Chhurpi* was found a high calorie value of 46.17 and 45.72 kcal/100g, respectively. This variable values may be due to species, breed, stage of lactation, seasonal variations, length of interval between milking, feed etc.

Crude Protein

The average protein content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 77.49±*0.37*, 73.77±0.01, 77.74±.23 and 74.92±0.29, respectively. The protein content of *Chhurpi* was found to be higher in control product of cow milk *Chhurpi* than the prepared cow milk *Chhurpi* and high in prepared buffalo milk *Chhurpi* in comparisons to control product of buffalo milk *Chhurpi*.

This protein content fluctuation might be due to the factors such as species, breed, nutrition or milking interval. The protein contain of prepared cow and buffalo milk *Chhurpi* was 77.49% and 77.74%, respectively. This value was greater in comparison of the control products. The prepared cow and buffalo milk *Chhurpi* was found a high calorie value of 309.96 and 310.96 kcal/100g, respectively. This study indicated that the estimated protein content was 78.5% and remaining 19% drain out in whey which was given by Walstra *et al.,* 2005.

Total ash content

The average total ash content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 5.72±0.40, 5.16±0.28, 4.72±0.09 and 6.11±0.04, respectively. This value indicated that milk used for *Chhurpi* making did not affect final ash content of *Chhurpi*. Ash content generally indicates the minerals and inorganic matter present in the product. Ash is a complicated mixture containing a number of metallic in dry milk product. Salts are by no means equivalent to "ash" because ashing of milk causes loss of organic acids including citrate and acetate, because organic phosphorus and sulfur are transferred to inorganic salts during ashing (Eckles, Combs and Macy, 2001).

Lactose content

The average lactose content with standard error of prepared and control product of cow and buffalo milk *Chhurpi* were 3.1±*0.05*, 3.02±0.02, 3.08±0.03 and 3.1±0.03 respectively, which were quite similar. When milk is used for *Chhurpi* making, most of the lactose follows the whey. Each of these products contains a relatively high proportion of lactose (Eckles, Combs and Macy, 2001). Lactose content of

the *Chhurpi* was found 3.1% which is 64.6% lactose remained in the products. The remaining out of 35.4% drain out in the whey. The prepared cow and buffalo milk *Chhurpi* was found an energy value of 12.4 and 9.24 kcal/100g, respectively. This variable value could be due to individuals within the breed and breed itself is a small factor. The main flavour compounds of milk and milk product *Chhurpi* are lactose and the dissolved salts, which caused a sweet and salty taste, respectively.

Yield of Chhurpi on fat basis

Whey proteins are denatured by high-temperature pasteurization, which makes them less soluble and cause them to precipitate with casein during acid precipitation. Low-temperature pasteurization has little effect on the proteins. This means *Chhurpi* making from high temperature pasteurized milk gives a higher yield because a greater part of the milk proteins goes into the *Chhurpi.* The higher percentage yield occurred to the cow and buffalo milk was 3.98% and 4.06% (Table 3). The table pertaining to yield of *Chhurpi* revealed that increased the yield of *Chhurpi* as increases the percentage of fat in both cow and buffalo milk prepared *Chhurpi.*

Table-3: Effect of fat content of milk on the yield of *Chhurpi*

Parameter of 4 L skim milk with fat percent	Coagulant	Coagulant (ml) /L milk	Total yield in g (%)	
	Citric acid %			
			Mean±SE	%
Cow milk (0.2%)	2	12 ml	153.58±1.15	(3.83)
Cow milk (0.5%)	2	12 ml	153.74±0.20	(3.84)
Cow milk (0.8%)	2	12 ml	159.52±0.24	(3.98)
Buffalo milk (0.2 %)	2	12 ml	154.30±1.18	(3.85)
Buffalo milk (0.5 %)	2	12 ml	155.41±0.95	(3.88)
Buffalo milk (0.8%)	2	12 ml	162.54±0.95	(4.06)

Cost of production

The production cost of process prepared *Chhurpi* was calculated by considering the variable cost (cost of raw materials, fuel and labour etc.).The average selling price of prepared *Chhurpi* was found Rs.206.56 per kg and average market price of *Chhurpi* was found different from place to place Rs.210 to Rs.300 per kg in the study area.

CONCLUSION

Chhurpi is casein based Nepalese indigenous dried milk product, made by coagulation

with acid. The coagulated casein is pressed with heavy load overnight which expel the moisture to a minimum level. Then it is dried for a 14 days to reduce moisture to an acceptable level. The *Chhurpi* having 0.8 % fat content in cow and buffalo skim milk was highly acceptable in quality having gumminess and chewiness. The product yield obtained was higher in more fat concentration i.e. 3.98% and 4.05% of cow and buffalo milk as acceptable product. *Chhurpi* is main source of protein, fat, lactose and minerals. *Chhurpi* is solid hard casein containing 77 % protein and 5% fat in cow and buffalo and 9% in yak milk. *Chhurpi* is a Nepalese indigenous product, so it needs to be patented. This will provide basis for preventing other countries to take a benefits of ours laws and secure patents in their favour. Therefore *Chhurpi* need to be catalogued and proper action should be taken to obtain patents under the existing law. Implementations of Good manufacturing practice (GMP) by the *Chhurpi* makers can be made obligatory as it has direct concern with public health hazard.

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Fish Disease Problems and Health Management Practices in Pond Fish Farming of Terai Region, Nepal

A. Jha and S. K. Wagle

Fisheries Research Division, Godawari, Lalitpur

ABSTRACT

In order to examine the fish disease problems and health management practices in pond fish farming of Terai region of Nepal, a semi-structured questionnaire survey and participatory rural appraisal (PRA) tools and in-situ field monitoring were used in seven districts representing east, central and west part of Terai. A total of 74 fish farms were interviewed and monitored, and five PRA sessions were conducted. Most of the farmers (83.8%) had disease problems in their fish ponds and average prevalence rate varied with farm size and locations. Large size farms had significantly high (P<0.05) prevalence of fish disease (51.8%) and central Terai was most suffered from the disease (32.6%). The most prevalent disease was epizootic ulcerative syndrome (22%) followed by argulosis (11.4%), red spot (9.6%), tail and fin rot (9.1%) and nutritional problem (8.6%). The disease occurred mainly on winter and on-set of summer season. For fish disease treatment, 41.9% farmers used lime followed by 12.2% farmers used lime with potash. Health management problems such as poor technical knowledge, lack of assistance, poor diagnostic services and lack of therapeutic measures were identified. A continuous support from research and extension services in building capacity of farmers has been suggested to effectively manage fish disease.

