Nepalese Veterinary Journal

Vol. 28 **2005** **Regd. ZCBA 7/2025/26**

Editorial Board

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Annual Subscription:

SAARC Countries: Rs. 40.00 **Other Countries: US $ 10.0**

EDITORIAL

Dear Readers,

The twenty eighth volume of Nepalese Veterinary Journal is being published with the papers presented at 7th national conference of Nepal Veterinary Association and some new articles submitted for publication. This journal is aimed at disseminating the technologies and information available within the country to the wider audience within and abroad with the hope that these new information and technologies will be helpful not only to improve production and productivity of animals but also to improve the quality of animal products and thus to the human health. National livestock development requires continuous and dedicated efforts of veterinarians involved in this endeavor from diverse perspective and effective execution of programs at all levels. Our efforts so far has produced some tangible outputs which need to be strengthened further to contribute for national development through improving the health and production of national livestock and the livelihoods of the people depending upon these resources. Our common commitment and actions will achieve this goal.

Many individuals contributed for production and editing of this journal and the list is extensive to name them all. I would like to express my sincere appreciation to all individuals who contributed directly or indirectly for the publication of this journal. I am also grateful to the authors for their contributions, which will increase our knowledge and understanding for improving animals and human health of the country and our collective and collaborative effort will leas us there.

Editor in Chief

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Research article

ACROSOMAL DAMAGE DURING CRYOPRESERVATION OF CROSSBRED BULL SPERMATOZOA: ASSESSMENT BY FLUORESCENT STAINING AND ENZYME ASSAYS*

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ABSTRACT

Infertility has been a consistent problem with crossbred dairy cattle in the Indian subcontinent. The quality of frozen semen is partly responsible for this problem in the population of dairy cattle bred artificially. To understand some of the causes for poor quality of frozen semen of the crossbred bulls, an experiment was carried out to assess the damage to acrosome, an important sperm organelle having key role in the zona penetration event during fertilization. Thirty-two ejaculates of semen from four crossbred bulls (eight from each) were collected, fresh aliquot examined and the rest extended in tris-fructose- egg yolk–glycerol extender. Pre-freeze samples were examined and the rest filled in French medium straws (50 million sperm/ straw) and stored in liquid nitrogen adopting the routine protocol. Fresh, pre-freeze and frozen-thawed semen samples were processed for fluorescent staining and for determination of acrosin and hyaluronidase enzyme activities.

Livability and acrosome reaction status were detected simultaneously using acrosomal probe FITC-PSA counterstained with Propidium iodide. Activities of acrosin and hyaluronidase enzyme per10^9sperm/min. were determined by colorimetry using benzoyl-arginine-para-nitroanilide andhyaluronic acid as substrates respectively. Morphological alterations in acrosome evident from fluorescent staining are described and supplemented with microphotographs. Mean values for hyaluronidase and acrosin activity were 68.2±3.3 and 126.0±4.07 mIU/10^9 sperm/min/ respectively. Cryopreservation induced damage to acrosome was severe as reflected by reduction in hyaluronidase activity by 34 and that of acrosin by 54 per cent in the frozen-thawed spermatozoa of crossbred bull. Prevention of enzyme leakage could be one of the approaches to improve fertilizing ability of the frozen semen of crossbred bulls.

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* Part of The Ph.D. Thesis of The First Author

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INTRODUCTION

Acrosome is known to contain the enzymes like hyaluronidase, acrosin, neuraminidase, b-N acetylglucosaminidase, acid phosphatase, phosphatase A, aryl sulfatase, aryl amidase, adenyl pyrophosphatase, cholinesterase and many other proteinases. Various enzymes that are generally not present in the seminal plasma but present in the sperm and leak when membrane permeability is altered can be estimated to assess the extent of cryodamage. Hyaluronidase and acrosin are two such enzymes. Intactness of these enzymes in the acrosome is essential for the events prior to and during zona penetration. The presence of acrosin has positive correlation with sperm penetration of *in vitro* matured bovine oocyte. Cryopreservation procedures are known to cause acrosomal damage, therefore, loss of acrosomal enzymes. Assessment of sperm acrosin activity before and after cold shock shows loss of this enzyme (Harrison and Flechon, 1980). Figures for intracellular acrosin and hyaluronidase activity for crossbred cattle spermatozoa seem to be lacking. Most of the past studies are based on extra cellular measurement and lack simultaneous structural assessment. This experiment, therefore, was carried out to assess the acrosomal damage in terms of structural alteration and enzymes leakage during cryopreservation.

MATERIALS AND METHODS

Thirty-two ejaculates of semen from four crossbred bulls (eight from each) were collected, fresh aliquot examined and the rest extended in tris-fructose-egg yolk- glycerol extender. Pre-freeze samples were examined and the rest filled in French medium straws (50 million sperm/straw) and stored in liquid nitrogen adopting the routine protocol. Fresh, pre-freeze and frozen-thawed semen samples were processed for fluorescent staining and for determination of acrosin and hyaluronidase enzyme activities.

Acrosome reaction status: fluorescent staining

The status of the spontaneous acrosome reaction along with viability was studied simultaneously adopting the technique described by Sukardi *et al*. (1997) with some modifications. The modifications included use of tris buffer instead of HEPES and removal of excess propidium iodide by diluting the contents several fold and centrifugation instead of filtration.

Preparation of reagents

i) Propidium iodide (PI) 500 µg/ml: 20.0 mg of PI was dissolved in 40.0 ml of tris buffer.

ii) Fluorescein isothiocyanate-*pisum sativum* agglutinin (FITC-PSA) 100 µg/ml: 1000 µg of FITC-PSA in 10.0 ml of tris-fructose buffer.

iii) p-Phenylaminediamine 0.1% in glycerol and PBS: 100 mg of p-phenylaminediamine was dissolved in 90.0 mL glycerol+10.0 mL PBS to make the final volume 100.0 ml.

Procedure

Semen sample containing 10^8 sperm was taken in a 15.0 ml centrifuge tube, centrifuged at 1000 rpm for 10 min. plasma removed carefully by pipetting, washed once again in tris-fructose buffer, supernatant removed and the final volume made to 1.0 ml with the addition of tris-buffer. To this was added 20 µl of PI solution (500 µg/ml) to get the final concentration of 10 µg/ml PI in the sperm suspension. The spermatozoa were allowed to interact with PI exactly for two minutes. The excess of PI was removed by adding 10 fold volume (10.0 ml) of Tris-fructose buffer, centrifuged gently for 5 min. and supernatant removed. The final volume was again adjusted to 1.0 ml. twenty µl of PI treated sperm suspension was smeared on a clean, grease free glass slide in duplicate and air-dried. Spermatozoa were permeabilized by flooding the slide with 100% ethanol for 5 min. The excess ethanol was removed by washing the slides with PBS. The permeabilized slides were then flooded with FITC-PSA working solution (40µg/ml) in tris-fructose buffer and kept in a dark cabinet for 10 min. The excess FITC-PSA was removed by agitating the slides with triple distilled water. A drop of p-phenylaminediamine reagent was placed on the slide to enhance the fluorescence, coverslip applied and its edges sealed with the nail varnish.

Within two hours the slides were examined under the fluorescence microscope (Nikon Microphot FXA EPI-FL3; Japan) with FITC filter set at 40-x magnification. A total of 200 spermatozoa were counted and categorized as follows.

PSA positive and PI negative: Acrosome intact live (AIL)

PSA positive and PI positive: Acrosome intact dead (AID)

PSA negative and PI negative: Acrosome reacted live (ARL)

PSA negative and PI positive: Acrosome reacted dead (ARD)

PSA positive stain showed a green to yellowish green fluorescence. PI positive stain showed red coloured nulcear material, indicating the damaged membrane (as intact membranes are impermeable to PI). The cells that showed a patchy fluorescence were grouped as acrosome intact since the majority of the acrosomal content was found still *in situ*. Cells which retained staining of the equatorial segment were considered fully acrosome reacted, as these cells were considered totally devoid of PSA staining.

Enzymes Estimation

Preparation of spermatozoa extract

An aliquot of semen containing 0.2 x 10^9 sperm was suspended in 2.0 ml of tris-HCl buffer (0.05 m, pH 7.4) and centrifuged at 3000 rpm for 10 minutes. The pellet was washed twice again in 2.0 ml Tris buffer by centrifugation for 10 min. Supernatant was

removed. The pellet was then suspended 1.5 ml detergent solution containing 1mM HCL and vortexed.The sperm suspension in the detergent mixture was sonicated for 2.0 min. providing break time to time. The tube containing the pellet was dipped in ice water to counter the heat generated while sonicating the sample.The sonicated sperm suspension was centrifuged at 10,000 rpm for 20 min. at 4^{o}C.The supernatant was collected and stored at-20^{o}C for the estimation of hyaluronidase and acrosin.

Estimation of hyaluronidase

Hyaluronidase in the spermatozoal cell extract was estimated according to the method of Linker (1984) using following reagents.

Reagents

i) Acetate buffer (acetate 50 mmol, NaCl 15 mmol; pH 4.0).
ii) Hyaluronic acid (Sigma Aldrich) 1.25 gm/L-prepared fresh each week
iii) N-acetylglucosamine (Sigma) standard solutions 20 µg/ml and 10 µg/ml– prepared fresh each week.
iv) Potassium tetra borate solution (Sigma) (pH 9.1).
v) Dimethyl aminobenzaldehyde (Sigma) 1% w/v in 100 mL acetic acid (Containing 12.5% v/v HCL, 10 M. Stock solution. The working solution was prepared just before use by diluting 1 vol. of stock solution with 9-vol acetic acid.
vi) All solutions were stored in a refrigerator at 4^{o}C.

The procedure involved two parts: enzyme reaction and colour reaction.

Enzyme reaction

In a test tube, 0.8 ml hyaluronic acid solution was pipetted and the solution was pre-incubated for 15 min. To this was added 0.2 ml of the sample (sperm cell extract), mixed well and incubated for exactly 20 min. Immediately 0.5 ml of this reaction mixture was transferred to colour reaction tube.

Colour reaction

The colour reaction mixtures were prepared in the order tabulated and described below:

Pipetted in test tubes (in the order)	**Standard 1 20 µg/ml**	**Standard 2 10 µg/ml**	**Sample**
i) Potassium tetra borate solution (pH 9.1)	0.1 ml	0.1 ml	0.1 ml
ii) Standard N-acetyl gluco saminse solution	0.5 ml	–	–
iii) Standard N-acetyl glucosamine solution 10 µg/ml	–	0.5 ml	–
iv) Incubation solution (reaction mixture)	–	–	0.5 ml

Mean time reagent blank and sample blank were prepared. Reagent blank consisted of 0.5 ml acetate buffer instead of the incubation solution and other reagents remained the same. Sample blank consisted of 0.1 ml tetra borate + 0.4 ml hyaluronic acid and 0.2 ml sample (sperm extract). The tubes were heated for three minutes in boiling water bath and then cooled with running tap water. Once cooled dimethylaminobezaldehyde reagent 3.0 ml was added to each including the reagent and sample blank. The mixture was shaken well and incubated for 20 min. in a water bath at 37^{O}C. Then the tubes were cooled, content poured into 1 cm wide cuvettes and absorbance measured immediately at 585 nm wave length. (Spectrophotometre; ECIL, India). The reliability of the assay was verified from the absorbance of both the standards, which had a relationship exactly or close to 1:2 (Standard 2 and 1).

Reference was made to the standards to calculate the amount of acetylglucosamine liberated in the incubation time (t = 20 min.). The reading of 10 µg/ml standard solution was taken for the calculation. One unit (U) of the enzyme activity was defined as one µmol N-acetylglucosamine liberated per min. The true sample absorbance was determined by subtracting the absorbance of the sample blank from the observed absorbance of the sample. Following formula was applied for the calculation:

$$\text{Hyaluronidase Activity} = 226 \times F \times \frac{A\ \text{sample}}{A\ \text{standard}} / \blacktriangle t \quad \text{mIU/109 sperm}$$

Where, F = dilution factor of the sample during pre-treatment, ▲t = 20 min.

Acrosin estimation

Before acrosin estimation, the time required for auto activation of proacrosin of spermatozoa was determined. For this the sperm extract was brought to pH 8.0 and incubated at room temperature for 60 min. From this, an aliquot was taken out at 0, 5, 10, 15, 20 and every 10 min. thereafter, for determination of the acrosin activity. Activation time was determined from a plot of percent proacrosin against incubation time. The maximum acrosin activity was considered as 100 per cent. The auto activation time was 10 min.

The acrosin activity was measured according to the methods of Froman *et al.,* (1984) and Kennedy *et al.,* (1989) with some modifications to suit to our laboratory conditions. Following reagents and the procedures were adopted.

Reagents

i) Tris buffer 0.05 M (pH 8.0)
ii) Calcium chloride 20 mM
iii)Benzoyl arginine-p-nitroainlide (BAPNA) 2.5 mM dissolved in DMSO and water.

Procedure

In a medium size test tube, 0.2 ml of the sample extract was incubated with 0.5 ml of tris buffer (pH 8.0) for 10 min. at room temperature. After incubation 0.4 ml of 20 mM calcium chloride solution was added. To this 0.5 ml of 2.5 mM BAPNA was added and immediately linear change in the absorbance was noted at 410 nm wavelengths for 3 min. recording the observation at 30-second interval.

Calculations

One unit of enzyme activity was defined as the amount of enzyme that hydrolyses 1 µmol BAPNA/min. at 27^{o}C. To obtain the whole numbers the acrosin activity was expressed in mIU/10^9 spermatozoa. The activity was calculated applying the following formula

$$\text{Acrosin activity} = \frac{\blacktriangle A}{\blacktriangle t} \times F \times \frac{1}{0.00995} \times 1.6 \text{ m IU}/10^9 \text{ sperm}$$

Where;

▲A = change in absorbance in 3 min. time

▲t = 3 min.

F = Dilution factor (i.e.)

9950 = molar extinction coefficient of BAPNA

1.6 = final volume at the time absorbance is observed.

RESULTS

Liveability and acrosome reaction status (fluorescent microscopy):

In the fresh semen, the overall mean (±SEM) per cent AIL, AID, ARL and ARD sperm were 67.8±1.08, 12.6±0.87, 13.9±0.88 and 5.7±0.81, respectively. The mean AIL cells, supposed to the functional cells in terms of fertility, were reduced by 11.95 and 53.5 per cents in pre-

freeze and frozen thawed semen when compared to their fresh figures. The microphotograph of simultaneous detection of liveability and acrosome reaction status of fresh semen is shown in Plate 1.

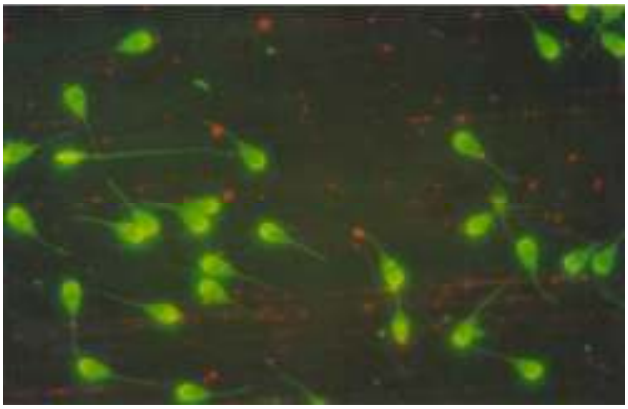

Plate 1. Photomicrograph of spermatozoa (x 400) from fresh semen (FITC-PSA + PI stained) depicting the intesne yellowish green fluorescence of acrosomal contents. Acrosomal content vary from cell to cell.

The acrosome intact (acrosome non reacted) live cells showed uniform contour of the acrosome with green fluorescence covering the entire sperm cell with maximum fluorescence intensity on the acrosomal area. Some of the cells also exhibited patchy fluorescence on the acrosomal region, however these were also considered AIL as most of the acrosomal content was intact *in situ*. The AIL cells were negative for PI staining therefore no conspicuous red colouration was noticed. The acrosome intact dead sperm (AID) also exhibited uniform acrosomal contour with green fluorescence covering the acrosomal region, but as these cells were positive for PI stain, exhibited red staining of the nucleus prominently discernible at the post-acrosomal region.

The acrosome reacted live cells (ARL) though are positive for FITC-PSA, did not manifest the intense fluorescence around the acrosomal region as acrosomal contents are already lost due to acrosomal membrane damage. However, these cells are negative for PI staining, therefore do not exhibit any red colour, as plasma membrane is still impermeable to the dye. These sperm cells are observed to be uniformly mildly green stained without any intense fluorescence on around the acrosomal region. Though some of the cells exhibited the intense fluorescent patch at the equatorial segment, as majority of the acrosomal content was lost, they were considered acrosome reacted.

The acrosome reacted dead cells (ARD), the fourth category of the cells observed in FITC-PSA+PI staining are the cells devoid of acrosome completely or some fluorescence patches seen at the equatorial segment and are positive for PI staining. The nucleus of these cells exhibited red staining.

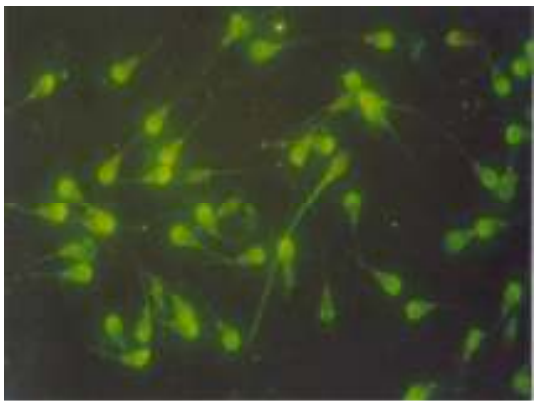

Plate 2. Photomicrograph of spermatozoa (x 400) from frozen-thawed semen stained with FITC-PSA+PI. Dead cells are stained red and many cells have lost their acrosome and/or its content (patchy acrosome).