Key Words: Disease prevalence, Fish disease, Health management, Survey, Terai region

INTRODUCTION

Aquaculture is often referred as the fastest growing primary production sector in Nepal in the last three decades, having witnessed an annual rate of growth of nearly 8.5 percent (Wagle *et al.,* 2011). However, the demand for aquaculture and fisheries to provide increasing fish needs in the country is estimated to be around 155000 tonnes by 2027 in which aquaculture has envisaged contributing by 56% (Mishra and Upadhyaya, 2010). Increasing demand for fish and competition for scarce natural

resources has promoted the development of intensive aquaculture. Intensification of aquaculture systems with carps, cichlids and catfish is occurring considerably in Terai region of the country in order to encompass increasing domestic demand for fish. With the expansion and diversification of aquaculture practices, disease after seed and feed has become one of the constraining factors for fish production in Nepal. Sustainable aquaculture production can only occur when fish are healthy and free from disease.

Mohan (2009) has emphasized that intensive aquaculture practices tended to provide a platform for the emergence of pathogens and the global trade in aquatic animals and their products offered avenues for trans-boundary spread of pathogens. He has also emphasized that the risk of pathogen transfer was greater for movement of live aquatic animals than for movement of dead product. Also, with the introduction of new exotic fish species in aquaculture, there is always a risk of pathogen transfer, disease incursion and subsequent outbreaks of disease in the existing aquaculture systems (Faruk *et al*., 2004a). Not only intensification of aquaculture is increasing day by day in Nepal but also live and dead aquatic animals are regularly being imported from various countries as well transported within country without significant quarantine measures (Mandal and Prasad, 2010). A lack of information on the status of fish disease and health management practices in rural aquaculture of Nepal exists.

Vast majorities of diseases and consequently economic loss in rural aquaculture became unnoticed, since most of the fish farmers do not understand the signs of diseases and incapable of reporting incidence of diseases. In these contexts, it becomes necessity to monitor the status of fish disease in Nepal, so that appropriate strategies can be formulated and implemented for the sustainable development of fisheries and aquaculture in country. This study was aimed to know the status of fish disease and health management strategies in aquaculture of Terai region of Nepal.

MATERIALS AND METHODS

Field survey on the status of fish disease and health management practices in seven districts, representing Western (Rupandehi), Central (Bara, Parsa, Dhanusha, Mahottari) and Eastern (Sunsari, Morang) of Terai, Nepal was conducted during July 2013 to June 2015. Data was collected through semi-questionnaire interview and participatory rural appraisal (PRA) with fish farmers. For the interview, simple random sampling method was followed. A total of 74 farmers (minimum of 10 in eastern Terai and maximum of 51 farmers from central Terai) having different farm size was interviewed. PRA tools including Focus Group Discussion (FGD) were conducted with fish farmers to get an overview of particular issues of fish health

and disease. A total of 5 FGD sessions, including fish disease camps organized, in different districts were conducted where each group sizes were between 15 and 45 farmers.

Information on the status of fish disease was also gathered through sampling and in-situ monitoring of fish health condition in ponds of each respondent farmers. Fish health conditions monitored through gross observation and microscopic examination was recorded in a data format. Water quality parameters including temperature, dissolved oxygen (DO), pH, ammonium, conductivity, turbidity and sechi disk visibility was measured using Vernier analogue meter (Model LABQUEST2) for each 74 representative ponds during fish sampling. Data were processed using Microsoft Excel and analyzed by using tabular and descriptive statistical methods. The technique of analysis included the classification of tables into consequential results by arithmetic mean, percentage and ratios.

RESULTS

All together, data from 74 fish farms were analyzed (Table-1). The farms were splited into four categories depending on the size of pond area. The first category comprised of small farms having less than 0.2 ha pond area which corresponded 10.8% of the interviewed farmers. The second category of fish farms had the pond area between 0.2 ha to 1.0 ha, third category was between 1.1 ha to 3.0 ha, and the fourth category was large farms comprised of pond area more over 3.0 ha. The second, third and fourth category fish farms represented 25.7, 28.4 and 35.1%, respectively in the survey.

Table-1: Summary of respondent in different part of Terai, Nepal

Farm category, ha	Western Terai	Central Terai	Eastern Terai	Overall	Percent
<0.2	2	6	0	8	10.8
0.2-1.0	2	15	2	19	25.7
1.1-3.0	4	12	5	21	28.4
>3.0	4	19	3	26	35.1
Total	12	52	10	74	100.0

Fish pocket areas of each district were selected for data collection mostly on the basis of having previous disease history. During the present survey, the majority (83.8%) of the farmers responded that they had disease problems during last years or year before. Although not significant ($P>0.05$), the average prevalence of fish disease in farmer's ponds was noticed highest (32.6%) in central Terai followed by eastern (21.7%) and

the lowest was in western Terai (18.8%). Average prevalence of disease in small farmers ponds was the lowest (5.5%) and the disease prevalence rate significantly increased (P<0.05) with the increase in farm size (Table-2).

Table-2: Prevalence of fish disease (%) in different fish farm categories of Terai, Nepal

Farm size, ha	**Western Terai**	**Central Terai**	**Eastern Terai**	**Mean ± SD**	
<0.2	0.0	16.6	0.0	5.5±9.6[a]	
0.2-1.0	0.0	33.3	0.0	11.1±19.2 [a]	
1.1-3.0	25.0	41.6	20.0	28.0±11.3 [ab]	
>3.0	50.0	38.8	66.6	51.8±14.0 [b]	
Mean ± SD	18.8±23.9	32.6±11.2	21.7±31.4	24.3±7.3	

The mortality percentage was determined by the approximate assumption of the respondent farmers. The mortality of fish was comparatively lower in small and medium farms (7.2% and 7.5%), and higher in semi-medium (9.7%) and large category farms (9.9%) (Table-3). Similar to the prevalence of disease, the highest mortality of fish was also found in central and eastern Terai (9.8% and 10.8%) and the lowest mortality was in western Terai (5.2%). Percent mortality of fish because of diseases across region and the category of farm was not significantly different (P>0.05).