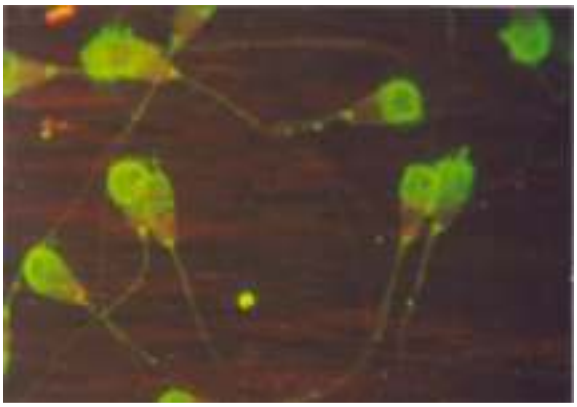

Plate 3. FITC-PSA+PI stained photomicrograph of frozen-thawed spermatozoa (x 1000). Moribund cells have begun to take PI stain, many cells depict patchy acrosome.

Due to freezing stress liveability of spermatozoa seems to be affected more adversely than the acrosomal integrity. This became obvious from comparison of the means of different categories of liveability and acrosome reaction status. There was an increase in AID, and ARD means both from fresh to pre-freeze and from pre-freeze to frozen-thawed stages (Table 1), implying the adverse effect of cryopreservation stress on sperm cell survival. When we compared the absolute figures instead of percentage points, the increase in number of sperm cells in AID and ARD categories are much higher than in ARL. Acrosome is one of the most important organelles altered during cryopreservation (Plate 2 and 3). When we add up the AIL and AID means for fresh and frozen-thawed spermatozoa, we find that about one-third of the acrosome intact sperms are converted into either ARL or ARD conditions.

Enzymes:

The cellular extracts derived from fresh, pre-freeze and frozen-thawed spermatozoa had mean (±SEM) hyaluronidase activities of 68.2±3.33, 57.0±2.92 and 45.3±1.80 m IU/109 sperm/min at 37oC, respectively. A reduction in the mean hyaluronidase activity by 16.42 per cent from fresh to pre-freeze and 20.5 per cent from pre-freeze to frozen thawed stages was noticed, thus resulting in a total reduction of 33.6 per cent from fresh to frozen-thawed stage (Table 1).Similarly mean acrosin activity of fresh, pre-freeze and frozen-thawed spermatozoa were 126.0±4.07, 99.8±4.34 and 57.3±2.87-mIU/109 sperm/min respectively, at room temperature (27oC). A reduction in mean acrosin activity of 20.10 per cent from fresh to pre-freeze and 42.6 per cent from pre-freeze to frozen-thawed stages was observed, resulting in a total reduction of 54.5 per cent during cryopreservation (from fresh to post-thaw stage) (Table 1).

Table 1: Mean (±SEM) value and their percentage alteration of some structural and acrosomal enzymatic attributes of crossbred bull spermatozoa cryopreserved in TFYG extender

Characteristics	Fresh Semen	Pre-freeze	Percent change	Frozen-thawed	Per cent Change from pre-freeze to frozen thawed	Per cent Change from fresh to frozen thawed
Acrosome reaction status						
AIL %	67.8 ± 1.08	59.7 ± 1.29	↓11.95	31.5 ± 1.34	47.2↓	53.3↓
AID%	12.6 ± 0.87	12.6 ± 1.03	-	24.4 ± 1.74	93.6 ↑	93.6 ↑
ARL%	13.9 ± 0.88	16.4 ± 0.77	17.98 ↑	18.4 ± 0.96	12.2 ↑	32.4 ↑
ARD%	5.7 ± 0.81	11.44 ± 1.09	100.00 ↑	25.7 ± 1.40	125.4 ↑	350.8 ↑
Hyaluronidase mIU/10^9sperm	68.2 ± 3.33	57.0 2.92	16.42 ↓	45.3 ± 1.80	20.5↓	33.6 ↓
Acrosin mIU/10^9sperm	126.0 ± 0.07	99.8 4.34	20.12 ↓	57.3 ± 2.87	42.6↓	54.5↓

DISCUSSION

Liveability and acrosome reaction status: The fluorescent staining exhibits more definable contour and distribution of acrosomal content. Patchy fluorescence is observed if part of the contents is lost. Therefore, this technique seems to be more reliable than the conventional techniques such as Giemsa. Moreover, it detects the liveability simultaneously. The morphological observations recorded in FITC-PSA+PI staining are similar to those reported by Sukardi *et al.* (1997) for ram and Januskauskas *et al.* (1999) for bovine sperm. The involvement of relatively high cost and the tedious procedures limit the use of this technique in routine semen evaluation work under our conditions. However, the technique seems to be faster and has the potential for its automation with flow cytometry (Januskauskas *et al.,* 1999).

With FITC-PSA staining, it was possible to distinguish between acrosome intact and acrosome reacted cells along with their liveability. Microscopic evaluation of sample revealed minute facts such as loosened acrosome still *in situ*, patchy acrosome, ruffled contour, more clearly than that in Giemsa stained smears. PI staining distinctly differentiates liveability, dead cells taking the red stain. Some moribund cells (slightly PI stained at certain region of the sperm cell) can also be observed. The limitations of this technique include its failure to subdivide AIL cells into capacitated and uncapacitated categories, as uncapacitated AIL cells are the cells we expect to be fully functional in the context of non-assisted fertilization i.e. artificial insemination.

Januskauskas *et al.,* (1999) recorded AIL, AID, ARL and ARD sperms to be 52.7±1.5, 38.8±1.3, 1.0±0.1, and 7.5±0.7 percent respectively in the frozen-thawed spermatozoa of Swedish dairy bulls. Our finding for AIL are lower than these results signifying the extent of problems with freezing crossbred bull semen successfully. Kumar (personal communication) observed AIL and ARL cells to be 38 and 16 percent respectively at-30^{o}C/min/freezing rate. No other reports in the literature were available to compare our results.

The greater loss in viability occurs during freezing than during equilibration and cooling as evident from 81.7 per cent liveability (AIL + ARL per cent figures) in fresh semen got reduced to 76.1 per cent during equilibration and cooling. However, at frozen thawed stage, the liveability is reduced to 49.9 with a substantial increase in ARL cell count. Holt and North (1994) experimenting with ram spermatozoa under cryomicroscopy and various staining techniques concluded that membrane damage manifested after thawing is the consequence of minimum temperature reached during freezing. Increased permeability induced by freezing is not necessarily accompanied by major structural damage implying that plasma membrane in frozen spermatozoa is damaged not only during thawing but also during freezing. Our methodology does not differentiate the damages occurred during freezing and thawing but the results reflect the total damage.

Enzymatic Activity:

Hyaluronidase is an important enzyme of the acrosome system synthesized during spermatogenesis in the testes. This study probably is the first of its kind to report the mean hyaluronidase activity in the fresh spermatozoa of crossbred bull. Lilliam *et al.* (1980) recorded a release of 344±33 nmole n-acetyl glucosamine per min per 10^{8} sperm cells from the bovine epididymal spermatoza treated with digitonin+2 mM calcium ion indicating the role of this enzyme in post-capacitation events of fertilization. The release of hyaluronidase by the spermatoza is required for the penetration of the ovum has been investigated in the studies on capacitation (Rogers and Morton, 1973).

A total loss of 33.6 per cent hyaluronidase activity from fresh to frozen-thawed state is in conformity with reduction in percent intact acrosome. This loss in hyaluronidase activity is attributable to damage to acrsomal membrane and alteration in its permeability. Release of acrosomal hyaluronidase following increased membrane permeability to calcium (can be comparable to cryo-capacitation) has been reported in bovine spermatozoa (Lilliam *et al.,* 1980). Foulkes and Watson (1975) detected an increase in hyaluronidase activity in the seminal plasma of bull semen after freezing and thawing indicating the leakage. In a similar study, Mall *et al.* (1997) reported a loss of about 45 per cent hyaluronidase activity in frozen thawed spermatozoa of buffalo attributable to cryodamages. The increase in hyaluronidase activity in goat spermatozoa in extra cellular medium after freezing has also been reported (Singh *et al.,* 1996). Acrosomal damage contributed to larger increase in percent of total hyaluronidase activity in the seminal plasma as reflected in our results by the decreased

activity of this enzyme in the sperm cell. The sperm with lower hyaluronidase activity might exhibit lower fertility. Its release into seminal plasma is an early and sensitive indicator of acrosomal damage during semen processing (Mancini *et al.,* 1964) and freezing (Foulkes and Watson, 1975).

The reduction in enzymatic activity of acrosin in frozen thawed sperm was more severe than that of hyaluronidase as reflected in our study by 54.5 per cent reduction in its activity from fresh to frozen-thawed state. The explanation for more abrupt reduction in acrosin activity than that of hyaluronidase under the same treatment might be attributable to differences in solubility in the extender, therefore differences in rate of release in the medium. The enzyme released in the medium is discarded and the part of the enzyme retained in the sperm cell was measured in our study. Freezing caused reduction in acrosin activity in goat sperms (Singh *et al.,* 1996). From these findings we recognize the need of measurement of acrosin activity of the spermatozoa as an additional independent parameter in determining the possible existence of a male factor of infertility in crossbred cattle. The test is quick and can be undertaken easily.

CONCLUSIONS

Cryopreservation induced damage to acrosome becomes severe as reflected from this study by reduction in hyaluronidase activity by 34 and that of acrosin by 54 per cent in the frozen-thawed spermatozoa of crossbred bull. Therefore, it is strongly recommended that in estimation of acrosin activity of the spermatozoa should be carried out as an additional independent parameter in determining the possible existence of a male factor of infertility in crossbred cattle. The test is quick and can be undertaken easily.

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Nepal Veterinary Association

Research article

PRODUCTION RESPONSES OF MIGRATORY SMALL RUMINANTS IN NEPAL WITH INTEGRATED APPLICATION OF HEALTH, NUTRITION AND FLOCK MANAGEMENT IMPROVEMENTS

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ABSTRACT

Studies on various approaches to and technologies on improvement on health, nutrition and flock management in the migratory flocks of Nepal showed that technologies on gastrointestinal parasite control, poisoning treatment, nutritional supplementation and predation control reduced the flock mortality, increased the body weight gain of young and yearling lambs and kids and advanced the mating and parturition by few months, which is better suited for survival and growth of young animals. Treatment of gastrointestinal nematodes with anthelmintics at monthly intervals during wet summer months improved the body weight gain of treated lambs and kids by 3.8 and 2.2 kg over the untreated controls. Similarly, oral drench of sodium thiosulphate solution @ 5-10 gm per animal was effective to save the animals from natural poisoning with aconite plants. The solar lighting and nylon net enclosure was very effective to control predation during night. The anthelmintic treatment and nutritional supplementation during winter was highly effective to alleviate stunting syndrome of yearling lambs, as well as inducing earlier mating and lambing/kidding in the adult ewes and does. All these approaches when introduced in an integrated manner were highly effective to improve overall flock productivity and income from the flocks. Introduction of these technologies in the flocks in a package of practices could support the viability and sustainability of the system and increase flock productivity. It is thus important to develop the mechanism to transfer these technologies to the migratory management system to protect it from declining, and support its continuity and contribution to the local and national economy.

INTRODUCTION

Migratory sheep and goats are an important component of mountain agriculture in the northern districts of Nepal. About 65% of sheep and 35% of national goat population are reared as mixed flocks under migratory management system since centuries utilizing the abundant natural resources available in the high alpine pastures of the Himalayas (LMP, 1993). These animals have special importance for hill and mountain communities as these animals are used not only for meat and wool/fibre production but also for maintaining soil fertility and even for pack use in some parts of the country.

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Despite the importance of the system, migratory management of small ruminants is facing several problems, which affect the overall existence of the system. These problems are associated with health, nutrition and management of the animals in the flocks. In addition, the managerial and socio-economic factors also play critical role to affect the viability of this system. The overall effects of these factors are additive and complimentary to each other, thus, all related constraints need to be addressed and mitigated for overall improvement in the system, which would result in productivity improvement, increased benefits from the flocks and enhanced viability of the overall production system.

Previous studies (Karki, 1985, Joshi, 1995) have shown that gastrointestinal parasites, plant (aconite) poisoning and predation are the main cause of animal mortality in the flocks. Similarly, poor growth rate in the yearling lambs (referred to as stunting growth) is another important cause of poor flock productivity (Karki, personal communication), while unavailability of adequate grazing pastures during winter, is responsible for bodyweight loss in lactating ewes which results to delayed mating and lambing and poor survival of the lamb crops (Karki, 1987).

In the migratory management system, access to modern veterinary care is non-existent and animal treatment system is traditional and mostly based on local herbs. Modern veterinary care are neither available nor adopted by the shepherds, thus heavy loss in animal population is the norm. It was recorded in the flocks of Darchula district, that mortality may be as high as 17% of total flock strength or 46% of the total new born animals in a year (Joshi, *et.al* 2004). Hence, high mortality of the animals in the flocks and poor winter nutrition are the main cause of productivity losses in the flocks.

Hence, some studies were conducted on the constraints related to health, nutrition and flock management so that strategies for productivity enhancement could be developed and implemented for overall improvement in flock productivity, which could help prevent the migratory system from declining in the country. The findings of some of these studies are presented in the paper.

MATERIALS AND METHODS

The following studies were undertaken on constraints related to health, nutrition and flock management.

Study 1: Control of gastrointestinal parasites in lambs and kids

Twenty four lambs and kids of both sexes born during November-December and aged between 5-6 months were matched for their body weight and randomly allocated to two groups; the treatment group received the preventive anthelmintic treatment at monthly intervals with fenbendazole @5 mg/kg body weight from JuneDecember. This group was

managed together with the control group managed under traditional practice (without any treatment). The remaining flock management practices were similar for both groups. Animals in both groups were weighed at monthly intervals to evaluate the responses on weight gain.

Study 2: Responses to gastrointestinal parasite control and nutritional supplementations in stunted lambs

Twenty-three stunted yearling lambs of both sexes with similar body weight and condition were randomly allocated to two groups. Animals in one group were treated with Fenbendazole @ 5mg/kg body weight and provided with concentrate feed (fortified with compound mineral mixture with vitamin A, D and E @ 5% of concentrate feed) @ 100 gm feed per animal/day for a period of 90 days during the winter months (December-February), while the other group was reared under normal flock management (un-supplemented). Body weight gain and body condition of animals in both groups was monitored for the study period.

Study 3: Control of gastrointestinal parasites and nutritional supplementation of ewes during winter months.

More than 100 lactating ewes and does in the flock were treated for gastrointestinal parasites once during December with fenbendazole @ 5 mg/kg body wt and then supplemented with concentrate feed fortified with agrimin forte at 5% of the feed. The feed was provided @ 100 gm/animal/day for 75 days. The mating and lambing/kidding pattern in these animals was monitored and compared with immediate previous year's lambing pattern of the flock.

Study 4: Treatment response of aconite plant poisoning cases.

Treatment response to natural poisoning cases with aconite plant found at the high altitude pastures was undertaken by treating the naturally poisoned animals with sodium thiosulphate solution orally @ 5 gm/young animal and 10 gm/adult poisoned animal. Recovery of poisoned animals was recorded as the positive response of the antidote on poisoning cases.

Study 5: Vaccination responses against 6-month disease in lambs and kids.

A total of 175 lambs born during October/November of the previous year in the flocks of Ghandruk village of Kaski district were allocated to two groups, one vaccinated with enterotoxemia vaccine (Raksha ET: Indian Immunological) at the rate of 2ml/sc during the month of March followed with booster dose one month later and the other managed as unvaccinated control under the similar management conditions. A total of 114 lambs were vaccinated and 61 lambs were monitored as unvaccinated control. The vaccination response was evaluated during the following year by recording the occurrence of six-month

disease in both groups. Recording was based on information provided by shepherds and recording the occurrance of disease and death in the vaccinated and unvaccinated groups. No post mortem or laboratory isolation was made on dead animals, and the cause of death was ascertained on symptomatic basis.

Study 6: Predation control from wildlife using solar lighting and nylon net enclosure during the nights

Mobile solar penal with 10 watt capacity were installed for night lighting at the two corners of the herded flock during the night and enclosed with one meter high nylon net having 6 cm pore size mesh and fixed around the flock with wooden poles and small iron pegs. The system was a simple solar penal with battery recharged during the daytime and used during the nights. This system was adopted especially during the winter months when the incidence of predation was found to be highest. The cases of predation during day and night were recorded for the intervened and non-intervened flocks of the area.

RESULTS

Study 1: Control of gastrointestinal parasites in lambs and kids

Weight gain in anthelmintic treated lambs and kids was significantly higher (Table 1) than traditionally managed animals under farmers management with the weight gain difference of 3.8 kg in lambs and 2.2 kg in kids within the period of 7-8 months. This increment in meat value is equivalent to about 200-350 rupees per animal with the expense of 36 rupees per animal on anthelmintics.

Table 1: Weight gain response of anthelmintic treated and untreated lambs and kids.

Groups	Initial wt	Final wt	Total wt gain	Daily wt gain	P
Lambs-treated	17.1	28.3	11.2	45.9	0.000
Lambs-control	17.1	24.5	7.4	30.3	
Kids-treated	12.4	22.5	10.1	41.4	0.04
Kids-control	12.4	20.3	7.9	32.4	

It was also recorded that anthelmintic treated animals maintained their superior weight gain trend throughout the study period, which was even more distinct during the post- monsoon months.

Study 2: Evaluation of responses to gastrointestinal parasite control and nutritional supplementation in stunted lambs

The response of stunted yearling lambs to parasite control and supplementary nutrition was highly significant in terms of the weight gain and improvement in body condition of animals (Table 2).

Table 2: Weight gain responses to supplementation in the stunted lambs

Experimental groups	Number of animals	Initial weight (kg)	Final weight (kg)	Total weight gain (kg)	P value
Supplemented	12	19.4	25.6	6.2	0.001
Un supplemented	11 (2 lambs died)	20.6	23.2	2.64	

In the un-supplemented group, two lambs died during the study period without any specific symptoms, thus it can be said that stunting was also associated with ill thrift of the animals. The weight response in the supplemented lambs was highly significant and the improvement in body condition was evident visibly.

Study 3: Control of gastrointestinal parasites and nutritional supplementation of ewes during winter months

The response to anthelmintic treatment and supplementary nutrition of ewes and does during the lean winter months induced two month earlier mating in ewes and does (Figure 1), resulting most of the lambing/kidding during October-November, which is considered as the most suitable months for survival and growth of new born lambs and kids under the migratory management.

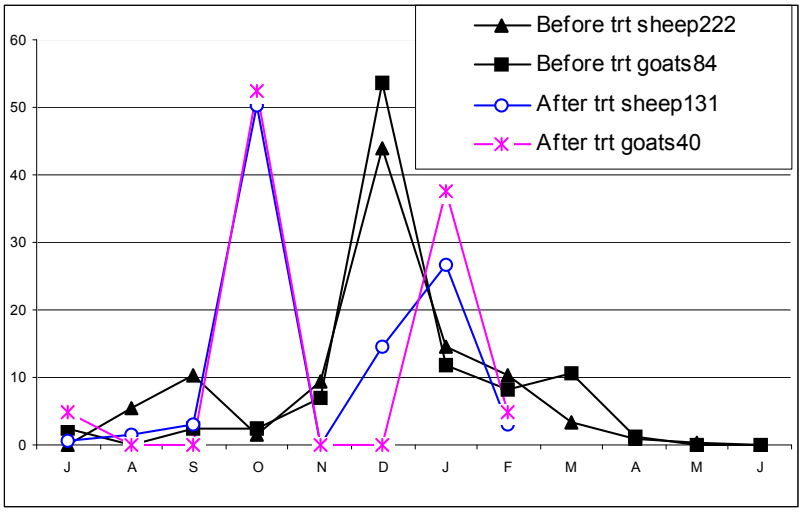

Figure 1: Change in lambing/kidding pattern in Sikles with supplementary nutrition and parasite control

Study 4: Treatment response of aconite plant poisoning cases

Among the 98 animals naturally poisoned with leaves and flower of aconite plant and treated with the sodium thiosulphate @5 gm/young animal and 10gm/adult animal as oral drench, 96 animals recovered from the poisoning effects, which indicates 98% efficacy of the antidote to treat poisoning cases under natural poisoning conditions.

Study 5: Vaccination response against 6month disease in lambs and kids.

Response to vaccination on the incidence of disease and mortality is presented as Table 3.

Table 3: Vaccination response of enterotoxaemia vaccine on six-month disease of lambs.

Groups	N	Death due to 6 month disease	Mortality % due to 6 month disease
Vaccinated	114	1	0.87
Unvaccinated	61	5	8.2

Only one vaccinated lamb died (0.87%) due to this disease in comparison to 8 percent mortality recorded in the unvaccinated animals, which clearly reflects the effectiveness of this vaccine against six-month disease in lambs. However, further study with confirmatory diagnosis is needed to evaluate the effectiveness of the vaccine more accurately.

Study 6: Predation control using solar lighting and nylon net enclosure during the nights

The night lighting and nylon net used in the flock prevented night predation in the intervened flock, where as in the non-intervened neighboring flocks night predation during the night was high. However, the day predation loss during grazing time was similar in both flocks (Table 4).

Table 4: Predation in intervened and non-intervened flocks.

Catagory	**Number of animals predated during**		**Total number of predated animals**
	Day	Night	
Intervened flock	10	0	10
Non intervened flock	11	13	24

Effectiveness of this technology for predation control during night flocking was very high and provided some new approaches for wildlife conservation and reduction of predation loss from wild life.

DISCUSSION

Series of studies conducted to alleviate the important constraints of migratory flocks especially for reducing the mortality and improving the nutritional status during the critical lean months provided some useful results, which will contribute for increasing the productivity of the flocks and to improve the viability of the system.

The higher weight gain in the parasite controlled animals by 3.8 kg in lambs and 2.2 kg in kids clearly shows the effects of gastrointestinal parasites on the growth and productivity of lambs and kids. This technology is highly profitable, as the economic value of the body weight gain was many times higher than the cost of medicine spent on the animals. Similar results were obtained in the previous studies (Joshi, 1995) with parasite control in the flocks of Ghandruk village of Kaski district, in which the responses were even higher when parasite control strategy was applied for longer period. The findings of this study also support the previous results and highlight the losses due to sub-clinical gastrointestinal parasitism and need to control this loss. Better growth rate in young animals would have other benefits on the overall flock productivity by subsequent better weight gain, earlier mating and lambing.The stunting syndrome seen in the yearling animals in some flocks though not studied for its specific cause or its magnitude in the population is an important cause of reducing flock productivity. The present study though could not identify the specific cause of stunting; it was clearly evident that the combined approach of gastrointestinal parasite control, nutrient and mineral/vitamin supplementation has significantly improved the condition of stunted animals. Thus this problem might be the effect of sub-clinical parasitism, or due to the deficiency of specific mineral (for example copper or cobalt) or other nutrients. Some long-term study involving larger sample size might be useful to understand this problem more clearly.

The supplementary winter nutrition and parasite control in adult ewes and does had significant beneficial response on improving the productivity of migratory animals. This intervention resulted earlier lambing/kidding from December month to October, which would result easier lambing management, better lamb growth and survival. It is possible because, during this period the grazing pasture is better for the lactating ewes and does leading the better lamb growth. Similarly, lambing during October will lead to better viability of the newborn lambs and kids as they would have developed the age related resistance against the gastrointestinal parasites by the next monsoon month and reduce the incidence of stunting syndrome as reported in the flocks.

The therapeutic approach against the natural cases of aconite poisoning in small ruminants has been found to be highly effective to save the animals in the field. Similar responses of sodium thiosulphate were not obtained in the experimental studies, in which the compound was used as antidote to lethal doses of aconite poisoning (Shrestha, 1995, personal

communication), however, its response under natural infection has been found to be very encouraging. The reason for this difference is unclear, but one possible explanation might be

the limited amount of poison consumed under the natural infection, and symptoms like drowsiness, off feed, salivation exhibited by the animals prevent the animals from grazing and further consumption of poison. In this situation, sodium thiosulphate helps to alleviate the symptoms and supports the survival of the poisoned animals.

Vaccination against six-month disease with enterotoxaemia vaccine, though yielded useful results, yet the findings need to be reconfirmed with accurate laboratory support and close monitoring, which was not possible during this study. However, for the field purpose, the present findings clearly indicate the protective responses of the vaccine against the six-month disease of lambs reported in the flocks.

Control of predation loss during the night shelter by using the solar lighting and the nylon net enclosure has been found to be very effective in avoiding this loss during winter months, when the flocks are kept on the fallow fields for *in-situ* manuring. Predation loss has been very serious in the flocks of Gandaki region, which accounts for about 19% of loss in flock population and about 70% of the losses in the newborn lamb crops (Karki, 2004). Hence, if this technology could reduce half of the predation losses, it would contribute significantly to the overall flock productivity.

It is this apparent that, with the introduction of these technologies in the migratory flocks in a package, the biological factors associated in reducing flock viability will be improved considerably, resulting overall flock productivity. However, in addition to the biological factors, it is also important to address the managerial or social factors, which affect this system considerably. Moreover, it is also important to develop mechanism for delivery of the technologies in the flocks, which at present is non-existent, as the public support services have not touched this system yet. Hence, some special efforts or program need to be developed which could deliver the available technologies and continue to investigate on the new approaches and technologies for the long term viability and sustainability of the system, so that the need of the people living in the remotest part of the country could also be addressed.

ACKNOWLEDGEMENTS

The authors are grateful to the shepherds and the highland community for allowing us to work in their flocks. We are also thankful to the field motivators for their careful recording. The financial support from DFID, UK through the HARP project and the managerial support from participating institutions is also gratefully acknowledged.

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Nepalese Vet. J. 28: ***26-32***
Nepal Veterinary Association

Research article

STUDY ON INFECTIOUS CAUSES OF INFERTILITY AND ITS MANAGEMENT IN CROSSBRED AND EXOTIC CATTLE IN NEPAL

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ABSTRACT

This study was aimed to evaluate the extent of infertility problem and to isolate, identify the specific and nonspecific agents causing infertility in crossbred and exotic cattle in Nepal. The treatment responses of antiseptic and antibiotics in repeat breeders and aborted cases have also been evaluated. One hundred and eighteen serum samples collected from the repeat breeder and aborted cows were subjected for antibody detection of Brucella abortus, Leptospira hardjo, Infectious Bovine Rhinotracheitis/Infectous Pustular Vulvovaginitis virus and Chlamydia pscittac which showed that 0.8% samples were positive for the presence of antibody for B. obortus, 9.3% for L. hardjo and 50.8% for Infectious Bovine Rhinotracheitis/Infectious Pustular Vulvovaginitis Virus. No sample was positive for C. pscittaci. The cervical and uterine samples tested for Trichomonads microscopically, revealed no positive case. Escherichia coli, Bacillus spp and Staphylococcus aureus were major bacterial species isolated from cows having cervicitis and endometritis. The treatment response of cows with pathological condition of endometritis, and/or cervicitis was found promising. Out of 28 cases treated, 19(67.8%) cows became pregnant. Therefore the treatment therapy applied in this study can be a promising therapy for the treatment of cases with infectious form of infertility of microbial origin.

INTRODUCTION

In Nepal infertility problem in crossbred and exotic cattle has been reported to be the most prioritized problem in dairy pocket areas in the country (Jha, 2000). A study conducted in Kathmandu valley in improved cattle revealed that among the various reproductive disorders the proportion of anestrus, repeat breeding and abortion was 45%, 27% and 5% respectively (Khanal, 1996). Reproductive disorders such as anestrus and repeat breeding was reported to be 21% and 36% respectively in breedable cows and heifers in Pokhara valley (Sankhi, 1999).

Infertility in animals is associated with microbial pathogens, anatomical abnormalities, hormonal imbalance, nutritional deficiency, hereditary defects and extreme climatic conditions. The infectious form of infertility could be due to *Leptospira*, *Listeria, Brucella, Mycoplasma, Ureaplasma, Campylobacter, Trichomonas, Chlamydia, Haemophilus, E.coli,*

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Streptococcus; various organisms vis: Viral agents such as Bovine Viral Diarrhoea and Infectious Bovine Rhinotracheitis and various fungus (Hungerfords, 1989). Pradhan (1996) found that 3.3% sera samples were positive for *Brucella* antibody in cattle in Chitwan district.

In this study attempts have been made to know the extent of infertility problem and to isolate and identify the specific and nonspecific agents causing infertility in crossbred and exotic cattle in Nepal. The treatment responses of antiseptic and antibiotics in repeat breeders and aborted cases have also been evaluated.

MATERIALS AND METHODS

In a close collaboration with Livestock Services Offices of Rupendehi, Kaski, Sunsari, Ilam, Lalitpur, Kathmandu, Morang and Chitwan districts; the infertility camps were attended and also the dairy farms having crossbred and exotic cows with infertility problems were visited. Clinical examination and rectal palpation were performed and where appropriate, pathological samples were collected for laboratory investigation.

The vaginal swabs and uterine mucus samples were preserved in the Amies transport media until the samples were subjected for *Campylobacter* and other bacterial examination in the laboratory. For *Trichomonads* the cervical and/or uterine mucus samples were examined on a slide under a microscope immediately after collection.

For Brucellosis, the test used was *Brucella abortus* C-ELISA manufactured by SVANOVA, Biotech AB, Sweden. For Leptospirosis the test used was ELISA for *L. hardjo* antibody manufactured by Cedi diagnostics, BV, Netherlands. For IBR/IPV the test used was ELISA IBR-IPV manufactured by Institute Pourquier, France. For Chlamydiosis the test used was Bovine *Chlamydia* AB test Kit, Immunocomb, Biogal Galed Lab, Israel.

At the time of visit to the farms, immediately after clinical examination the cows found to have endometritis and/or cervicitis were treated with following therapies.

Group 1: Povidone iodine solution 10-20 ml mixed with 10ml normal saline administered intrauterine.

Group 2: Antibiotics (Gentamycin 5ml or 1gm Cephalexin powder in 10ml of distilled water or Kanamycin 1 gm in 10 ml of distilled water) administered intrauterine.
Group 3: Povidone iodine 10-20 ml mixed with 10ml normal saline given intrauterine and oxytetracycline injection @ 4mg/kg body weight given intramuscular. In addition to the above treatment all treated cows were given minerals and vitamin A, D3 and E @ 50 gm/cow/day orally for 10 days.

RESULTS

The reproductive disorders in different breed types and age groups was recorded as follows (Table 1).

Table 1: Reproductive problems among the breed type

Breed	**Age group (Years)**	**No of cows with reproductive problems examined**		
		Anestrus	**Repeat breeding**	**Abortion**
Jersey	2-8 yrs.	24	27	8
Jersey cross	2-10 yrs.	43	68	11
Holstein	3-8 yrs.	5	11	0
Holstein cross	3-7 yrs.	7	11	1
Brown swill	2-3 yrs.	2	0	0
Haryana cross	3-4 yrs.	0	3	0
Total 221		81 (36.7%)	120 (54.3%)	20 (9.0%)

On physical examination the reproductive disease conditions recorded in the animals was as reported in Table 2.

Table 2: Disease conditions found during rectal examination of cows

Reproductive problem	*Disease conditions*							
	Endometritis	***Endometritis /Pyometra***	***Cervicitis***	***Vulvitis/ vaginitis***	***Poor development of reproductive organ/inactive ovary***	***Follicular cyst***	***Luteal cyst***	***No any disease condition***
Anestrus	***3***	***3***	***5***	***9***	***20***	***2***	***4***	***35***
Repeat breeding	***27***	***9***	***20***	***6***	***-***	***10***	***2***	***46***
Abortion	***2***	***3***	***-***	***3***	***-***	***-***	***-***	***12***
Total(221)	***32(14.5)***	***15(6.8)***	***25(11.3)***	***18(8.1)***	***20(9.1)***	***12(5.4)***	***6(2.7)***	***93(42.1)***

Figures in parentheses indicate percentage

Forty-nine vaginal swabs and 72 uterine mucus collected from the cows with pathological condition of cervicitis, endometritis vaginitis with a history of repeat breeding and or abortion were subjected to microbiological culture. The culture yielded the growth of following organisms (Table 3).

Table-3: Bacterial species isolated from the vaginal and uterine mucus samples

Bacterial species	No. of isolates (%)
Escherichia coli	49 (28.0)
Bacillus spp.	26(14.6)
Staphylococcus aureus	11(6.3)
Enterobacter spp.	10(5.7)
Pseudomonas spp.	10(5.7)
Streptobacillus spp.	9(5.2)
Corynaebacterium spp.	9(5.2)
Streptococcus spp.	8(4.6)
Micrococcus spp.	8(4.6)
Bacillus cerus.	8(4.6)
Acenetobacter spp.	7(4.0)
Staphylococcus epidermidis	6(3.3)
Vibrio spp.	5(2.9)
Proteus spp.	5(2.9)
Klebsiella oxytoca	4(2.3)
Campylobacter spp.	2(1.1)
Brahmnella spp.	1(0.57)
Total	175(100)

The micro organisms isolated were subjected to antibiotic sensitivity test, which showed sensitivity to different antibiotics as presented in Table 4.

Table 4: Antibiotic sensitivity test of some bacterial isolates

Isolate	No. of isolate tested	Antibiotics used & no. of sensitive isolates									
		A	**CL**	**C**	**CO**	**G**	**K**	**N**	**O**	**S**	**T**
Escherichia coli	10	1	10	1	2	10	10	10	7	8	10
Pseudomonas spp.	5	0	2	0	0	5	5	2	5	2	3
Staphylococcus aureus	5	0	5	2	2	5	5	5	4	4	4
Bacillus cerus	3	1	3	1	1	3	3	3	3	3	3
Streptococcus spp.	3	1	3	1	2	3	3	3	3	1	2
Acenetobacter spp.	2	2	2	2	1	2	2	2	2	2	1
Enterobacter spp.	2	1	2	1	2	2	2	2	2	2	2

A= Ampicillin, CL = Chloramphenicol, C= Cloxacillin, CO= Cotrimoxazole,, G= Gentamycin, K= Kanamycin, N= Nitrofurantoin, O= Oxytetracycline, S= Streptopenicillin, T= Tetracycline

Serum samples collected from the animals were subjected to serological tests against *Brucella abortus, Leptospira hardjo*, *C.pscittaci* and IBR/IPV antigen: The result of the test is presented in Table 5.

Table 5: Results of serological tests

Type of Infertility case	No. of serum samples tested	No of serum samples positive for antibody to			
		Brucellosis	**Leptospirosis**	**IBR/IPV**	**Chlamydiosis**
Anestrus	21	0	0	4	0
Repeat breeding	77	0	8	46	0
Abortion	20	1	3	10	0
Total	118	1 (0.8%)	11 (9.3%)	60 (50.8%)	0

The responses to the therapentic intervention with different therapentic approaches is presented as Table 6.

Table 6: Treatment effects on cows with disease condition of endometritis and/or cervicitis.