Table-3: Mortality % of fish due to disease in various pond categories in Terai, Nepal

Farm size, ha	**Western Terai**	**Central Terai**	**Eastern Terai**	**Mean ± SD**
<0.2	0	7.3	14.4	7.2±7.2
0.2-1.0	8.6	11.2	9.3	9.7±1.3
1.1-3.0	5.8	5.1	11.7	7.5±3.6
>3.0	6.3	15.5	7.8	9.9±4.9
Mean ± SD	5.2±3.6	9.8±4.6	10.8±2.9	8.6±3.0

A range of disease and conditions was reported by the farmers of different parts of Terai according to their occurrence. The diseases occurrence was also confirmed by gross observation and clinical examination of fish during the survey period (Table-4).

Relative diversity and incidence of fish diseases was high in central Terai where fish farms are densely located. The most prevalent disease was epizootic ulcerative syndrome (EUS, 22.0%) followed by argulosis (11.4%), red spot (9.6%), tail and fin rot (9.1%) and nutritional problem (8.6%). Other diseases like trichodiniasis, dropsy, white spot, gill necrosis and fungal infection were also reported by the farmers but with lower incidence. Farmers considered EUS, red spot and argulosis to be a major problem during winter and the onset of summer season. EUS was the major problems in all parts of Terai, most prominently in central Terai. Trichodiniasis and white spot disease with prevalence of 6.9% and 7.7%, respectively, across Terai were the most commonly occurring disease in fish hatcheries and nurseries affecting fish fry in surveyed area. Farmers reported that a significant percent of fish disease (9.3%) are not known or the symptoms could not recognized by them. Disease related to nutritional problem were found very severe (8.6% occurrence) in all parts of Terai.

Table-4: Types of fish disease problems (%) in carp fish farming of Terai, Nepal

Fish diseases	Western Terai	Central Terai	Eastern Terai	Mean ± SD	Status of disease in gross observation and clinical examination		
					Severity*	Fish Size, g	Season**
EUS	18.8	29.8	17.4	22.0±6.8	++	>100	W
Red spot	12.5	7.6	8.7	9.6±2.6	+++	>75	W+S
Tail & fin rot	6.3	8.1	13.0	9.1±3.5	++	>200	W+S
Argulosis	12.5	8.5	13.0	11.4±2.5	++	>500	W
Trichodiniasis	6.3	5.7	8.7	6.9±1.6	+	<5	R
Dropsy	6.3	3.8	4.3	4.8±1.3	+	>200	W+S
White spot	12.5	6.2	4.3	7.7±4.3	+	<5	R+W
Nutritional problem	6.3	10.9	8.7	8.6±2.3	++	>50	W
Fungal infection	6.3	4.7	4.3	5.1±1.0	+	>100	W
Whirling	0.0	2.4	0.0	0.8±1.4			
Gill necrosis	0.0	5.7	8.7	4.8±4.4	+	>10	S
Other	12.5	6.6	8.7	9.3±3.0			
Total	100	100	100	100.0			

* + denotes low, ++ moderate, +++ high

** W=winter, S=summer, R=rainy

Out of 74 respondents 26 (35.1%) farmers reported that they found disease during winter and onset of summer season, 18.9% reported winter season, 12.2% reported summer season, 5.4% reported rainy season and 28.4% farmers reported that they do know about this (Table-5). Observation of fish on the outbreak of disease in different seasons during the survey period was also revealed that 70% disease occurred in winter and both the winter and summer season followed by 20% in rainy and both the rainy and winter season and the least (10%) occurred in summer season (estimated from Table-4).

Table-5: Disease occurring season in carp fish farming of Terai, Nepal

Season	**Western Terai**	**Central Terai**	**Eastern Terai**	**Total**	**Average (%)**
Summer	2	5	2	9	12.2
Winter	2	10	2	14	18.9
Winter & summer	3	19	4	26	35.1
Rainy	1	3	0	4	5.4
Disease not reported or don't know	4	15	2	21	28.4
Total	12	52	10	74	100.0

On the causes of severe outbreak of diseases during winter and onset of summer, farmers responded that degraded environmental conditions which include reduced water temperature, low water depth, off-flavor of pond water, excessive plankton bloom and reduced feed intake by fish might make fish susceptible to disease. Water quality parameters measured in different seasons during the survey period supported the farmers experience and observations (Table-6). Mean water temperature was lowest during winter (23.2 ^{o}C) and summer (24.3 ^{o}C) compared to that of the rainy season (32.7 ^{o}C). Relative to rainy season, elevated ammonium level (0.8 to 0.9 mg/L), increased turbidity (78.5 to 94.6) and low sechi disk visibility (20.4 to 20.8 cm) during winter and summer season could have resulted in stressful condition for fish.

Table-6: Water quality of fish ponds in different seasons (range, mean±SD) in carp fish ponds of Terai, Nepal

Water quality parameters	**Wet (Jun-Sep) N=55**	**Winter (Oct-Jan) N=50**	**Summer (Feb-May) N=62**
Temperature, °C	29.2-36.3	19.2-27.2	21.8-29.0
	(32.7±2.0)	(23.2±2.2)	(24.3±1.4)
Dissolve oxygen, mg/L	2.1-11.5	3.7-12.9	3.2-9.8
	(6.5±2.9)	(7.7±3.1)	(6.5±1.4)
pH	6.6-9.3	6.8-9.0	6.5-8.8
Ammonium, mg/L	0.3-0.6	0.3-1.1	0.6-1.3
	(0.4±0.2)	(0.8±0.3)	(0.9±0.2)
Conductivity, µs/sec	27.2-82.0	84.0-112.0	19.0-399.0
	(63.5±24.6)	(94.6±13.3)	(78.5±106.2)
Turbidity, NTU	45.5-322.0	46.8-472.0	18.0-236.9
	(123.8±78.1)	(166.6±108.3)	(159.9±85.0)
Sechi disk, cm	20-27	18-24	17-23
	(22.7±2.1)	(20.8±2.2)	(20.4±2.4)

Farmers were asked during FGD about their ability to recognize fish disease. Majority of the farmers said that they could recognize some disease, while only few said that they could not recognize any disease. None of the farmers say that they could recognize/identify all or most of the diseases. The ability to recognize disease was based on number of clinical signs experienced by the farmers, which include mortality, abnormal behavior, abnormal appearance, reluctant to accept feed, reduced growth etc.

Most of the farmers (87.8%) took some measures to prevent the outbreak of disease in their ponds. The preventive measures included pond drying, addition and replacement of water, use of lime before disease outbreak, water drainage, grading of fish and

stocking of uniform size fish, etc. Farmers often got advice from neighbor farmers, fertilizer and pesticide sellers, veterinary drug suppliers and extension workers for advice when disease occurred in their ponds and applied range treatments. Out of 74 respondents, 61 (82.4%) fish farmers treated their fish after occurrence of fish diseases in their ponds.