Treatment applied	No. of animals treated	No of pregnant	Effectiveness
Group 1	10	6	60 %
Group-2	7	5	71 %
Group-3	11	8	72.7 %
Overall	28	19	68

DISCUSSION

The bacteriological examination of 121 vaginal and uterine mucus samples revealed that 86.7% samples were positive for bacterial infection and 13.3% had no bacterial growth. It can be seen in Table3 that the most common isolates from the vaginal and uterine mucus were *Escherichia coli* followed by *Bacillus spp*., *Staphylococcus aureus*, *Enterobacter spp*., *Pseudomonas spp*., *Streptobacillus spp*. and *Corynebacterium spp*..

The pattern of bacterial isolates found in this study indicates that most of the isolates, which do not seem to be pathogenic in healthy condition, may be pathogenic when the reproductive organs are under stress or injured. Isolation of *Pseudomonas spp*. from the reproductive tracts is warranted because it is usually reported to cause infertility in cows. Singh *et al* (1996) reported that the *Bacillus spp*. was more frequentlyisolated followed by *Escherichia coli* and *Staphylococcus aureus* from cervico-vaginal mucus of repeat breeder cows. Deshmukh and Markandeya (1995) reported that ascending types of microbial infections and residual microflora of genital tract of cows under certain conditions lead to low grade endometritis resulting into repeat breeding.

Two samples were found to be positive for *Campylobacter spp.*. The *Campylobacter fetus subspecies fetus* can produce sporadic abortions and can be responsible for repeat breeding problems in cows (OIE Manual, 1996).

Among the isolates examined for antibiotic sensitivity tests, most of the isolates were susceptible to gentamycin, chloramphenicol, kanamycin, nitrofurantoin and tetracycline. Majority of the tested isolates were found to be resistant to ampicillin and cloxacillin. Based on this finding the gentamycin, chloramphenicol, kanamycin, nitrofurantoin and tetracycline can be promising drugs to treat the infectious cases of infertility caused by bacterial infections.

The cervical and uterine samples tested for Trichomonads microscopically, none of the sample was found positive.

One hundred and eighteen serum samples collected from the repeat breeder and aborted cows were subjected for antibody detection for *Brucella abortus*, *Leptospira hardjo*, Infectious Bovine Rhinotracheitis/Infectous Pustular Vulvovaginitis virus and *Chlamydia pscittaci,* 0.8% samples were positive for the presence of antibody of B.abortus 9.3% for L.hardjo and 50.8% for infectious bovine rhinotracheitis/Infectious pustular vulvovaginitis and none for C. psittaci.

This result clearly indicates that leptosirosis and IBR/IPV diseases are possibly playing a great role in causing infectious form of infertility problems in dairy cows of Nepal. Because it is well known that *Leptospira hardjo* can cause abortion, reduced conception rate and low fertility in cows, the positive serological result for the presence of leptosirosis in Nepal alarms for further investigation and develop an effective control strategy because it has public health significance as well. The presence of antibodies against IBR/IPV in such a high percentage (50.8%) indicates the presence of this disease in cattle population of Nepal. It is a viral disease responsible mainly for poor conception with worldwide prevalence (Sewell and Broklesby, 1990).

The treatment response of the cows with pathological condition of endometritis, and/or cervicitis was found promising. Out of 28 cases treated, 19(67.8%) cows were found to be pregnant at follow up after 5 months. Therefore the treatment therapy applied in this study can be a promising therapy for the treatment of infectious form of infertility particularly infertility due to microbial origin.

Further indepth study needs to be done on the identification of various causes of infertility including infectious causes and development of its strategic control measures.

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Nepalese Vet. J. 28: 33-42
Nepal Veterinary Association *Research article*

STUDIES ON AFLATOXIN LEVEL IN POULTRY MEAT AND MEAT PRODUCTS MARKETED IN KATHMANDU AND CHITWAN VALLEYS

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ABSTRACT

A study was conducted for a period of one year on the aflatoxin levels in poultry meat and meat products marketed at Kathmandu and chitawan Valleys of Nepal showed that 5 percent samples were positive for aflatoxin B_1. These findings are discussed in this paper.

INTRODUCTION

There are more than two dozens of mycotoxins, which are classified as hepatoxins, nephrotoxins, neurotoxins, cytotoxins, estrogenic mycotoxins and other mycotoxins according to the main organ/system involvement. Aflatoxins are one of the major mycotoxins. Aflatoxins are produced by different species of fungi such an *Aspergillus flavus, A. parasiticus, A. nomius* but *A.niger, A.oryzae, A.versicolour*, etc fungi are also capable to produce aflatoxins. These toxins provoke even fatal aflatoxicosis in man, animals and poultry. *Aspergillus species* are ubiquitous in nature and these molds can grow rapidly and become so much in grains or feed stored under aerobic conditions when moisture content of the feed stuff or food exceeds to 15% at optimum temperature of 24 to 25 centigrade. **A. flavus** 58417 produces all toxic components but *A. flavus oryzae* produces only AFB_1 and AFB_2. *A.niger* produces only AFB_1 and *A.parasiticus* produces B_1, B_2, G_1 and G_2 metabolites.

Rationale of present study:

Aflatoxins are hazardous to human health and have been detected in human milk, blood, urine, tissues etc. Many human deaths, cirrhosis in children, hepatic carcinomas and colon adeno-carcinomas in human beings have also been reported from aflatoxicosis in many countries of the world. Aflatoxicosis in poultry cause severe loss in poultry industry due to reduction in body weight, changes in blood parameters, and various pathological changes in different organs of the chickens (Sedai, 1999). Aflatoxin also increases susceptibility towards various poultry diseases such as Marek's disease, Salmonellosis, coccidiosis, Gumboro disease etc. About 1.3 times more feed is required to gain similar weight as that of non-infected normal chickens. Half- life of single oral does of AFB_1 in hen is about 67 hours (Sawhney *et al*., 1973).

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In broilers, metabolites of dietary AFB_1 and B_2 reach highest concentration in gizzard, liver and kidney (Chen *et al*., 1984). Report on prevalence of aflatoxin in meat and meat products have been reported by earlier scientists. In India maximum permissible limit of mycotoxin in feed is 20 parts per billion (ppb) for broiler feed and 30 ppb for layer feed but in certain parts of the country (e.g. Uttar Pradesh State) up to 2250 ppb mycotoxins have been recorded (Devegowda and Jamkhedkar, 1994).

Consumption of aflatoxin-contaminated diet is the main source of aflatoxin in body fluids and tissues and aflatoxin poses serious health hazards in human beings and animals (Mishri *et al*., 1999). Sinha *et al* (1997) reported the wide distribution of aflatoxin in the staple foods/feeds in Bihar state. In Nepal, most of the feed ingredients for poultry feed are imported either from Uttar Pradesh or from Bihar state of India where the prevalence rate of aflatoxins is too high.

Status of aflatoxin contamination in Nepal:

WHO (1979) has reported that almost all plant products can serve as substrate for fungal growth and mycotoxin production, thus providing potential for direct contamination of human food. When farm animals used for food production are fed on feed contaminated with mycotoxins and aflatoxins, it not only causes direct toxic effect on animals, but also carries aflatoxins into milk and meat, thus creating a medium for human contamination to mycotoxins. Occupational exposure may occur through other media such as air. Aflatoxins have proved to be hazardous to human health due to their presence in varieties of staples food and feed ingredients. These toxins have been detected in urine, milk and blood samples of human beings (both maternal and foetal blood). Metabolite P_1 is considered as a major human urinary metabolite of AGB_1.

Alpert *et al.,* (1971) reported that in different parts of Uganda increased frequency of detectable aflatoxin contamination of food samples (range 10.8 to 43%) were associated with increased incidence of primary liver cancer (range 1.4-15 cases per 100,000 total population per year) and Amla *et al*. (1971) suggested the ingestion of AFB_1 as a possible cause of cirrhosis in children in India.

Degar (1976) also found aflatoxins as causative agents of colon adenocarcinomas.Philips *et al*., (1976) reported that aflatoxin B_1 at an estimated level of 520 ug/kg fresh weight was detected in the liver of a resident of the U.S.A, suffering from carcinoma of liver and rectum. They further reported that AFB_1 had been found in the liver or other tissues of human subjected at levels of up to 500 micro gram/kg or more.

Wray and Hayes (1980) reported that aflatoxins were found in the human tissues, either in the free form in the liver or bound to DNA in the liver. Olsen *et al*. (1988) studied occupational exposure of aflatoxins among the Danish workers engaged in animal feed production and found that seven of 45 workers exposed to AFB_1 contaminated feed at the

level of 0 to 26 microgram/kg of aflatoxin B_1 and serum albumin of these seven workers contained less than 5 picogram per milligram (pg/mg) to 100 pg/mg albumin. They further reported that dust samples collected at different sites showed the presence of aflatoxin with the level of non- detectable to 8-microgram/kg dusts. Aflatoxins can be accumulated in different organs or tissues of animals, birds, and man. Mintzlaff *et al*. (1974) reported that when poultry were fed with ration-containing aflatoxin at the rate of 25-5000 micro gram/kg for 8 weeks, residues of AFB_1 were found in the liver and in the muscle tissues. They further reported that the liver contained the highest concentration with a mean value of 15 micro gram/kg weights at the highest exposure level. Aflatoxin residues were also detected in kidneys, muscles and adipose tissues.Rodricks and Stoloff (1977) found aflatoxin residues in the animal tissues, eggs and poultry following the experimental ingestion of aflatoxin-contaminated feed. Chen *et al.* (1984) reported that in broiler, the metabolites of aflatoxin B_1 and B_2 reached highest concentration in gizzard, liver and kidneys.Richard *et al*. (1986) fed a diet containing 50 or 150 ppb aflatoxin to turkeys for 11 or 13 weeks and reported that afltoxin B_1 and M_1 were found in the liver, kidneys, gizzard and faeces of poultry.Agacdelen and Acet (1993) fed AFB_1 to 12 hens at 500 femtogram (fg)/day for 7 days and detected aflatoxin first in eggs at 0.013 ng/gm on 3rd day of administration. They further reported that AFB_1 levels in eggs were 0.031, 0.051, 0.082 and 0.117ng/gm on 4^{th,} 5th, 6th and 7th day respectively. AFB_1 was no longer detected in the eggs on the 4th day after stopping aflatoxin administration.

The prescribed permissible level of aflatoxin by HMG/Nepal is 30 ppb in cereal grains and 50 ppb for milking cattle feed. Karmacharya (1989) reported that out of 69 maize samples taken from hills of Nepal 12 were contaminated with AFB_1 and three with AFB_2 having the aflatoxin level of up to 110 ppb. Similarly, out of 41 maize samples taken from Terai areas of Nepal, nine were contaminated with AFB_1 and 2 (22%) with AFB_2 with the level of aflatoxins up to 321ppb.

Karki and Sinha (1991) reported that *A.flavus* isolated from Terai area produced higher amount of aflatoxin B_1 (6334 ppb) than from the hills and mountains. They further reported that out of 95 poultry feed samples, 49 (51.57%) samples were contaminated with aflatoxin at the level of up to 1100 ppb. They had also identified *A.flavus* fungi in eight corn samples (out of 52) from Katmandu valley and in 17 corn samples (out of 52) from Terai/inner Terai of our country.

Karki *et al*., (1994/95) reported that out of 3 samples of *pea nut* cake, one samples was found to be contaminated with AFB_1 (181 ppb) and AFB_2 (70 ppb). Similarly, Khadka (1999) reported the presence of aflatoxin AFB_1 in 37 poultry feed samples in a total of 50 samples

colleted from Katmandu, Chitawan and Biratnagar. It indicates that proper care is not taken during storage of feed at farmer's field and at factory level.Aflatoxins residues have been found in animal tissues, poultry meat and eggs following the experimental ingestion of

aflatoxin-contaminated feed this has been tested or reviewed by some earlier workers abroad. Due to continuous feeding of contaminated feed these toxins are accumulated in different tissues of poultry and also transferred to eggs and the hatched progeny. But studies on concentration of aflatoxins in poultry meat and meat products have not been carried out in Nepal, hence this study was carried out to estimate the prevalence of aflatoxins in the poultry meat, meat products and some visceral organs of poultry marketed in Katmandu and Chitwan valleys and to evaluate the quality and quantity of aflatoxins present in the collected samples so that policy recommendations could be made for quality poultry production and meat marketing for human consumption in the country.

Statistical analysis

Simple statistical tools were used for the calculation. The test of significance was not done after getting the total results of the tested samples, since out of 100 samples only five samples were found positive for aflatoxins.

MATERIALS AND METHODS

Due to some the unavoidable circumstances the study started later than expected, hence strict seasonal pattern for sample collection could not be maintained however, a seasonal collection schedule was maintained as far as practicable. A total of 100 poultry meat/liver and meat products samples were collected by random sampling from Kathmandu and Chitawan valleys. The samples were collected from the poultry carcasses weighing not less than 1.5 Kg. body weight. From the carcass, 100 gm of liver and or 100 gm of other meat samples were collected to represent the sample size. Similarly, meat product samples were also collected from the heaps of meat products. Seasonal collection was done to test the levels of aflatoxins in the tissues/meat and meat products according to seasons. The detail design of the collection of samples is given in the Annex-1.

Extraction and precipitation of aflatoxin

Extraction and precipitation of aflatoxin was done by the method described by Thomas *et al*. (1975). The materials so collected were minced finely with the help of the mincer/grinder wherever possible. Then they were kept in the conical flask (250 ml/500ml) separately and were treated with the double volume of methanol-water (70:30). After 24-36 hours of treatment the mixture-containing flask was shaken for 30 minutes using the magnetic stirrer or by hand shaking technique. Then, the liquid portion was filtered through Whitman filter paper number 1. This procedure was repeated for two times to extract the whole toxins from the sample. The filtrate so collected was mixed with equal volume of hexane and shaken well for separation of lipids from the medium. Hexane treatment was repeated and separated using the separating funnel unless the upper layer became transparent. Then, medium was mixed with equal volume of chloroform and aflatoxin containing extract was separated with the

help of separating funnel and the lower layer of chloroform was collected passing through the Whatman filter paper number 1 containing sodium sulphate anhydrous and evaporated using water bath to obtain the yellowish precipitate of aflatoxin. The aflatoxin so obtained was wrapped with aluminum foil (both mouth part and tube length) and black-paper sheet and stored at low temperature and away from the sunlight.

Thin layer chromatography for elution aflatoxin fraction

Qualitative estimation of extracted aflatoxin was done by thin layer chromatography (TLC) techniques as per the procedure described by Pons and Goldblatt (1965). The analysis of individual components was carried out by TLC method. For this, TLC plate was prepared by uniformly spreading the silica gel-G slurry on chromatoplate. But in most cases, readily available TLC plates were also used in this study.

Spotting of unknown extracts and standard aflatoxin on TLC plate

The suitable quantity of extracted aflatoxin obtained from liquid medium was dissolved in 5 ml chloroform. Fifty misrelate of extracted solution was spotted in a line 2 centimetre (cm) from bottom of chromatoplates leaving at least margin of 2 cm from either side with the help of micropipette. The micropipettes were reused after washing with sodium hypochlorite solution (5%) followed by distilled water and finally with acetone. The solution containing known quantity of aflatoxin B_1 were spotted along with the sample extracts. The solution was allowed to spread more than 5 mm in diameter during application on plates.

Developing TLC plate

The developing solution was prepared in chromatography glass-tank containing Toluene: Isoamyl alcohol: Methanol (TIM) with ratio of 90:32:2 (v/v/v/) as described by Nabney and Nesbitt (1965). Solvent mixture was kept up to a depth less than 1 cm. The plate was developed by standing them in chromatography tank. The solvent front was allowed to reach 10-12 cm. After developments the plate was dried in horizontal position. 9:1 chloroform: acetone solvent was also equally effective to develop the TLC plate.

Qualitative estimation of aflatoxin extracts

The TLC plate was viewed under long wave ultra-violet lamp (peak emission 365 μ) in a dark cabinet. The intensity of fluorescent of the extract spot was compared with that of the standard. The spot, which matched with, that of standard, gave the approximate concentration for further calculation. In case, if spot was not matched then either extracted solution was diluted or the concentration of standard spot was increased or decreased.

Confirmatory test for AFB_1

Confirmatory test was carried out to rule out any possibility of the presence of fluorescent substance other than aflatoxin by Spraying with hydrochloric acid (HCl). The aflatoxin spots showed fluorescence of light-blue or yellow-green under U.V**.** light. The same was again visualized after 4.0 hours. The spot again gained back the original fluorescence under U.V. light. This confirmed the presence of aflatoxin.

Quantitative estimation of AFB_1

The final quantification was done using the method given by Romer/as described by Jones (1972). The formula used was as below: -

$$\text{Amount of aflatoxin } B_1 \text{ (In parts per billion)} = \frac{S * Y * V}{W * Z}$$

Where,
S = Microlitre (µl) of aflatoxin B_1 standard equal to that of material being evaluated on the plate.
Y = Concentration of aflatoxin B 1 standard in ug/ml.
V = Microlitre (µl) of solvent required to dilute the final extract.
W= Weight (in gm) of the original sample contained in the final extract.
Z = Microlitre of sample spotted to give fluorescence intensity equal to S, the aflatoxin standard.

Calculation:

In the present study following were the basis for calculating the quantity of aflatoxin B_1: -
Samples spotted on TLC were 5 ul (Microlitre), 10 ul. and 20 ul.
S = 5,10,15 and 20 ul
Y = 0.93 microgram/ml
V = 100 ul (i.e.0.1 ml)
W= 25 gm
Z = 5 ul, 10 ul.15ul and 20 ul.

RESULTS

Materials were collected in the month of May, August, September and December 2003. Out of a total of 100 samples (Poultry liver-50 and Meat samples-50) only 5 (Five-3 liver and 2 meat samples) samples were found positive for aflatoxin B_1 .The detail of the results is given in the Table 1.

Table 1: Result of Aflatoxin analysis in samples.