Farmers use a diverse number of treatments, many in multiple combinations (Table-7). Liming was the most common treatment followed by application of potassium permanganate (potash), natural zeolites, salt, antibiotics and pesticides. Highest number of farmers (41.9%) used lime followed by combination of lime and Potassium Permanganate (12.2%), natural zeolite (Toximar, 6.8%) and salt (5.4%). Few farmers (13.5%) do not treat their diseased fish at all. Some farmers used pesticides in combination with lime (4.1%) to treat the external parasites (eg. Argulus). Out of 74 respondents 13 (17.8%) reported that they use unspecified Indian products (Aqua Health, Toximar, Sokrena, Cifax, Wasorich) to treat the fish against various disease as per the advice of local veterinary drug and pesticide sellers.

Table-7: Chemicals used in disease treatment in carp fish pond in Terai, Nepal

Chemicals	**Western Terai**	**Central Terai**	**Eastern Terai**	**Total**	**Average (%)**
Lime	7	20	4	31	41.9
Lime and potash	2	7	0	9	12.2
Salt	0	3	1	4	5.4
Lime and Vitamins	0	1	0	1	1.4
Bleaching powder	0	3	0	3	4.1
Lime and malathion	0	3	0	3	4.1
Aqua Health	0	3	0	3	4.1
Toximar (Natural hydrated sodium calcium aluminium silicates)	0	3	2	5	6.8
Sokrena-WS (bactericidal, fungicidal)	0	2	1	3	4.1
Cifax	0	1	0	1	1.4
Wasorich & Mittha	0	1	0	1	1.4
No chemicals	3	5	2	10	13.5
Total	12	52	10	74	100.0

Assistance of fish farmers on disease management from government organizations and NGOs ranked very low. In the present survey, 40.5% of the 74 respondents reported that they did not receive any kinds of assistance from GOs and NGOs for the management of fish disease (Table-8). Other problems faced by the farmers were lack of training and demonstration (24.3%), poor technical know-how (18.9%) and unavailability of appropriate drugs (16.2%).

Table-8: Problems faced by the farmers in controlling the fish diseases

Chemicals	**Western Terai**	**Central Terai**	**Eastern Terai**	**Total**	**Average (%)**
Lack of assistance	3	23	4	30	40.5
Poor or lack of technical know how	4	8	2	14	18.9
Unavailability of chemical & drugs	2	9	1	12	16.2
Lack of training & demo	3	12	3	18	24.3
Total	12	52	10	74	100.0

DISCUSSION

The result of the study indicated that the prevalence of fish disease was approximately 24.3%. Large-scale farms suffered from highest prevalence of fish diseases than medium and small-scale farms. In relative terms, this suggests large-scale farmers are more vulnerable to disease shock. Large-scale farms in Terai are gradually shifting fish farming towards intensification through high stocking density and intensive feeding. Fish mortality and economic loss is likely to increase as aquaculture expands and intensifies (Faruk *et al.,* 2004b). The prevalence of fish disease in central Terai was highest because of high stocking density of small sized fry, poor input, poor understanding of fish health management and large number of undrainable ponds present in this part of Terai. Diseases outbreak in pond aquaculture due to stress induced by these conditions including weather-related environmental stress have been well reported by (Tang and Nelson, 1998; Mazid and Banu; 2000; Faruk *et al.,* 2004a). On the other hand, western Terai had the lowest fish disease problems. This might be due to the presence of relatively younger ponds with water exchange and drainage facility, awareness of farmers on fish culture, low stocking density with bigger sized fish, proper feed supply and pond management.

Present study recognized a range of disease and conditions in pond fish farming with carps in Terai region of Nepal as was reported by the farmers according to

their occurrence and general field observations. The most prevalent disease was EUS followed by argulosis, red spot, tail and fin rot and nutritional diseases.

EUS is considered to be a major problem for pond aquaculture in Nepal. *Aphanomyces invadans,* is an invasive oomycete or water mould, is regarded as a primary fish pathogen of EUS (Baldock *et al*. 2005). A number of other saprophytic oomycetes are known to be opportunistic pathogens and may be present as secondary invaders on surface lesions caused by *A. invadans* in fish (Lilley and Roberts, 1997; Sosa *et al*., 2007). The first outbreak of EUS occurred in 1989 in Nepal and 15-20% of total fish production due to initial outbreak was reported (Phillips, 1989; Shrestha, 1994). Since the initial outbreak it has had a serious effect on aquaculture which is still in the early stages of development. The result of the present study indicated that EUS was still causing significant fish losses in the country. The fish farmers of the survey area informed that at the beginning of the winter season, this disease usually appeared and caused severe mortality of fish. Field observation made during the survey was also indicated that in most of the cases low temperatures are an important determinant of EUS outbreaks (Table 4). Rodgers and Burke (1981) and Lilly *et al*. (1998) reported that EUS outbreaks in freshwater fish in Asia have occurred during periods of declining and/or unstable temperatures. In a study conducted in Chitwan, Nepal revealed that the high prevalence of EUS in fish usually associated in ponds having rapid fluctuation of temperature, limited or no exchange of water and poor application of liming material (Baidya and Prasad, 2013).

Argulosis was the most common disease problem in grow out fish reported by the farmers. Gross observation revealed that infections from the freshwater branchiuran crustacean fish louse *Argulus*, represents a major threat to fish health both in farm hatcheries and in grow-out systems. The survey results showed that fishes mainly affected during the winter season and the perceptions of the farmers were that *Argulus* hinder their farming species when adult indigenous major carps and other carps were kept continuously in the same water body for longer period. Few farmers used malathion, a systematic phosphoric esters, to control the *Argulus*. Due to the indiscriminate use of pesticides, pond environments are deteriorating as well as adversely affecting pond productivity. Use of bamboo splits and lime (Ahmed, 2004), wooden boards treated with lime and turmeric mixture (Saha, 2008) and boards painted with Chlovar, Netrex (Parvez *et al*., 2013) could be suggested as an environmentally sustainable *Argulus* control strategy in hatchery system, grow-out system and homestead fish ponds. Effective measures for the control of *Argulus*

infections should be taken from early spring onwards before the infection establishes (Mikheev *et al.,* 2001).

Farmers in the present study frequently reported red spot and tail and fin rot on their fish. Red spot disease is characterized by the presence of red spot at the base of the fins and over the skin, dermal ulceration, hemorrhagic septicemia and scale protrusion disease. Motile aeromonad *(Aaeromonas hydrophila)* is mainly responsible for this disease. Low water quality, high stocking density and other stress factors enhanced this disease (Roberts, 1997). Certain algae (Kawakami and Hashimoto, 1978) and other protozoa (Chang and Huang 1981) that are grazed upon by fish, also harbor *A. hydrophila*. Aeromonads, Pseudomonads and Cytophaga-like bacteria may involve in tail and fin rot disease. In addition to the presence of bacteria, low pH and hardness of water, traumatic damage, pollution and inappropriate nutrition enhance outbreaks of this condition (Faruk *et al.*, 2004a).

Trichodiniasis and white spot disease was also found common in all parts of Terai. Trichodidiniasis caused by *Trichodina* sp was most common in small sized fish in hatchery and fish nursery during monsoon and prolonged cloud cover. Excess mucus production and removal of the skin epithelium are the major symptoms which results in sluggish movement, loss of appetite, emaciation, loss of condition with larger head and darker skin than normal. Present study revealed that the prevalence of white spot disease was high in western Terai. This disease is caused by ciliate protozoan *Ichthyophthirius multifiliis*. The stress resulting from suboptimal environmental conditions (high nitrite concentration, lower temperature, higher chlorine concentration) is, probably, important contributing factors that cause the loss of protective mucus, compromise the integument and stressed the fish immune system. Delaying in the ichthiophtiriasis diagnosis leads to a severe clinical infestation which drastically affect gill tissue and respiration capacity (Sandita *et al*., 2011).

Nutritional disease is the dietary imbalances from the presence of disproportionate levels of components presents in a fish diet. The most frequent clinical signs associated with this condition are in appetence, associated with darkening of skin and poor growth (Brown, 1993). In the present survey, nutritional problems were more severe in central and eastern Terai. Most of the farmers in these parts of Terai reported that feeding to fish was practiced sporadically with oil cake or rice bran and few practiced combination of both ingredients. Some farmers stated that the growth of their fishes was not satisfactory and the head became comparatively larger than

the body. This might be because of under feeding and improper balance of feed. Such situation is indicative that the efforts are required increasing farmers profile in this particular area.

Present survey and corresponding field observation showed that most of the disease mainly occurred during winter season. During this time the water quality also became very poor (Table- 6). As a consequence of low temperature and sub-optimal environmental condition during this time immune system of fish is suppressed and the fish become more vulnerable to disease (Lilly *et al*., 1998). Farmers could be suggested to take some preventive measures at the beginning of and during winter season which include application of lime, disinfection equipment, addition or replacement of water, minimum handling fish, etc.

In the present study, farmers were mostly found lack of knowledge on health management and have inadequate opportunities to improve management skills. Their ability to respond effectively to fish disease problem is also very limited. As a result, they suffered from fish loss and associated financial losses due to disease. Farmers response to disease problems was generally application of chemicals with little understanding of their effectiveness. They largely depend on advice received from neighbor farmers and local veterinary drug and chemical stores for the treatment. Several chemicals such as Toximar, Sokrena, Cifax and Wasorich and Mittha used by the farmers for the treatment of a specific disease problem have not yet been tested, verified and recommended to the local environment. This indicates that continual research efforts are necessary to verify the effectiveness of these chemicals and other commercial products claimed for fish disease treatment.

In conclusion, this study demonstrated that there is a substantial fish disease problem in fish farming of Terai region of Nepal. The fish losses due to diseases are associated with the farmers inadequate understanding of basic fish culture and heath management techniques. Therefore, there is need to develop strategies for primary fish health management packages, training program on simple diagnostic procedure and effective treatment and creation of awareness amongst farmers on fish health management. Also, research on potential environmental and farming conditions responsible for the outbreak of fish disease is needed to create awareness among framers. Tests and verification of effectiveness of different drugs and chemicals in local environment to control disease outbreak is equally essential.

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Epilepsy in Companion Dogs: An Overview

K. Roy[1], P. C. Shukla[1] and I. C. Datta[2]

1. The Nanaji Deshmukh Veterinary Science University

2. Jawaharlal Nehru Agriculture University, Jabalpur, M.P., India

ABSTRACT

Seizures could be a nightmare for the pet lover. The multi-faceted disorder, canine epilepsy with recurring episodes of seizures challenges the clinical acumen of the pet physician. Some dogs may experience seizures only once in their entire life time, but others may experience several episodes daily. Hyperthermia, poisoning, hypoglycaemia, hypocalcaemia after whelping, nutritional deficiencies, distemper, intestinal parasites, liver and kidney disorders, and thyroid problems may be the underlying factors. The conventional therapy involves the judicious use of phenobarbital, potassium bromide, and other anti-convulsant drugs, eg. primidone, valproic acid (VPA) and gabapentin, developed primarily for human epileptic patients. Veterinarians place more reliance on the time tested convetional anti-seizure medications. However, the newer preparations are mandated if the traditional drugs do not control seizures adequately per se. In this eventuality, the blood chemistries to check out possible impaired hepatic/ renal function and drug blood levels need to be monitored regularly. Dietary and environmental assessment and necessary adjustments followed by nutritional supplementation, improved hygiene and sanitation, and increasing use of potent herbal oreparations are part of the contemporary holistic long-term approach.

Key words: canine epilepsy, seizures, dogs, CNS, neurons, anticonvulsive agents, radio imaging

INTRODUCTION

Epileptic seizures somewhat resemble an electrical storm. To any pet lover, having to watch the companion dog in acute agony during an episode of seizures is a traumatic experience. Therefore, the attending physician needs to be proficient to promptly and effectively deal with the challenging situation and bring relief to the patient and the owner. According to Lowenstein (1990), a paroxysmal event seizure originates from continuous hyper-synchronous discharges from neurons in the central nervous

system (CNS) with the precise cause often remaining undetected. The underlying factors may be per acute reaction to drugs/ toxins, allergens, pyrexia or a secondary complication to some other malady. Alternatively, altered histoarchitecture of the specific brain area following tumor growth, hydrocephalus, or a bleeding/ circulatory disorder may be responsible (Podell, 1996). Canine epilepsy represents a complex pathoclinical syndrome and not a specific disease entity; the afflicted dog patient exhibits recurring bouts as a consequence of patchy degenerative changes in the brain neurons. Abnormal electrical activity triggers massive bursts of unsynchronized nerve impulses carrying scrambled messages to the skeletal muscles, resulting in deranged motor activity (Parent, 1991). Because of the multi-lateral underlying factors, canine epilepsy is clinically manifested in different forms (Chugani, 1999). Objective clinical evaluation of the patient on presentation with the carefully recorded anamnesis is, therefore essential for rational diagnosis and positive outcome.