Month	Location	Samples obtained from	Number of samples collected	Samples positive for Aflatoxin	Remarks
May	Kathmandu	Liver	7	0	
		Meat/Meat products	8	0	
	Chitwan	Liver	5	0	
		Meat/Meat products	5	0	
August	Kathmandu	Liver	6	1	Trace
		Meat/Meat products	6	0	
	Chitwan	Liver	7	1	Other has AFB_1(3.72 PPB)
		Meat/Meat products	6	0	
September	Kathmandu	Liver	6	2	AFB_1=3.72(PPB), other =Trace
		Meat/Meat products	6	1	AFB_1=3.72(PPB)
	Chitwan	Liver	7	0	
		Meat/Meat products	7	0	
December	Kathmandu	Liver	6	0	
		Meat/Meat products	7	0	
	Chitwan	Liver	6	0	
		Meat/Meat products	6	0	

The concentration of AFB_1 was found 3.72 ppb in two livers and one meat samples. As the prevalence of aflatoxin in the poultry tissues was found only in the poultry tissues was found only in the rainy season the test of significance was not carried out. Due to unavailability of standards of other aflatoxin fractions their estimation was not carried out.

DISCUSSION

In Nepal, probably, this is the first report on aflatoxin contamination in the meat tissues. Only two or three reports are available from India. Some western reports showed the tissue concentration of aflatoxins in the poultry organs/meat after feeding aflatoxins in their diets. Mintzlaff *et al.* (1974) had reported 15 microgram of aflatoxin /kg of liver.Similarly, Arshad *et al.* (1993) found 1.32 to 4.95 ppb of aflatoxin in the chicken livers after feeding different levels of aflatoxins for a period of 8 weeks.Yadav *et al*., (1995) reported that aflatoxin B_1 was found 4.7 ppb in the liver and 2.0 ppb in the muscle tissues of the broiler chickens fed with the feed containing 0.5 ppm of AFB_1. In the present study, the analysis of the results showed that sample collected only in the month of August and September showed the positive values i.e. trace to 3.72 ppb both in the livers and muscles of the commercial poultry. Because of the very small sample size, samples from the local poultry were not taken in this study. This result simulates with the findings of Arshad *et al.* (1993) and Yadav *et al***.** (1995). Except in the rainy seasons, no aflatoxins were detected both in the livers and muscles samples of the poultry. This might be due to the presence of lower concentration of aflatoxins

in the feed. Because, Kan *et al. (*1989) could not find the residues of aflatoxins in the breast muscles of broilers and laying hens feeding them 150 and 750 microgram AFB_1 /kg for 6 or 3 weeks, where the detection limit was less than 5 nanogram/kg.

CONCLUSIONS AND RECOMMENDATIONS

Aflatoxins are carcinogenic, immunosuppresant, teratogenic and hepatotoxic in nature. They are accumulated in the tissues of the living individuals. These toxins are responsible for the stunted growth of the poultry leading to the economic losses. Although the present study showed only 5% positive for aflatoxinB_1, there might be even more percentage of contamination in the samples if the study could have been carried out using ELISA or High Performance Liquid Chromatography (HPLC) technique. Dashain and Tihar are our main festivals and people use maximum amount of meat during these festivals. So there is more chance of contamination with aflatoxin during these days**.** Although the prescribed permissible level of aflatoxin during these days beyond the prescribed permessible level of aflatoxin given by HMG/Nepal, which is 30 ppb in cereal grains and 50 ppb for milking cattle feed respectively.

ACKNOWLEDGEMENTS

I am indebted to my supervisors namely, Dr. S.N. Mahato, Director General, Department of Livestock Services; Dr. Tika Bahadur Karki, Director General, Department of Food Technology and Quality Control, Kathmandu and Dr. R.M. Shrestha, Chief, Central Veterinary Laboratory, Tripureswor, Kathmandu, for their valuable guidance, hearty encouragement and support and allowing me to work in their laboratories.

My sincere thanks go to Mr. Kundan B.Shrestha and Ms. Jayanti Amatya of Central Food Laboratory, Kathmandu who helped much during the qualitative and quantitative estimation of aflatoxins. My sincere thanks go to Mr. Dhana Raj Rai of Central Vety. Lab., Kathmandu, who helped me much during the sample collection, extraction and also in the estimation of aflatoxin in the laboratories. Other staff members of CVL who were directly or indirectly involved in this study are also acknowledged. I would like to express my sincere thanks to my wife, Bindu and daughters for their support, patience and motivation during this study period.

Last but not the least; I am indebted to the Royal Nepal Academy of Science and Technology (RONAST) and its faculty members for the financial as well as moral support, without which this study would not have been possible

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Nepalese Vet. J. 28: 43-50
Nepal Veterinary Association

Research article

ASSESSMENT OF PATHOGENIC PROPERTY OF *FUSARIUM GRAMINEARUM* [1] IN BALB/C MICE

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ABSTRACT

An experiment was conducted to investigate the immunologic property, pathogenicity and treatment of Fusarium graminearum infection. Several groups of mice were randomly selected for the following groups: (PC, T_1 and T_2 were groups of mice that respectively received a 1:1, 1:100 and 1:100,000 fungal dilution while T_3, T_4, and T_5 groups of mice respectively received the same concentration but each were treated with Diethylamine acetarsol (Acetylarsan). A group of mice was included as negative control (NC).

In vitro assays were used to examine the ability of F. graminearum to produce enzymes, which are thought as important virulence indicators. Results revealed the ability of the pathogen to produce collagenase and elastase. In addition, histopathological examination indicated vascular congestion and mild triaditis of the liver. Pulmonary congestion and lymphoid hyperplasia in the spleen were noted. The fungi were recovered from the liver, lungs, spleen and skin of legs of some experimental mice. Likewise, weight of spleen was doubled as early as the second week and continued up to the fourth week where it tapered off in the untreated group. Similar increase in the weight of spleen was observed in the treated group but not as much as in the untreated group. Hematological findings showed an increase in lymphocytic count from 1.83 to 3.356, monocyte count from 0.47 to 0.981 and neutrophils count from 0.399 to 1.698 in untreated groups. Lymphocyte count in the treated groups increased from 1.8 to 3.64, monocytes increased from 0.068 to 0.325 and neutrophils increased from 0.223 to 1.056. High incidence of death was observed in animals that did not receive treatment (PC, T_1, and T_2) while relatively fewer death incidences were exhibited by groups that received diethylamine acetarsol (T_3, T_4 and T_5).

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INTRODUCTION

Fusarium Infection had been associated with Deg Nala disease mostly in buffaloes and cattle in India, Pakistan and Nepal, which affects the productivity of the buffaloes and cattle in South Asia, thus affecting the financial resources of village people. The precise mechanisms underlying the observed symptoms of Deg Nala disease is not known. In this study, investigative efforts had been focused on the pathogenicity, ability to produce immune response and efficiency of diethylamine acetarsol as effective therapeutic agent.

Infections that may be debilitating in nature can cause significant economic losses as a result of decreased production confounded by reduced growth rate, mortality and poor animal performance. An effort to improve animal production in the village calls for suitable control or therapeutic measures of any disease. There is insufficient information describing the pathogenicity of the fungus *Fusarium* in livestock in different parts of the globe. Experimental studies of the immunologic properties and treatment of *F. graminearum* infections should be considered, to understand the pathogenicity, immunological characteristics and treatment of *F. graminearum* infection.

METHODOLOGY

Fusarium graminearum Test Strain

The fungus *Fusarium graminearum* was obtained from the National Culture Collection of Microorganisms, Institute of Molecular Biology and Biotechnology, University of the Philippines at Los Baños, Laguna. The stock culture was inoculated in Sabouraud's Dextrose Agar and was kept at room temperature for two to five days.

Test for Pathogenicity

Elastase Production

Inoculation and Incubation of the Culture Medium: Sabouraud's dextrose agar was supplemented with 0.3% elastin (Sigma No.E 1625). Test strains (F. graminearum) and the positive control (P. aeruginosa) was inoculated as streaks in Sabouraud's dextrose agar supplemeted with 0.3% elastin. Three plates were set as replicates for the test strain and the positive control. A negative control consisted of an uninoculated Sabouraud's dextrose agar supplemented with 0.3% elastin (also in three replicates). Plates were incubated for two weeks at 37ºC.

Observation of Elastase Production: Clearing of zone around fungal colonies or bacterial colonies in case of positive control indicates elastase production. Designated scores were set forth to describe elastase production. These were presented as follows: 0.0, no clearing around colonies; 1.0, 20-30% of colonies on the surface of the medium are surrounded by clear zones; 3.0, 40-70% of colonies on the surface of the medium are surrounded by clear zones; 5.0, 80-100% of colonies on the surface of the medium are surrounded by clear zones. Observation of positive reaction was undertaken daily for two weeks. This procedure was conducted twice to confirm the result of the first experiment.

Collagenase Activity

Inoculation and Incubation of the Control Medium: Nutrient broth (5 ml) was supplemented with type I collagen from Bovine Achilles Tendon (5 mg). The media were dispensed in tubes and autoclaved at 115°C for 15 minutes. The tubes were inoculated with the test strain (*F. graminearum*) and *Clostridium perfringens* (positive control). Three tubes were set as replicates for the test strain and positive control. An uninoculated Nutrient broth supplemented with type I collagen from Bovine Achilles Tendon (also in three replicates) was included as a negative control. These were incubated at 37ºC for two weeks.

Observation of Collagenase Activity: The tubes were examined grossly for indication of collagen digestion during entire incubation period. Designated scores were set forth to describe collagenase production. These were used as follows: 0.0, no indication of collagen digestion; 1.0, 20-30% of the collagen in the medium is digested; 3.0, 40-70% of collagen in the medium is digested; 5.0, 80-100% of collagen in the medium is digested. Observations of positive reaction were undertaken daily for two weeks.

RESULTS AND DISCUSSION

The colonies of *F. graminearum* were initially white in color and cottony at the surface of Sabouraud's dextrose agar. As the colonies matured, they became greenish to brown in color. This is in conformity with the morphological studies on *F. graminearum* conducted by Segal *et al.* (1998).

Elastase and Collagenase Production

Table 1. Shows the ability of *F. graminearum* to produce elastase and collagenase. The ability of *F. graminearum* to produce elastase was observed to be the same as that of the positive control (*Pseudomonas aeruginosa*).

F. graminearum produced collagenase at a lower level when compared with positive control (*Clostridium perfringens*). The degree of collagenase production manifested by both test strain and positive control was not significantly different. The effect of the above test suggests that enzymes like collagenase and elastase produced by *F. graminearum* may enhance its damaging effect on tissues. The characteristic lesions of the Deg Nala disease like drying and sloughing of tail tips (Irfan, 1971) and injuries of the extremities could be possibly related to the damaging effect of these enzymes on tissues in chronic cases of infection.

Table 1. Elastase and collagenase production of *F. graminearum*

Criteria	***F.* graminearum**		**Positive Control**		**Negative Control**	
Elastase	5.00	$(0.000)^{ns}$	5.00	(0.00)	0.00	(0.00)
			P. aeruginosa			
Collagenase	4.30	$(0.942)^{ns}$	5.00	(0.00)	0.00	(0.00)
			C. Perfringes			

Data presented as mean (± standard deviation) response of three replicates.

Designated scores were set as follows to categorize responses: 0.0-no indication of positive responses as described in the materials and methods; 1.0-20-30% of the colonies on the surface of the medium show positive response; 3.0-40-70% of the colonies show positive response; and 5.0-80-100% of the colonies on the surface of the medium show positive response. The criterion for elastase production was the presence of clear zones around the colonies while collagenase production was indicated by collagen digestion. There was no significant diffence compared with the positive control.

Mortality

Data on the mortality profile shown in Table 2 shows that there was high mortality rate (20%) in the group that received the undiluted or concentrated fungal inoculum (PC).

Table 2. Weekly mortality profile (M/Total) of mice infected with *F. graminearum*

Weekly Interval	Treatment Groups						
	PC	T_1	T_2	T_3	T_4	T_5	NC
1	4/20 (20)	2/20 (10)	1/20 (5)	1/20 (5)	1/20 (5)	1/20 (5)	0 (0)
2	3/16 (18.75)	3/18 (16.6)	1/19 (5.26)	1/19 (5.26)	0 (0)	0 (0)	0 (0)
3	1/13 (7.69)	1/15 (6)	1/18 (5.5)	0 (0)	0 (0)	0 (0)	0 (0)
4	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Data represent the ratio of the number of dead animals (M) over the total of animals (T) in a given treatment group at indicated time intervals. Values in parenthesis are mortality rates (%).

This trend tapered down to 18.75% on the second week and reached a lower level (7.69%) on the third week. Relatively lower mortality rate (10%, 16.6%, and 6%) was recorded in T_1 while T_2 exhibited the lowest rate of mortality percentage from the first week to the third week of observation. These results were different from those of treatment groups that received the diethyl amine acetarsol. Treatment 3 had mortality rates of 5.0% to 5.2% on the first and second week and no death was noticed thereafter. Treatments 4 and 5 manifested 5% mortalities only on the first week. No mortality was recorded in the negative control. Data on mortality profile show that infection with *F. graminearum* induced immediate death of experimental animals within the first four days post-challenge. No existing literature reveals elicited responses similar to this. The death recorded in the first few weeks post-challenge with *F. graminearum* indicated that the defense mechanism of the animals was inadequate to mount immune protection.

Histopathological Examination

Based on histopathological findings, various changes in the liver, lungs and spleen were observed. In the liver, vascular congestion and portal triaditis were noted.

There were reactive nuclear changes; slight enlargement of the nuclei but no alteration of chromatin distribution was noted.

Some livers exhibited mild to moderate dysplastic changes which included prominent nuclear enlargement, hyperchromasia, pleomorphism and chromatin clumpings. The amount of dysplastic changes in some hepatocytes gives the possibility of some of the cells that develop hepatocellular carcinoma. No indication of malignant cells was seen.

In the lungs, most of the changes seen were non-specific vascular congestion. Some emphysematous changes were seen but were sporadic and non consistently present.

In the spleen, lymphoid hyperplasia and vascular congestion were observed. These were considered as being non-specific changes.

This study offers a more detailed histopathological finding related to *F.* graminearum. Previous literatures revealed gross lesions that involved the skin, lungs, sinuses, spleen, kidney, muscles, CNS, and liver, heart, eyes, joints, and toe nails of infected animals (Boutati and Anaisse, 1997).

Splenomegaly

Splenomegaly (Table 4) was observed in the positive control. The initial weight of 49 mg increased to 125 mg in the fifth week. The group that was given lower concentration of pathogen had the lowest spleen weight (43 mg) and reached weight of 115 mg in the fifth week. In the groups that were treated with antifungal drug, the lowest weight of the spleen was 60 mg and highest weight was of 200 mg in the fourth week.

This pattern indicated that in the control group and even in mice that received lower concentration of fungi, the body system took a longer time to develop the capacity to build up the body immune systems. These results are similar to the findings of other authors relating to splenomegaly in human beings diagnosed with systemic infection due to *Fusarium* (Guarro and Gene, 1995).

Table 3. Weekly weight (in gm) of spleen in experimentally infected mice with *F.* graminearum (mean ± sd)

Weekly Interval	*Treatment Groups*						
	PC	T_1	T_2	T_3	T_4	T_5	NC
1	0.0499 (0.009)	0.0435 (0.085)	0.0660 (0.006)	0.0395 (0.005)	0.0405 (0.0025)	0.0405 (0.0045)	0.0380 (0.003)
2	0.073 (0.0015)	0.073 (0.005)	0.0775 (0.005)	0.0675 (0.005)	0.126 (0.014)	0.063 (0.0015)	0.064 (0.002)
3	0.075 (0.005)	0.165 (0.055)	0.085 (0.005)	0.11 (0.01)	0.165 (0.055)	0.125 (0.005)	0.0415 (0.035)
4	0.08 (0.002)	0.0825 (0.0025)	0.055 (0.005)	0.105 (0.005)	0.2 (0.01)	0.1275 (0.0075)	0.045 (0.003)
5	0.125 (0.005)	0.115 (0.005)	0.0365 (0.0015)	0.079 (0.001)	0.175 (0.005)	0.1225 (0.005)	0.0455 (0.005)
6	0.0985 (0.005)	0.099 (0.001)	0.035 (0.000)	0.062 (0.004)	0.105 (0.005)	0.099 (0.005)	0.0485 (0.005)

Based on the findings gathered in this study, *F. graminearum* is a pathological agent, which has the ability to infect vital organs of the body, which would cause impairment of organ functions. This pathogen possesses the ability to digest elastin and collagen that may be seen in body tissue which could be attributed to the manifestation of the disease. This study also indicated that *F. graminearum* caused substantial pathological damage to liver, lungs, and spleen. The pathogen was found to induce leukocytosis and marked increase of lymphocytes, neutrophils and macrophage. It was found that when infection was induced, the mortality ranged from 20% in first week and declined to 7.69% in the third week in positive control group and untreated group. While in treated group, mortality was only 5% from the first to third week.

Relating the findings of this study to Deg Nala disease in Nepal, further studies should be conducted to confirm the direct involvement of other species of *Fusarium* on Deg Nala disease, which would require application of fungal isolation and identification procedures. Finally the application of diethyl amine acetarsol or its derivatives as a treatment for *F. graminearum* infection in domestic animals might also be considered for evaluation.

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Nepalese Vet. J. 28: 51-57
Nepal Veterinary Association

Research article

GENETIC AND NON-GENETIC FACTORS AFFECTING LITTER TRAITS OF PAKHRIBAS BLACK PIGS IN NEPAL

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ABSTRACT

Pakhribas pig is a black colour pig and is highly preferred by the people in the eastern hills of Nepal. This breed has been developed in Nepal by three way crossing of exotic breeds (Saddle back, Fayuen and Tamworth) at Agricultural Research Station (ARS), Pakhribas, then Pakhribas Agricultural Centre (PAC). The data on 317 pigs born over a period of fourteen years (1989 to 2002) were used to study the effect of non-genetic factors on litter traits and estimate their genetic parameters. The overall litter size at birth, litter size at weaning, litter weight at birth and litter weight at weaning was 9.4±0.19, 8.3±0.18, 9.32±0.25 kg and 50.6±1.45 kg respectively. Parity of birth affected the litter traits. Heritability estimates of litter size at birth were low but the estimates for litter weight at weaning was high (0.68±0.314) suggesting selection based on litter weight at weaning would bring the improvement.

INTRODUCTION

Pakhribas pig is a result of three-way crossing of exotic breeds (Saddle back, Fayuen and Tamworth) developed at Agricultural Research Station (ARS), Pakhribas, then Pakhribas Agricultural Centre (PAC) (Aryal *et al*., 1992). The breed is very popular in the eastern hills of Nepal because of its black colour and a good litter size at birth and weaning. For religious purposes black colour is preferred over other colours. It is medium in size and can be maintained by the farmers in the hills.

The performance records indicate that they are good in litter size and other reproductive parameters. A study carried out at On-farms aiming to compare the productivity of Pakhribas pigs with the local and its crossbred with Pakhribas pigs showed that the Pakhribas pigs produced 90% more meat than the local and 60% than its crossbreeds at the farmers feeding and husbandry system (Oli, 1986). This indicated that Pakhribas pigs are more productive and well suited to local environments.

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Information on the non-genetic and genetic factors affecting production traits is however, not available for Pakhribas pigs. The study was therefore, undertaken to estimate the non-genetic factors affecting litter traits and estimate their genetic parameters. In order to accomplish the study, a retrospective study was done and for this data obtained over fourteen years were analysed.

MATERIALS AND METHODS

The study was conducted at Agricultural Research Station (ARS), Pakhribas, then Pakhribas Agricultural Centre (PAC), Dhankuta district, in the eastern midhills of Nepal. The area is located at an altitude of 1740 m above sea level. The geographical location is 27^o 17'N and 87^o 17'E.

Sixteen sows and four boars have been kept at the Station for the production and distribution of the piglets to the farmers. They have been replaced periodically by good stock. They are fed with a commercial ration at a rate of 3 kg/day/adult pigs in individual pens.

Pigs with piglets were kept at separate farrowing pen. Piglets were provided heat by electric bulbs. In farrowing pen rice straw was kept in order to make bed for the mother and also to maintain the temperature. Piglets were weaned at 6 weeks of age and at this age they were distributed to the farmers. Piglets were drenched for internal parasites before they were weaned. Records monitored were litter size at birth and weaning and litter weight at birth and weaning.

Data recorded over fourteen years (1989 to 2000) for litter traits of Pakhribas pigs at the Station were analysed for the estimation of genetic and non-genetic factors affecting the traits. For this least-squares analysis techniques based on unequal subclass (Harvey, 1990) were performed using the model:

$Y_{ijk} = u + a_i + b_j + c_k + e_{ijkl}$

μ is overall mean
a_i is the effect of ith parity of dam (i = 1, 2,…..14)
b_j is the effect of jth season of birth (j= 1, 2, 3)
c_k is the effect of kth year of birth (k=1, 2,…..14)
e_{ijkl} is a random element assumed to be normally and independently distributed

An overall analysis, ignoring sires and dams, was used first to examine environmental effects. Then sires and dams were included in the model as random effects to estimate the genetic components of variance and to eliminate non-significant fixed effects.

RESULTS AND DISCUSSION

Non-genetic factors

Litter traits (Litter size and weights at birth and weaning)

Least-square means of litter traits are presented in table 1.

Table 1. Least-squares means of litter traits of Pakhribas pigs for different non-genetic factors

Factors	LSB (N)			LWB (Kg)			LSW (N)			LWW (Kg)		
	No	Mean	SE	No	Mean	SE	No	Mean	SE	No	Mean	SE
Overall	317	9.39	0.19	251	9.32	0.25	307	8.31	0.18	238	50.6	1.45
Parity of dams												
1	36	7.99	0.50	30	7.82	0.54	36	7.14	0.46	30	41.7	2.80
2	34	9.02	0.52	28	9.60	0.56	33	7.94	0.49	25	51.5	3.12
3	28	10.2	0.56	23	10.8	0.61	28	9.42	0.52	22	60.9	3.14
4	29	9.75	0.56	23	10.9	0.63	28	8.90	0.54	21	56.5	3.41
5	25	10.8	0.61	20	10.9	0.66	25	9.69	0.56	19	55.6	3.40
5	22	9.9	0.64	18	10.1	0.70	22	8.39	0.59	16	50.9	3.77
7	23	10.3	0.62	17	10.4	0.73	22	8.98	0.59	17	56.6	3.74
8	22	9.95	0.64	17	9.76	0.73	22	8.99	0.59	17	58.9	3.73
9	21	10.0	0.66	16	9.11	0.76	20	8.68	0.62	15	49.2	4.00
10	19	9.79	0.69	14	9.30	0.80	17	9.08	0.67	13	52.2	4.21
11	17	9.86	0.74	13	9.27	0.86	17	8.55	0.69	13	48.2	4.38
12	15	9.70	0.77	11	8.92	0.89	12	8.56	0.79	9	56.1	4.98
13	13	7.38	0.83	9	6.25	1.00	13	5.95	0.77	9	32.3	5.09
14	13	7.06	0.86	12	7.25	0.87	12	6.08	0.82	12	37.6	4.39
Significance		**			***			***			***	
Season of birth												
Early dry (Dec-Feb)	60	9.21	0.41	52	9.18	0.45	59	7.96	0.39	52	50.2	2.37
Late dry (Mar-May)	96	9.43	0.32	71	9.14	0.40	90	8.48	0.31	63	50.9	2.26
Wet (Jun-Nov)	161	9.51	0.25	128	9.62	0.29	158	8.50	0.23	123	50.7	1.64
Significance		NS			NS			NS			NS	
Year of birth												
1989	13	8.92	0.83	-	-	-	12	7.93	0.80	-	-	-
1990	14	9.89	0.81	-	-	-	12	8.69	0.81	-	-	-
1991	19	10.2	0.70	6	8.24	1.21	19	8.77	0.65	6	52.2	6.08
1992	15	7.18	0.78	6	8.19	1.21	15	6.44	0.73	2	16.0	10.3
1993	20	9.30	0.68	13	7.68	0.83	20	8.42	0.63	12	55.2	4.37
1994	25	9.46	0.62	20	9.06	0.69	25	8.39	0.58	19	52.7	3.54
1995	29	10.1	0.57	27	10.3	0.59	29	8.21	0.53	25	48.9	3.07
1996	30	9.44	0.56	30	9.46	0.55	30	8.82	0.51	30	59.6	2.75
1997	32	9.91	0.53	31	10.6	0.53	31	8.91	0.50	30	64.0	2.71
1998	30	8.13	0.56	29	9.26	0.55	29	6.99	0.52	29	46.4	2.79
1999	27	9.18	0.58	27	9.82	0.57	27	8.04	0.54	27	53.2	2.88
2000	23	9.47	0.63	23	10.0	0.62	20	8.77	0.63	20	60.0	3.36
2001	25	10.5	0.60	24	9.09	0.60	24	9.19	0.57	24	53.7	3.03
2002	15	9.77	0.77	15	9.95	0.75	14	8.94	0.73	14	45.3	3.91
Significance		NS			NS			NS			***	

LSB: Litter size at birth; LWB: Litter weight at birth; LSW: Litter size at weaning: LWW: Litter weight at weaning

The least square mean of litter size at birth (LSB) in the herd was 9.4±0.19. This was similar to the record average of 9.43±0.272 by Rasali and Penalba (1993), which included the herd average of three genotypes (Landrace, Yorkshire and their crosses) from Pokhara, Nepal. Aryal *et al.* (1992) using the Pakhribas black pig reported the similar value of 9.3±0.81 in the eastern hills of Nepal. The figure was slightly higher than the average records of 8.96±0.35 by Dhaubdel and Pokharel (1997) using the same three genotypes in Khumaltar, Kathmandu, Nepal. This is slightly higher than the records of 8.6±0.7 for Landrace and 8.4±0.7 for Hampshire pigs in Nepal but slightly lower than the record of 10.2±0.5 for Yorkshire in Nepal (Annual Report, 1992). Wattenakul *et al*. (1997) reported similar value for litter size at birth of 9.27 and 9.33 for Landrace and Yorkshire population in the UK.

The least square mean of litter weight at birth (LWB) in the herd was 9.32±0.25 kg and this was slightly lower than that of 10.6±0.393 kg reported by Rasali and Penalba (1993) using the pig herd in Nepal. However the values obtained was close to the records of 9.4 and 9.26 kg reported by Fahmi and Bernard (1972).

The least square mean of litter size at weaning (LSW) in the herd was 8.3±0.18. This showed the Pakhribas black pig is remarkable in terms of weaning size. If we calculate the pre-weaning mortality it comes to be 11.7% and this is lower than the reports by several authors in pigs (Fahmy and Bernard, 1972; Annual Report, 1992; Rasali and Penalba, 1993; Dhaubdel and Pokharel, 1997). They all reported a higher pre-weaning mortality and a lower weaning size (ranges from 5.1 to 7.5) than the results of the study. This indicated that the breed has better maternal ability than the other breeds including white and black colour breeds of pigs.

The least square mean of litter weight at weaning (LWW) in the herd was 50.6±1.45 kg and this was higher than the report of Rasali and Penalba (1993) using the three genotypes of pigs in Nepal (44.0±0.55 kg). This showed that the Pakhribas pig has better maternal ability.

Season and year of birth were not important source of variation for litter traits (except at litter weight at weaning where year was significant) (table 1). Parity of dams affected all four-litter traits ($P<0.01$; $P<0.001$). In general piglets born from first, thirteen and fourteen parity were poorer than those born from other parity in terms of higher litter size and heavier litter weights.

The overall pre-weaning mortality was 11.5% suggesting that the Pakhribas pigs are good in terms of higher survival rate at weaning age. Dams of parity first, thirteen and fourteen yielded lower litter size and weights than those of other parity. This suggested that dams after twelve parities would not be productive.

Genetic Factors

Heritability estimates of litter traits are presented in table 2.

Table 2. Heritability (h^2) estimates of litter traits of Pakhribas pigs

Traits	Heritability	Standard error
Litter size at birth	0.05	0.095
Litter weight at birth	0	0
Litter size at weaning	0.17	0.141
Litter weight at weaning	0.68	0.314

This showed that heritability of litter size traits was low to moderate and high for litter weights. Rasali and Penalba (1993) working with pigs in Nepal reported a similar kind of estimates for LSB (0.03), LWB (0.04) and LSW (0.06) but the estimates of heritability for litter weight at weaning was lower (0.29) than the present study. Strang and Smith (1979) working with British Large White pigs reported a low estimate of heritability for litter size (0.05). Mercer and Crump (1990) working with Landrace pigs in the UK reported low estimates of heritability for litter size at birth (0.09±0.043) but moderate estimates of heritability for litter weight at birth (0.135±0.047). Haley *et al.* (1988) reported low estimates of heritability for litter size at birth (0.09). Similarly Kerr and Cameron (1995) reported low estimate (0.06) for litter size at birth and moderate estimates of heritability of litter weight at birth (0.11) in Large White Yorkshire pig. In the same line, Crump *et al.* (1997) and Sorenson (1990) both reported similar types of estimates of 0.11 and 0.12 respectively. Estimates of heritability for litter weight at birth were 0.11 (Crump *et al*., 1997) and 0.11 (Kerr and Cameron, 1995) for Landrace and Large white Yorkshire pigs. The estimates obtained in the study are close to the values reported in the literature. In general the estimates of heritability for litter size are low and moderate for litter weights. However, in the present study, litter weight at weaning had high heritability estimate. This showed that mass selection might be effective for making genetic improvement for litter weight at weaning.

Low (litter size) to high estimates (litter weight at weaning) of heritability indicated that some genetic variance is available for these traits. High heritability of litter weight at weaning and moderate heritability of litter size at weaning indicate that these two traits if used as selection criteria may bring genetic improvement in these traits and consequently improve productivity.

CONCLUSIONS

Pigs should be kept up to 12th parity as the productivity in terms of litter size and weights declined after 12th parity. Dams should be selected based on the litter size weight at weaning, as the estimates of heritability were medium to high.

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Nepalese Vet. J. 28: 58-67
Nepal Veterinary Association

Research article

MITIGATING MILK HOLIDAY PROBLEM IN NEPAL: DIAGNOSIS AND PRESCRIPTION

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ABSTRACT

Production and supply of milk has seasonal trend in Nepal with high production during July to November. During this period production exceeds beyond the processing capacity of dairy industry, resulting in drop in milk purchase during certain days of week, referred to as holidays. A study was undertaken in Dhankuta, Ilam, Makawanpur and Kaski districts of Nepal in order to investigate the severity and economic losses of the problem. Situation of milk collection and supply of 4 different Milk Producers' Cooperatives (MPCs) studied, and 129 consumers from Fikkal, Dharan, Hetauda and Pokhara markets were interviewed. Production of milk during flush season fluctuated from 313 to 1529 lit per day whereas in lean season it went down to 47 to 141 lit per day in studied MPCs. March to June are the critical months for milk production and productivity of dairy animals, during which, supply of milk to Dairy Development Cooperation (DDC) is significantly reduced. Milk holidays were observed for up to 11 days in a month causing high economic loss to dairy farmers and cooperatives. Among the available milk products in the market, the five most demanded dairy items were whole milk, yoghurt, ghee, cheena and khuwa, and the least preferred were cheese, chocolate and ice cream. Preference of consumer on indigenous milk products necessitates the production of these particular products at farm level; where the problem of surplus milk or milk holiday is severe.

INTRODUCTION

Animal husbandry is a tradition of Nepalese farming, mainly for milk, manure and draught power. Milk and milk products are essential components of daily diet and often are the source of daily income to the rural poor. In the past, most portions of the produced milk and milk products were utilized by the farmers themselves due to remoteness and unavailability of market, except only in some cases where farmers could sell their products in nearby districts head quarters. After 1952, with the establishment of a Dairy Industry in the country, milk collection and processing was initiated formally and dairy business established as an enterprise.

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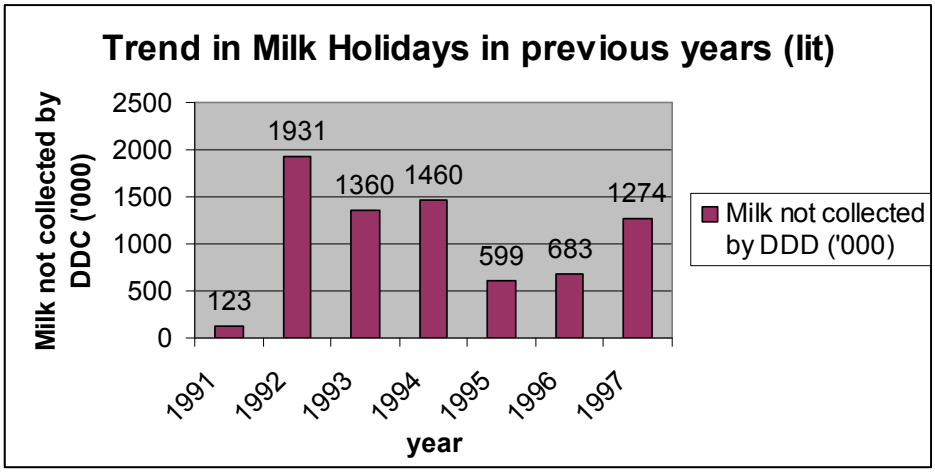
Boosting of milk production through farmers' cooperatives helps to improve the rural economy by sale of milk and milk products and employment at different layers of the society. However, as the processing capacity of the dairy industry is limited, all milk produced by the farmers during the flush season could not be procured by dairy industry thus the days without milk sale has been referred to as milk holiday, and this trend has been observed since 1991.

The trend in milk holidays is presented in Figure 1. The trend shows the pattern of milk holidays, which began modestly in 1991 and peaked during the following years (Upadhyay, *et. al*, 2000).

Fig. 1. Trend in milk holidays during past years (lit)



Source: (Upadhyay, *et. al*, 2000).

At present, dairy industries (Public and Privates) are not in a position to absorb the ever-increasing production of fluid milk, which is further creating milk holidays, causing enormous economic loss to milk-producer farmers involved in milk business as regular income generating activity.

Rural dairy farmers are trying to process surplus milk and thereby minimize the economic losses, but poor knowledge on processing and lack of study at the farm level production makes the traditional milk products incompatible in the market due to taste, nutritional value and shelf-life. A study made by New ERA on the consumer preferences for milk and milk products in major towns of Nepal reported that, more than 95 per cent of urban households and institutional consumers of milk and milk products expressed their ignorance about the milk products produced at the farm level and the rest showing awareness gave average or poor rank to the quality of the products (New ERA, 2000). It means that most of the rural farmers do not process milk and the few involved in processing produce the by-products of poor quality.

In remote areas of the country, district headquarters provide some markets, but these markets are too small to consume larger quantities of milk products. Besides that, during the rainy season, the roads are damaged by landslides disrupting the transportation of milk to milk plant (Shrestha and Bhattarai, 1994). This situation also leads to milk holidays causing loses to dairy farmers and entrepreneurs.