Clinical Presentation

Canine epilepsy may be classed as idiopathic or primary/ symptomatic or secondary from the aetiolgical perspective. Canine epilepsy is named idiopathic in the absence of any definitive brain abnormality. Most of the affected dogs experience the first bout of seizures between one to five years of age. A genetic predisposition is suspected in certain breeds including the Beagle, Daschund, British Alsatian, Labrador retriever, Golden retriever and Collie (Chandler, 2011). Symptomatic epilepsy refers to seizures arising as a consequence of localized brain lesion or some other specific cause: traumatic injury or degenerative changes following deranged metabolism.

Alternative classification is based on the dog patients' pathoclinical profile. Thus, generalized tonic-clonic epilepsy, formerly named grand mal or major epilepsy, begins with contraction of groups of skeletal muscles in different body areas leading to loss of consciousness. The afflicted dog lies prostate on the floor with stretched out limbs and retracted head. The severely compromised respiratory rhythm may cause apnoea. The tonic phase is usually transient lasting only 10 to 30 seconds, and is accompanied with involuntary vocalization and facial twitching. Excessive salivation, dilatation of the pupils, urinary and bowel incontinence are also observed.

The clonic phase, characterized by rhythmic movements is associated with clamping of the jaws and jerking movements of the limbs (Parent, 1991). Petit mal or minor epilepsy is very rare, or remains undetected. The symptoms include a brief period of unconsciousness, muscle flaccidity, blank staring stance, and occasionally rotation of the eyes upwards (Thomas, 2000). Status epilepticus, clinically manifested as persistent seizures (lasting 30 minutes or more), or a cluster of multiple seizures

within a short span of time, without any detectable intervening spell of consciousness, often leads to a life threatening situation. Such patients may also exhibit generalized tonic-clonic seizures.

During a bout of seizures, the dog lies montionless, but eventually gets up on the feet and appears to be normal (Loweinstein and Alldredge, 1998). In partial seizure, the animal remains conscious.

Pathobiochemistry

The normally functioning brain neuron carries a net negative intracellular charge, relative to the external milieu; the resting membrane potential is maintained by the sodium-potassium ATPase pump. Upon excitement the nerve cell becomes increasingly +ve until the threshold value is reached, gets depolarized and an action potential is generated. Seizure occurs when the brain neurons depolarize spontaneously. Excessive excitement/ de-inhibition of the depolarization mechanism promote seizures. Thus, in hypoglycaemia (blood glucose level falling < 40 mg/ dl), the ATPase pump is deprived of the energy substrate. As a consequence, the resting potential in the neurons becomes more +ve with increased susceptibility to spontaneous depolarization. In diseases like hepatic encephalopathy, the bioactivity of the inhibitory transmitters such as γ-amino butyric acid (GABA) is markedly impaired permitting rapid depolarization. Since abnormal electric activity in the brain is usually confined to a restricted area, for rapid propagation of seizures a group of cells must depolarize simultaneously. If depolarization is of sufficient magnitude, the impulse generated is carried to the entire brain cortex culminating in generalized seizures.

It is noteworthy that in dogs, seizures are often encountered during mid night. Decreased activity in the reticular formation permits reverberating circuits between the thalamus and the cortex to synchronize. Additionally some groups of neurons which are marginally hyperactive when the animal is awake are more excitable during sleep. Why seizures terminate as rapidly as they begin remains a mute question. There may be specific areas in the cerebellum, caudate nucleus, thethalamus and reticular formation that inhibit impulse generation, and thereby play a role in termination of seizures. However, this is purely speculative and remains to be established.

Diagnosis

A systematic diagnostic protocol is recommended for planning effective remedial

strategies depending on the clinico-pathobiochemical profile of individual patients.

1. **Cardinal clinical symptoms are**

- Stiffness of the body
- Loss of consciousness
- Fore and hind limbs extended and visibly rigid
- Vocalization such as screaming loudly
- Excessive salivation
- Sudden, violent shaking
- Muscular twitching or gentle shaking of a limb
- Staring, often with a vacant look
- Vomiting
- Urinary and bowel incontinence

2. **Anamnesis**: Documentation of information from the owner on the pattern of seizures is very useful (Singh, 2002). Special attention needs to be focused on the medical history including trauma sustained during birth/ earlier head injury or protracted febrile condition that could affect the brain gray matter in the post-natal life of the patient. The onset, frequency and the course of development of seizures must be carefully recorded. The physician cannot detect any physical abnormality in the pets that have idiopathic epilepsy when they are not in seizure. A well-structured questionnaire in the local vernacular would be very handy.

3**. Neurological evaluation**: Record of the neurological symptoms would clearly indicate the presence of cerebral lesions for further corroboration by advanced radio imaging diagnostic procedures.

4. **Video recordings**: Videotaped episodes document the precise nature and magnitude of

5. **Electroencephalograph (EEG)**: The EEG is used for corroboration of tentative clinical diagnosis of epilepsy in the dog patient, differentiation between partial and generalized abnormal electrical discharges arising in the brain neurons, and localizing the focal point of seizures (Brendt *et al*., 2005).

6. **Imaging techniques**: CT scan/ MRI profile of the brain cortex are very useful in definitive diagnosis of the suspected intracranial lesion as the primary cause of epilepsy. CT-scan identifies the gross structural aberrations, and MRI-scan pinpoints the brain lesions.

7. **Cerebrospinal fluid analysis**: Pathobiochemical profile of the CSF reflects lesions in the CNS and serves as an important aid in diagnosing conditions like granulomatous meningo-encephalitis, GME (Lothman, 1990). CSF is tapped aseptically from the epidural space in the cervical region just behind the head and subjected to exfoliative cytology and protein analysis. Microbial culture and serological testing for determination of antibody titre may also be undertaken.

Differential Diagnosis

Canine epilepsy with seizures may be carefully differentiated from certain other abnormalities which are:

- Brain tumor: Primary or metastatic tumor may be malignant. By exerting pressure on the brain area as it grows in size, it may induce loss of vision, motor incoordination and seizures. MRI and CT-scan are the most dependable diagnostic procedures.
- Head injury: The severe mechanical impact in scull injury may cause minute intracranial abnormalities, identifiable clearly only in the MRI scan.
- Hepatic/ renal dysfunction.
- Toxicity: Environmental pollutants like chlorinated hydrocarbon or organophosphate pesticides, lead, arsenic, etc. endotoxins elaborated by *Staphylococcus/ Clostridium spp*.
- Lissencephaly: a congenital disorder affecting the patency of the cerebral cortex.
- Hypoglycaemia/hypocalcaemia associated with malnutrition or endocrine anamolies.
- Addison's disease
- Toxoplasmosis
- Distemper

First Aid Protocol in Seizures

1. First and foremost precaution: During the episode the pet physician/ owner should never put the hand inside the dog's mouth to avoid getting bitten badly since the patient is totally unaware of the surroundings (Wyllie, 1997).

2. The animal should be kept in a safe place away from sharp or hard objects to avoid traumatic injury to the head.

3. No attempt should be made to restrain the body since involuntary muscle contractions because of violent convulsions might cause serious internal injuries if the animal were not permitted to move or jerk around freely.

Treatment

The Salient features of some effective traditional and newer anti-epilepsy drugs (AED)

are presented in Table-1.

Table-1: Salient features of some effective traditional and newer anti-epilepsy drugs (AED)

Name of the drug	Indications	Dose schedule	Duration	Comments
1. Phenobarbital (PB)	Seizures, idiopathic epilepsy	Intial dose @ 2-3 mg/ kg b.wt. twice daily	Clinician's discretion (dog patient remaining seizure-free 12 - 24 months)	Sedation, ataxia, restlessness, hepatic dysfunction (Oral sylimarin 5 ml b.i.d./4 weeks)
2. Potassium bromide	Drug of choice in idiopathic epilepsy well tolerated in hepatic dysfunction	Initial dose with PB combination @ 20 mg/kg/ day or else @ 30 mg/kg/day; loading dose @ 90 mg/kg/ day with food	In long-term use KBr serum titre needs to be monitored twice/ year	old anticonvulsant Br^- replaces Cl^- *in vivo*. KBr used in congestive heart failure/ NaBr in hyperadrenocorticism
3. Felbamate	Different types of seizures including focal	Initial dose @ 15 mg/kg t.i.d. with like increment/ every 2 weeks, plateauing off at 60 mg/kg t.i.d.	Long-term use permissible but is costly. Also frequent dosing is required daily	Benificial in patients refractory to bromide and PB: LFT (ALTassay) is advised.

4. Diazepam liq/ gel	Seizures in cluster with idiopathic epilepsy	2 mg/kg with PB, or else 0.5-1.0 mg/ kg rectal administration bi.d. or t.i.d.	Useful only for managing life-threatening status epipepticus (30-60 minutes)	Rectal administration of tablet by the owner issafe home remedy till vetcare becomes avialable
5. Primidone (pharmacological action similar to Phenobarbital)	Seizures, idiopathic epilepsy	Initial dose @ 14.3 mg/ kg b.i.d. then increase judiciously to control seizures	Clinician's discretion (dog patient remaining seizure-free 12 - 24 months)	Sedation, ataxia, rest-lessness, hepatic dysfunction (Oral sylimarin 5 ml b.i.d./4 weeks)
6. Valproic acid (VPA)	Generalized seizures (in combination with PB)	Starting dose @ 5-70 mg/kg t.i.d. with food	Only for short-term use	Because of short half life (1.2-3.7 hr) used as add-on therapy in some patients, refractory to other AED
7.Carbamazepine (CBZ)	Seizures	Starting dose @ 1-3 mg/kg/ day b.i.d.	Only for short-term use	Half life is only 1.5 hr and tapers off to 0.6 hr upon 8 days continued use
8.Gabapentin	Seizures	7-15 mg/kg ti.d.	Only for short-term use	May be used as add on therapy with PB/ KBr, if not effective *per se*
9.Clorazepate	Cluster seizures	2 mg/ kg b.i.d. or t.i.d.	Need-based therapeutic use decided by the Clinician	Adjunctive anti-convulsant with PB

Management of status epilepticus

The clinical emergency may be resolved successfully by following the recommended sequential protocol. Start off with 50% dextrose solution @ 2 ml/ kg slow i.v. infusion → 10% calcium gluconate solution @ 4 ml/ slow i.v. infusion → diazepam oral @ 5 mg/ kg b.wt., repeated at 10 minutes intervals up to 3 times → phenobarbitone @

3-16 mg/hr i.v. → diazepam @ 0.5 to 2 mg/ kg/ hr in 5% dextrose-saline solution slow i.v. drip, administered up to 36 hr → lorazepam LA i.v. general anaesthesia @ 2.5-10 mg single dose, effective for 12-24 hr. Ice packs may be employed for controlling hyperthermia.

Dietary supplements

Neutraceuticals play an important role in prevention of repeated bouts of seizures aggravated by dietary deficiencies in the companion dogs. These include thiamine (Vit. B_1), essential for normal nerve function, omega 3 and omega 6 essential fatty acids (EFA), vitamins, minerals and probiotics like live yeast cells. Dietary supplementation of 100-300 µg betaine-HCl, 50-500 mg dimethylglycine (DMG), 0.2-1.0 g taurine and 10 - 200 mg propanthrocynadin is stated to be beneficial to the epileptic dogs.

CONCLUSIONS

Optimal management of canine epilepsy and seizures includes judicious selection of the appropriate anti-convulsive drugs, to be employed singly or in combination, counseling for suitable lifestyle changes, improved environment and positive outlook of the owner with caring interaction with the pet dog, long-term planning of the remedial therapeutic strategy and anticipated outcome. It is important for the pet physician to formulate a rational initial management strategy, based on the clinical presentation of the individual dog patient. Diagnosis may not be apparent initially, and a detailed pathoclinical and EEG follow-up may be necessary to complete the final diagnostic work-up. Recognition of the possible triggering factor (s) is also essential for effective comprehensive therapeutic management of the patient. Canine epilepsy and seizures sharing common features with the human malady is not intractable. Rapid advances in medical bioengineering and instrumentation shall permit prompt diagnosis, and thereby facilitate improved remedial therapies.

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A Case Report

Successful Treatments of Wasting Form of Type 1 Primary Clinical Ketosis in a Buffalo of Chitwan.