Milk by its nature is a highly perishable commodity and as such needs cold chain to maintain its quality and needs to be marketed quickly. Existing processing facilities and methods in farmers' management conditions are not adequate to handle flush season milk production and left over milk due to milk holidays. The informal dairy processing sectors in rural areas use traditional and simple processing technology and the products of such processing are not always compatible with the demand of modern society. Informal dairy products like *ghee, curd, chhurpi* could not always meet the quality of recent standards (LMP, 1993) and this is one of the reasons behind the importation of milk products from abroad, which is evident by the import of 47.2 MT. butter and 5.5 MT. *Cheese/Paneer/Khoa* during 1996/97 (Upadhyay *et al*, 2000). Further more; rural processing technology adopted at the farm level incurs significant nutrient losses during the course of processing and product conservation, which might be one of the reason of malnutrition and low level of food self-sufficiency in the country.

This study highlights the severity of the problem of milk holidays at the farm level and its proper management approaches. Besides that, it provides information on milk and traditional milk products and their supply trend by season, their quality standard and market demand. It also provides some effective recipes for the rural farmers for tackling the surplus milk situation at their homestead.

MATERIALS AND METHODS

Four major milk-producing districts of the country namely Ilam and Dhankuta in the Eastern Development Region, Makawanpur in the central and Kaski in the Western Development Region were selected as study sites. In general, dairy pocket areas are the major sufferers from milk holidays. From these areas, 2 to 3 milk producer cooperatives (MPC) were studied for the problem of milk holidays, milk surplus, number of permanent and temporary shareholders in the cooperative, average daily milk collection, road accessibility, and other related information. Information gathered from different MPCs and the group discussions, four cooperatives were selected for project implementation. These were: Hattikharka MPC from Hattikharka VDC-5 in Dhankuta, Shree Krishana MPC from Fikkal-8, Ahale in Ilam, Chetansil MPC from Chisapani-Hatiya in Makawanpur and Nangdanda MPC from Dhikurpokhari in Kaski.

Secondary data for the last three consecutive years (1999, 2000 and 2001) were collected from cooperative files, which were computed in SPSS 10.0 software computer package and statistical analysis made accordingly.

Semi-structured questionnaire survey was made in nearby markets of the studied sites for milk products. Demand of milk and milk products was collected during different seasons and preference of 129 consumers was recorded from different markets.

Sample of traditional milk products like *Ghee, Khoa,* Yogurt, *Churpi, Peda, Paneer, Lolypop* were collected from different local markets around the study sites. Chemical analysis of the products was carried out at Central Laboratory of Agricultural Research Station, Pakhribas. Organoleptic analysis of the milk products was made using the manual published by NDDB/Nepal (2001). The methodology used was moisture determination, titrable acidity (titration method with 0.1 N NaCl solutions with 0.5 % Phenolphthalein as indicator), total ash (using Muffle Furnace), protein (by Spectrophotometer) and fat percent (Gerber Method).

RESULTS AND DISCUSSION

Overall recording system in most of the visited MPCs and farm households were poorly organized. Farmers were unaware about its importance at the farm level. Both the dairy farmers and cooperative personnel were not serious about these aspects. Actually, their income from milk business basically depends on the recording system. They get payment based on their daily milk sale based on fat and SNF percent of the milk. Milk collection and supply situation of 4-MPCs are presented in Table 1.

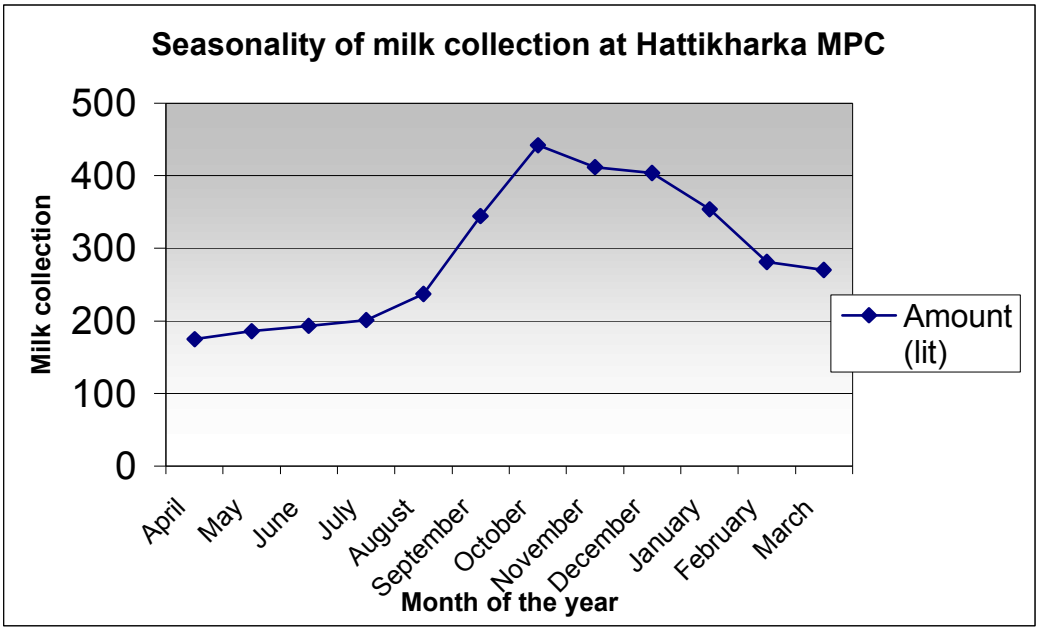
Table 1. Milk collection and supply trends of 4 studied MPCs

Statistics	**Frequency (days)**	**Percent**
Milk Collected	2541	89.9
Milk Holidays	286	10.1
Total	2827	100.0

The result showed that, about 10 per cent collection was influenced by milk holidays. During these days, farmers were compelled to use milk themselves or sell in far below market prices. Only the way to use left over milk at the farm level is making curd from traditional method and produce butterfat, the recovery percent for which from the traditional method is quite low. Remaining whey is either consumed in the family or fed to the animals. In flush season, severity of holidays becomes more acute as the milk production goes around 9 folds above the lean season.

The range of milk production in flush season increases to 1529 to 313 lit/day from the respective lean season values of 141 to 47 lit/day, which was evident from the mean flush season production of 755 lit/day from 85 lit/day during the lean season in the studied MPCs. Month of March-May are the lean months, whereas September-January is the flush season. Milk production is basically governed by the availability of forage to the animals, which ultimately results to lean or flush season. The dynamics of milk production at dairy cooperative level, average monthly milk production recorded at Hattikharka MPC shows that peak milk production was during October, which then gradually declines and September to January are the critical months for milk holidays as the milk production exceeds the milk processing ability of the plants (Fig 2).

Fig. 2. Dynamic of milk production by month at MPC



During the study visit, it was observed that besides milk holidays, dairy farmers were loosing their income from milk returned, as quality of milk do not match with alcohol test standard set by the dairy processors. Moreover milk clotting that generally occurs in summer season is another factor for loss. In hilly region of the country, walking distance between milk collection centers and chilling points is generally 2-3 hours. Increased temperature and humidity during this period causes milk spoilage. The range of losses from these problems is 5 to 317 lit/day. In Hattikharka MPC, these problems are found to be more critical and recorded around 83 days in the studied period, likewise 7 days in Chisapani and 6 days in Fikkal MPC. Overall it caused 3.4 per cent milk loss to the dairy farmers.

Losses incurred by the dairy farmers from milk holidays and alcohol tests accounts to be 13.5 per cent of the total milk production, which amounts to 110.5 million liters annually processed through national milk grid (Kantipur, 2003).

Among the 4 studied MPCs, Chisapani and Nangdanda supplied milk to the local consumers as well directly from their collection point as they have Hetauda and Pokhara markets nearby. However, major portion of the collected milk by these two MPCs are supplied to the dairy plants. In general, all MPCs supply their milk to the collection points of the DDC. Amount of milk collected and supplied from studied MPCs is presented in Table 2.

Table 2. Average daily collection and supply trend of Milk between MPC and DDC

Site Name	Milk (lit)/day		Fat (%)		SNF (%)	
	MPC	DDC	MPC	DDC	MPC	DDC
Hattikharka	292	282	5.1	4.4	8.4	8.1
Chisapani	232	219	5.7	5.4	8.4	8.1
Nagdanda	-	-	-	-	-	-
Fikkal	246	241	-	3.1	-	8.1
Overall	257	247	5.4	4.3	8.4	8.1

From the above table, it is evident that percent of fat and SNF tested at cooperative level and DDC did not match properly, which might be due to lack of fat testing knowledge of dairy farmers or other reasons. As the payment is based on fat and SNF%, dairy farmers are loosing their income due to this difference. Next to milk holidays this is another severe problem, which directly affects dairy farmers to their income. There should be special focus on the analyzing capacity of basic milk parameters and knowledge about it in the milk producer farmers, MPCs technicians as well as local chilling center personnel.

Milk byproducts collected from different markets were chemically analyzed and results are presented in Table 3.

Table 3: Mean Chemical composition of the indigenous dairy products (%)

Products	Moisture	Acidity	Total Ash	Protein	Fat
Churpi (Hard and Soft)	9.02	1.28	5.19	54.46	-
Khoa	27.17	1.06	3.35	23.52	8.6
Yogurt (Buff)	-	1.48	-	1.63	4.3
Yogurt (Cow)	84.78	0.25	-	3.69	3.97
Butter (Cow)	16.38	0.27	4.08	0.70	-
Butter (Buff)	21.94	1.44	2.15	1.04	-
Ghee (Cow)	0.17	2.03	0	0.21	-
Ghee (Buff)	0.08	3.59	0	3.75	-
Lolypop	9.86	0.79	2.94	8.28	-
Peda	11.17	0.52	2.97	18.14	8.1

Based on the laboratory analysis, it was found that moisture content in soft churpi was greater than hard. Average moisture proportion of soft and hard *churpi* was found to be 10.0 to 4.9 per cent. Maximum moisture content found in soft *churpi* was 11.79 per cent. Protein content in *churpi* varies from sample to sample and the range lies between 38.59 to 61.89 for soft and 49.16 to 62.59 per cent for hard. Basically soft *churpi* is the product of Sikkim and Bhutan and is well known to the Nepalese consumer of the eastern region of the country. It is white in color and sweet in taste and comparatively soft than hard *churpi* found in the country. Size is somewhat smaller from hard *churpi* available in local markets.

The chemical structure of *Barmajhia Peda* (famous place in Saptari district for quality Peda) has moisture content 19.69, acidity 0.47, total ash 3.12, protein 18.48 and fat 7.6 per cent. The other famous traditional milk product is *Lollypop*. The *Lollypop* mainly used in the eastern Nepal resembles the chemical properties of general *Peda.* It is in cylindrical shape around 8 cm long and wrapped with colorful plastic wrapper.

Comparison of traditional milk by-products quality with standards seems difficult, as the national norms on these products do not exist. So, in order to make the traditional product competitive and viable in the market, a thought should be given from concerned agencies on quality standard.

Organoleptical analysis of milk products has been made based on its appearance, consistency, flavor and texture. For yogurt, appearance at the surface of product, color, visible purity, and presence of foreign matter, spots of mould and seepage of whey are important. In sense of consistency thickness, stickiness and coarseness have been studied. For flavor evaluation smelling and tasting of the product were made. Analysis has been performed for the yogurts made from cow, buffalo and mixed milk (cow- buffalo). In most of the samples collected from farm level-separation of whey, sour in taste, whitish or light brown color, defective aromatization of the product were evident. Generally one night culture media available locally was used for yogurt making. Traditional *Theki* is the popular utensil for making yogurt. It was experienced that the lack of knowledge on by-product development is the root cause of poor quality dairy products of the farm level.

Among the traditional milk products studied, *churpi* was found appropriate in quality aspects (physically) and marketing stand point (demand). It was in different color and fat contents. Main proportion of the churpi found in markets is made from Yak milk; however cow and buff milk *churpi* are also common.

A large variety of milk products are available in the markets and used by the people. Among these ghee, panir, *khoa, gudpak, peda, dahi, mohi, chena, churpi,* cheese (hard and soft), butter, *rasmalai, lollypop,* milk powder, whole milk, 3 and 5 per cent fat milk, ice cream, and chocolate are the widely used ones. Majority of the products are easily available in the market except *panir, gudpak, mohi, soft churpi,* cheese, lollypop, chocolate and ice cream. It was recorded that *panir*, *Khoa, gudpak, dahi*, in Fikkal area are in short supply. Highly demanded dairy products were *ghee*, whole milk, *dahi, khoa/peda* and *chena*, in the surveyed areas. However, other dairy products were also in demand. The sources of availability of these products were sweet traders, dairy dealers and farmers themselves. Among three sources, most of respondents answered that they bought milk products from local sources. Out of 129 respondents, some people complained of not getting milk on time.

Season wise consumer demand on milk products was studied in the nearby markets of the studied sites. The result is presented in Table 4.

Table 4: Use of Milk and milk products by season (%)

Product	*Winter*	Summer	No difference
Whole Milk	-	-	100
Ghee	72	7	21
Yogurt	1	97	2
Khoa	9	8	83
Peda	4	3	93
Paneer	-	2	98
Soft churpi	6	-	94
Hard Churpi	2	3	95

From Table 4, it is clear that the demand of almost all dairy products is year the round except yogurt and ghee. The consumption of yogurt is higher in summer whereas demand of ghee is higher in winter. Other products are constantly in demand year the round. It gives clear message that milk and milk products could be marketed at any time and season of the year. Average weekly consumption of whole milk, *ghee*, yogurt, *paneer* and *khoa* by a Nepalese family in the studied sites was found to be 8.8, 0.6, 4.4, 0.6, and 0.8 kg respectively.

CONCLUSION

Milk holiday no doubt is a vulnerable issue to all dairies concerned. Though, its post-harvest aspects seem more crucial in recent context, pre-harvest activities should also get due attention. Period of overflow of milk need to divert in dry season with managerial interventions like changing in conception and parturition period of buffalo and nutritional management during lean season for constant production of milk round the year. Clean milk production at the farm level, storage, transportation, and dairy animal health are some of the pre-harvest aspects where more focus is required. Managing milk holidays through product diversification at the farm level is another and probably the most important aspect in current national context. In this connection proper thought should be given in quality and shelf life of the value added products. Besides all these, efficient marketing channel need to be established particularly with newly urbanized areas and markets and the capital city, and adjoining markets of the neighboring countries.

ACKNOWLEDGEMENTS

The project team is thankful to Dr SB Mathema - HARP Manager for regular support and encouragement to run the project activities smoothly under difficult field conditions. Staff from Department of Livestock Services Office-Makawanpur and Kaski, Central Campus of Technology-Dharan, Agro Enterprises Center-Dharan, National Dairy Development Board-Harihar Bhawan and ARS-Pakhribas is sincerely acknowledged for their assistance and support during the site selection and survey works.

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Nepalese Vet. J. 28: 68-72
Nepal Veterinary Association

Research article

SERO STATUS OF JAPANESE ENCEPHALITIS VIRUS INFECTION IN PIG IN NEPAL

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ABSTRACT

Japanese Encephalitis is a mosquito-borne viral disease and causes encephalitis in human as well as in horses and abortion in pigs. Pigs are considered as the most important natural amplifying animal for Japanese Encephalitis virus. A random sero-survey was conducted in Nepal from September 2002 to March 2003 by collecting 270 serum samples from pigs, ducks and horses covering 16 districts of the country. Out of 270 samples tested, 55.18% were found positive for the presence of antibodies against JE infection.

INTRODUCTION

Japanese Encephalitis (JE) is an emerging disease of animals and humans in Nepal. First epidemic of JE was recorded in Nepal in 1978. Since 1978, Nepal health authorities have reported more than17, 000 cases and 4000 deaths in human beings due to acute encephalitis. Epidemic occurs usually with the onset of monsoon rain in July and ends in October. Occurrence of JE has been reported up to 62% in the age group of 12 months to 15 years children (Bista *et al*; 2001). Annually 2000-3000 cases and 200 to 400 deaths occur due to JE. Now it is prevalent in 24 districts of terai and inner terai of the country and 11.5 million people living in these districts are at risk. Recently, the incidence of this disease was recorded in Kathmandu valley in September 2003 and 7 people died. Three viral strains namely B-2524, B9548 and Nep-1/90 have been isolated from Nepal. Laboratory diagnosis facility for JE by using IgM capture ELISA is available for human beings (Epidemiology and Disease Control Division, 2001).

Studies on the status of JE infection in their host animals have not been carried out in detail in Nepal in the past (Joshi and Gaidamovich, 1981-1982). Realizing the importance of pigs as carriers of this disease Central Veterinary Laboratory (CVL) under the Department of Livestock services, has lunched a JE investigation program since 2002. The main objective of this investigation is to study the status of JE virus infection in pigs and other susceptible animals.

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METHODOLOGY

A total of 270 serum samples were collected randomly from September 2002 to March 2003 from pigs, ducks and horses by visiting various farms of different districts. These sera were labeled and stored at-20 ^{0}C until tested. Among 270 sera, 200 were inactivated for 30 minutes at 56 ^{0}C before shipment to Australia. Then these sera were refilled into serum vials supplied by Australian Animal Health Laboratory (AAHL). Vials were disinfected by wiping with 0.01M Citric acid, packed well and sent to AAHL for test. Out of 270 sera, 70 were tested in Kathmandu after the establishment of JE diagnostic capability at CVL according to standard procedure described by WOAH (1996). All sera were tested for the detection of antibodies against JE virus by following the protocol of competitive enzyme linked immunosorbent assay (C-ELISA) developed by AAHL (2002).

RESULTS AND DISCUSSION

The numbers of positive and negative sera were differentiated on the basis of percentage inhibition on the development of color in each well. Sera resulting in level less than 40% inhibition were considered negative where as sera resulting at level greater than 40% inhibition were considered positive. Antibodies against JE virus were detected in 149 sera out of 270. C-ELISA results are summarized in Table 1&2. In this way 55.18% of tested sera were found positive for JE. Percentage of positive result in pigs, horses and ducks was revealed 57.25, 50 and 9.09 accordingly.