C. N. Kharel

ABSTRACT

A anorexia case of Buffalo was reported in Bharatpur, Chitwan. History and physical examination and laboratory diagnosis confirmed the case to be wasting form of type I primary clinical ketosis. Cybow 9 urine reagent strips (DFI Co.,Ltd) were used to demonstrate ketone bodies in urine of affected buffalo. It was treated with glucose replacement therapy. Dextrose and glucocorticoid was used treatment. Appetite resumed, body condition improved and milk production regained. So glucose replacement therapy with glucocorticoid for sustained response can be used successfully in primary wasting form of type 1 clinical ketosis of buffalo as used in cattle.

Key words: Anorexia, Ketosis, Buffalo, Treatment.

INTRODUCTION

It is a disease of high producing dairy cow during early lactation with worldwide distribution. Clinical ketosis is accompanied by obvious clinical signs like anorexia, drop in milk production, loss of body condition, sweat smell in breadth and occasionally nervous signs in nervous form of disease. Primary ketosis is not associated with other disease conditions producing anorexia. Wasting form of ketosis is initiated with gradual reduction in appetite and milk production and resulting in rapid loss of body condition and diagnosis can be confirmed by positive urine ketone test (Radostits, Gay, Hinchcliff, & Constable, 2007).

Ketosis occurring around the peak lactation is called type 1 ketosis. Underfed animals with high production are energy deficient and progress into type 1 ketosis as a result of reduced gluconeogenic precursors (Herdt, 2014). Kumar *et al.* (2015) showed vital clinical parameters such as temperature, ruminal motility and respiration and heart rate within normal range.

Clinical findings and urinary ketone bodies confirm the diagnosis in buffalo (Maiti and Sharma, 2004).

Case history

A buffalo case with anorexia and drop in milk production, weight loss and depression was reported in Dharapani Chock; ward number five of Bharatpur sub-metropolis, Chitwan, Nepal in a small commercial dairy farm owned by Prakash Poudel on 12 August 2015. The buffalo had decreased body condition score of around 2.5. It was on day 37 of its lactation with average production of around 11 liters a day before the start of this clinical condition and milched 2 liters a day when attended and stopped milking during start of treatment. Symptomatic treatment for anorexia with liver tonics and rumenotonrics clid not respond but in vain.

Diagnosis

Physical examination: Buffalo was lethargic with rectal temperature of 102^{O}F, ruminal motility one per minute, Conjuntival mucus membrane was normal, mild dehydration as revealed by dry and slightly wrinkled skin and sunken eyes.

Laboratory examination: Cybow 9 urine reagent strips, manufactured by *DFI Co., Ltd, Korea,* were utilized that were dip-and-read test strips for in vitro diagnostic use. Reading was taken 60 seconds after strip was dipped in urine in a test tube by comparing the color of test paper attached to a plastic strip with the color chart blocks printed on the vial label. The result urine examination is presented in Table-1.

Table-1: Reading taken based on the color change of urine reagent strips.

SN	Parameter	Case value
1	PH	6.5
3	Protein	Trace
4	Ketone bodies	40 mg/dl

This laboratory result combined with above mentioned clinical findings and history confirms the diagnosis of wasting form of type 1 primary clinical ketosis.

Treatment

Treatment was started on the next day (13 August, 2015) after confirmatory diagnosis. 25% dextrose 200 ml intravenous, Belamyl (liver tonic plus vitamin B-complex) injection 10ml Intramuscular, Curadex (Dexamethasone sodium injection) 5 ml intramuscular, drenching of 200ml glycerine mixed with 1 liter of water, drenching of 200ml Anabolite gluconeogenic precursors with vitamin B-complex and minerals by (Virbac Animal Health, India) were prescribed and administered. Animal started

eating two hours after treatment but the anorexia resumed the next day. Same treatment was repeated the next day replacing 25% dextrose with Rintose 500 ml (a product of Vetoquinol, India with 20% dextrose and electrolytes) there was no improvement. So on the third day the treatment was modified Dextrose 25%-300 ml, Destrose 10%-500 ml, Dextrose 5%-200ml intravenous, Curadex @2.5 ml intramuscular and 2.5ml intravenous, and 200 ml Anabolite and 200 gm molasses daily for 5 days as follow up treatment. Animal responded this treatment and got recovered within a week.

RESULTS AND DISCUSSION

Treatment of two buffaloes of clinical ketosis with Dextrose – 20% (0.5 gm/kg body weight) for 2 days, Inj. Insulin 200 i.u. subcutaneous (0.5i.u./kg b.wt) for a single occasion and glycerin 200 gm daily for 5 days resulted in recovery (Maiti and Sharma, 2004).

Herdt in 2014 prescribed bolus administration of 500 ml 50% dextrose solution as a common glucose replacement therapy combined with glucocorticoid for more sustained response in cattle. But in this case 25% and 5% dextrose were used.

In spite of initial good response of first day treatment, case relapsed the day and second day treatment also didn't respond. Suspecting the impurity of drenched glycerin and potential methanol toxicity, glycerin was replaced by molasses in the third day prescription resulting in successful treatment. Cost didn't permit the use of insulin.

Milk production record starting 2 days before ketosis, during the disease and treatment process and up to 3 weeks after starting the treatment was taken which can be represented as per the following self-explanatory graph.

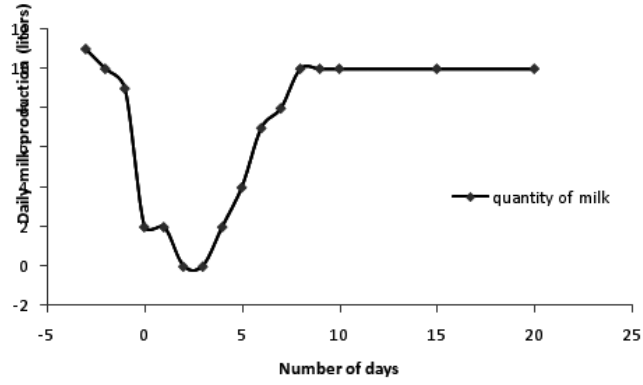

Fig. 1: Changing milk production during disease and treatment

Daily milk production of the buffalo was 11 liters which was dropped up to nil during the disease and milk production started to improve after third day treatment and it was 10 liter at the end of prescribed follow up treatment and remained constant thereafter. Appetite was regained. Body condition score of the buffalo at the end of treatment was observed to be 3.75.

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