Antibodies against JE infection have been detected in Nepal in pigs and ducks in the past (Joshi and Gaidamovich, 1981-1982) by performing hemagglutination test. However this study is the first report detecting antibodies against JE virus in horses in Nepal. Only four horse sera collected from Parsa districts were tested. A total number of 11 duck sera were collected from Bara, Parsa and Banke districts but only one duck serum from Banke district was found positive. The evidence of this laboratory finding strongly indicates the persistence of JE virus infection in Nepalese pigs, ducks and horses. All these tested sera were collected from 16 districts of terai regions, where high incidence of JE disease has been recorded and samples were collected only from unvaccinated pigs against JE disease.

Table 1. Region wise percentage of positively for JE

Region	District	Positive/Tested	% of positive	Mean
Far-western				**93.18**
	Kailali	19/22	86.36	
	Kanchanpur	6/6	100	
Mid-western				**72.98**
	Banke	7/7	41.17	
	Bardiya	7/9	77.77	
	Dang	5/5	100	
Western				**33.48**
	Kapilbastu	4/15	26.66	
	Nabalparasi	8/14	57.14	
	Rupendihi	2/12	16.66	
Central				**28.71**
	Makwanpur	15/43	34.88	
	Bara	5/16	31.25	
	Parsa	2/10	20	
Eastern				**68.35**
	Jhapa	4/10	40	
	Morang	13/13	100	
	Sunsari	26/38	68.42	
	Saptari	14/24	58.33	
	Siraha	12/16	75.00	
Total		**149/270**		**55.18**

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The highest incidence of JE in human beings was recorded in Far western as well as in Mid-western region and lowest in Central regions in the past (Epidemiology and Disease Control division, 2001). This study has also shown the highest percentage of sero-positive cases in Dang, Kanchanpur and Kailali districts of these regions. Therefore, the result of this study co-relates with the incidence of human infection reported by Epidemiology and Disease Control division, 2001.

Table 2. Species wise percentage of positive for JE

Species of animal	Total sera	Positive sera	Positive %
Pig	255	146	57.25
Horse	4	2	50
Duck	11	1	9.09

Use of C-ELISA for the detection of specific antibodies (IgG) against JE virus infection in pigs and other susceptible animals has been established standardized at CVL, Kathmandu. Reproducibility of this test was also confirmed by re-testing 10 sera, which were already tested at AAHL. It is considered very sensitive and specific diagnostic method which could be used for sero-surveillance and sero-monitoring of JE in all animals including human. Diagnosis of animal infection by JE virus isolation is difficult due to short period of viraemia usually lasting for 2 to 4 days (Daniels, 2001) and most JE infection in pig is asymptomatic. In this situation C-ELISA is considered to be very suitable test to detect specific antibodies produced in response to JE infection.

RECOMMENDATION

This study has given some information about the sero-status of JE in host animals. However, numbers of sera tested in this study were not enough and statistically significant to represent the overall situation of the country. It is essential to continue JE investigation program lunched by CVL, to study in depth the epidemiological aspect of this disease for developing control strategy and to adopt control measures. Control of JE in human beings by vaccination would be more effective if it would be planned considering the disease status in host animals. Development of JE vaccination and mass immunization of pigs would be the best option in future for sustainable pig industry and control of this disease. Further collaborative work with national and international institution is needed to control JE.

ACKNOWLEDGEMENTS

Authors would like to thank to the Department of Livestock services, His Majesty's Government of Nepal, CSIRO, Australian Animal Health Laboratory and Crawford Training Fund, Australia for providing their help, technical support and fund to establish diagnostic capability at CVL and performing this study. Sincere thanks goes to all veterinarians, technicians and staff involved in this study.

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Nepalese Vet. J. 28: 73-77
Nepal Veterinary Association

Research article

MAPPING OF LIVESTOCK TICK FAUNA OF DIFFERENT AGRO-CLIMATIC ZONES OF NEPAL

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ABSTRACT

In the year 2002/2003, various tick species infesting different farm animals from different agro climatic zones of Nepal were collected for identification and mapping. Altogether, five genera were identified viz. Boophilus, Hyalomma, Rhipicephalus, Haemaphysalis and Ixodes. Among them Boophilus microplus was the most abundant in all the agro climatic zones and in most of the farm animals namely, cattle, buffalo, goat, pig and rabbit.

INTRODUCTION

Ticks are important as vectors of disease throughout most part of the world. They are among the most efficient arthropod vectors: they can transmit viruses, rickettsiae, bacteria, and protozoa, and are able to cause paralysis through neurotoxic salivary secretions. They possess a number of qualities, which account for their vector potential: they attach firmly, suck blood, feed slowly, and may go unnoticed for a lengthy period of time. Many species are quite resistant to environmental stresses and may live for years. They have few natural enemies and have a wide range of hosts. Some species are able to transmit pathogens to their offspring through the egg (transovarian transmission), from the larval to the nymphal stage, or form the nymphal to the adult stage (transstadial transmission). The females lay great numbers of eggs. All these factors combine to give ticks great potential as disease vectors. (James and Harwood, 1969).

Hence, it is very important to know the tick fauna of different Agro-climatic zones of the country so that epidemiology of various tick borne diseases could be established both in human as well as in animals.

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METHODOLOGY

Ticks were collected from different agro-climatic zones of the country. The ticks were hand picked from different parts of animal body viz. ears, neck, shoulders, dewlap, forelegs, ventral abdomen, inguinal region, perennial region, hind legs, and tail. The ticks were preserved in 70% alcohol containing 5% glycerine until further laboratory analysis. The preserved ticks were examined for their morphological characteristics under stereomicroscope and identified according to the figures and key described by Acarology Division, IMR (1995), Kaiser and Hoogstraal (1964), Morel (1989) and MAFF (1986).

RESULTS AND DISCUSSION

Ticks were collected and identified from 21 districts of the country. The total number of ticks examined were 1268 of which female ticks were 1001, males 210 and nymphs 57. A total of five genera of ticks were identified from livestock in 21 districts (Table 1). Of the five genera, *Boophilus microplus* was the most abundant tick common in all districts and in all livestock species. It is likely that high infestation rates of *B. microplus* ticks could maintain enzootic stability of babesiosis in local cattle. Mahoney (1980) reported that infestation rates of 40 *B. microplus* ticks per animal per day are required to ensure stability for *Babesia bovis*. In this study, almost in all animals, the number of tick infestation per animal was more than hundred,

Table 1. Tick species identified

Ticks species	Districts	Livestock species
1.Boophilus microplus	All	Cattle, Buffalo, Goat, Rabbit, Pig
Boophilus annulatus	Jumla, Kathmandu	Cattle
2.Hyalomma marginatum issaci	Morang, Sunsari, Jhapa	Cattle
3. Rhipicephalus haemaphysaloides	Morang, Chitwan	Cattle
Rhipicephalus sanguineus	Dhanusa, Lalitpur	Goat, Cattle
4. Haemaphysalis bispinosa	Jumla, Argakhachi, Siraha, Saptari	Cattle
5. Ixodes ovatus	Myagdi, Kathmandu	Cattle, Goat

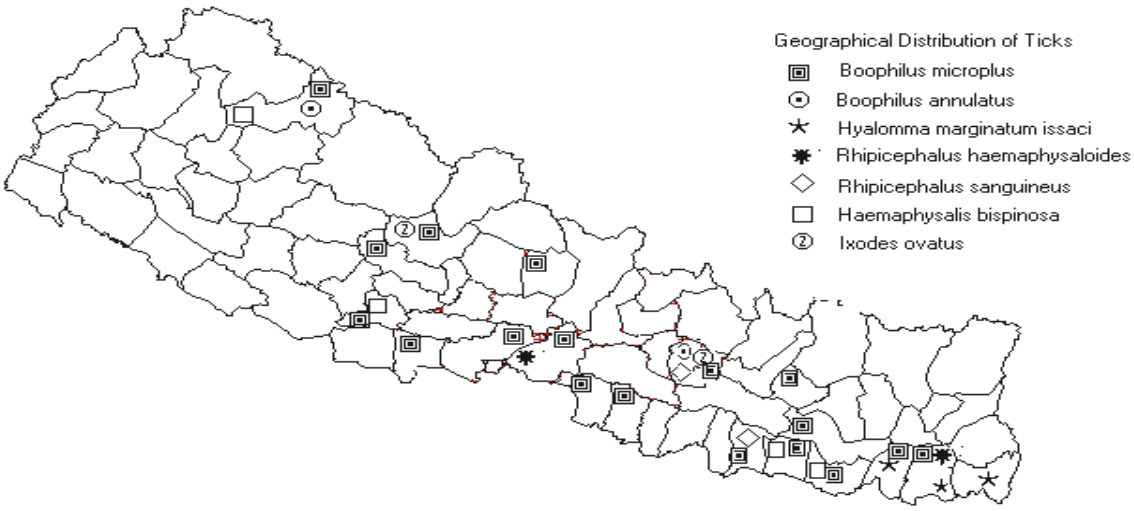
Hyalomma marginatum issaci, the vector tick for theileriosis, was identified from three eastern Terai districts. In the presence of parasites as well as the vectors, it can be said that eastern Terai region is endemic for theileriosis. *Haemaphysalis bispinosa*, a vector tick for *Theileria sergenti*, which is only common in Japan and Korea, may also transmit the disease in our context; however, the protozoan parasite from Nepal has not been identified. Field observations showed that buffaloes were less infested with ticks. The reason behind this was that they were given more care and managed mostly under stall fed conditions. *Ixodes ovatus* tick was identified from Myagdi and Kathmandu from cattle and goat respectively. The districts wise identification of ticks is given in Table 2.

Table 2: Tick species present in different districts.

Mountain	**Region**	
1. Jumla:	West	: *Boophilus microplus*
		: *Boophilus annulatus*
		: *Haemaphysalis bispinosa*
2. Okhaldhunga	East	: *Boophilus microplus*
Hills		
1. Baglung	Central	: *Boophilus microplus*
2. Myagdi	"	: *Boophilus microplus*
		: *Ixodes ovatus*
3. Kathmandu	"	: *Boophilus microplus*
		: Boophilus annulatus
		: *Ixodes ovatus*
		: Rhipicephalus sanguineus
4. Bhaktapur	"	: *Boophilus microplus*
5. Arghakhachi	"	: *Boophilus microplus*
		: *Haemaphysalis bispinosa*
6. Lalitpur	"	: *Boophilus microplus*
		: *Rhipicephalus sangui*neus
7. Kaski	"	: *Boophilus microplus*
Terai		
1. Morang	East	: *Boophilus microplus*
		: *Hyalomma marginatum issaci*
		Rhipicephalus haemaphysaloides

2. Sunsari	East	: *Hyalomma Boophilus microplus* : *marginatum issaci*
3. Jhapa	"	: *Boophilus microplus* : *Hyalomma marginatum issaci*
4. Siraha	"	: *Boophilus microplus* : *Haemaphysalis bispinosa*
5. Saptari	"	: *Boophilus microplus* : *Haemaphysalis bispinosa*
6. Dhanusa	Central	: *Boophilus microplus* : *Rhipicephalus sanguineus*
7. Nawalparasi	"	: *Boophilus microplus*
8. Rupendahai	"	: *Boophilus microplus*
9. Chitwan	"	: *Boophilus microplus* : *Rhipicephalus haemaphysaloides*
10. Bara	"	: *Boophilus microplus*
11. Parsa	"	: *Boophilus microplus*
12. Udaypur	"	: *Boophilus microplus*

Fig. 1 Map Showing District wise Geographical Distribution of Identified Ticks



This study is the first attempt to identify the distribution of tick species in the domesticated animals of the country and provides some information on their distribution but the information obtained are still preliminary because of the small geographical coverage, inadequate representation of animals species and sampling technique. Moreover, tick collection methods were not accounted for, hence, to obtain the full picture of the national situation, a proper well-planned study would compliment the findings of the present study.

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Nepalese Vet. J. 28: 78-94
Nepal Veterinary Association

Review article

NUTRITIONAL VALUES OF GRASSES FOUND IN NEPAL

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ABSTRACT

The nutritive values of grasses in Nepal have not been evaluated comprehensively. In this study, altogether 686 samples of different grass species were collected from 28 districts (Comprising of 9 mountain, 11 hills and 8 terai districts) of Nepal during past twelve years were evaluated for their nutrient content. Average dry matter, organic matter, total ash and crude protein content of different grass species were found to be 21.96, 87.87, 12.13 and 12.0 percent respectively. Similarly, average neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, hemicellulose and cellulose content of different grass species were recorded to be 62.53, 46.49, 13.55, 15.76 and 32.85 percent respectively. Likewise, average calcium and phosphorous content of different grass species were recorded to be 0.78 and 0.32 percent respectively. Highest content of dry matter (36.67%), organic matter (97.97%), total ash (30.2%) and crude protein (26.91%) was recorded for Dush, Myang Myang, Karimari, and Sunhemp species, where as, highest NDF, ADF, lignin, hemicellulose and cellulose content of different grass species were recorded for Sonchus spp, Chirabhuje, Marbindo, Bichro and Chirabhuje which was 81.86, 71.96, 39.32, 51.42 and 60.03 percent respectively. Similarly, grasses like Myang Myang and Kikyu were superior in phosphorous content (0.65%) and Bethe for calcium content (2.8%).

INTRODUCTION

Livestock productivity in Nepal is low primarily because of poor nutrition as a result of increasing livestock pressure on limited fodder resources. Poor nutrition is a constraint to animal production in Nepal as an increasing number of animals are competing for existing feed resources. It is estimated that only about ¾ of total feed requirements for ruminants are met resulting malnutrition of animals in all part of Nepal. In some areas, such as mid hills, the level of feeding is insufficient for most of the animal's life, while in other locations substantial underfeeding during the dry season is common (LMP, 1993).

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It is well known that pasture plays a significant role in sustaining the whole farming system in Nepal. There is no tradition of cultivating grass in the cultiviable private land for forage production. However, grasses growing naturally in and around the farmland are cut and carried to feed the animals. Green forage during the rainy season is not a problem because of abundant growth of local grasses and shrubs in and around the farmland. Perennial grasses like setaria, molasses and napier have been successfully disseminated to farmers and can be found growing in terrace risers in many places. Annual forage oats is the most popular forage grown by farmers. Improved, high yielding, more palatable legume and grass species are becoming widely distributed in permanent pastures as a result of continuing research and pasture improvement programs. Forage legumes not only provide feed for animals but also furnish large quantities of nitrogen for the growth of companion grasses. New cultivars having higher yield, improved palatability, increased winterhardiness and improved disease resistance are being developed which will increase both forage yield and utilization by animals.

Nutritive value of forage is characterized by its chemical composition, digestibility, and nature of digested products. Chemical composition is a factor associated with only plant and its environment. Since a great variety of forages are used for animal feeding, studies on the nutritive values of the forages are important. Therefore, series of studies were undertaken in past years to evaluate different forages used for livestock feeding.

METHODOLOGY

Sample collection

This review compiles the results of 686 samples of 136 different grass species collected from 28 different districts of Nepal during 1990-2001.

These samples were dried to a constant weight under laboratory condition (Aman and Hesselmen, 1984) and were grinded approximately 2-mm size as suggested by Wu.*et.al*. (1994) and Luhallo *et.al*. (1996). Sample were subjected to laboratory analysis for proximate components (AOAC, 1980), detergent fibres (Goering and Van Soest, 1970) and minerals (Ca and P). Calcium content was determined by titrimetric and Phosphorous by spectrophotometry methods.

Data analysis

Obtained data were analysed by using SPSS Statistical Package (SPSS, version 9.0)

RESULTS AND DISCUSSION

Proximate components

The results of proximate analysis are given in Annex 1.

Dry Matter (DM)

Among the 136 grass species collected, dry matter was evaluated for 48 species. Dry matter content varied between 9% in Panaki to 36.7% for Dush. In general, the dry matter content ranged between 15-25% with very few species lying outside this range (Annexure 1).

Total Ash (TA)

Total ash content was evaluated for 136 grass species and the ash content ranged between 3 percent in Boss grass to a very high value of 30.2 percent in Karimari grass species. It was found that 53 grass species were having the total ash value between 5-10%, 46 species having 10-15% and 20 species having 15-20% total ash content.

Crude protein (CP)

Crude protein content was evaluated for all 136-grass species and it ranged between 3.7% for *Hydrangia rebusta* (Pibekhar) to 26.9% for sunhemp. Among the species evaluated, 15 species were found to have the crude protein value of more than 20 percent, 68 species having CP between 10-20% and rests having the CP below 10 percent.

Detergent fibres

Fibre and lignin content of different grass species is given in Annex 2.

Neutral detergent fibre (NDF)

NDF content of grasses is given in Table 2. Table 2 showed that NDF content of 37 grass species ranged between 70-80% and a similar number of species were having 60-70 and 30 species having 50-60%. Similarly, it was also noted that NDF

content of 17 grasses was found in the range of 40-50%, and 10 grass species were with 30-40% NDF. There were 4 grasses that had NDF content in the range of 80-90%. Average NDF content of different grass species was found to be 62.53%, with highest NDF content found in Daha (81.68%) followed by Dam and Phurke (81.04 and 80.25% respectively). The least NDF content was found in Sunhemp grass (30.99%).

Table 1: ***NDF content of grasses***

S.N	Range of content	Number of sample
1	30-40	10
2	40-50	17
3	50-60	30
4	60-70	37
	70-80	37
5	80-90	4
Total		135

Acid detergent fibre (ADF)

ADF content of grasses is given in Table 2.

Table 2 showed that there were 55 grass species, which had ADF in the range of 40-50% followed by 36 species with 50-60% and 29 grass species having ADF between 30-40%. Eight grass species had ADF content more than 60 percent and 7 species in the range of 20-30%. ADF content of different grass species ranged from 22.75 to 71.96%, with an average of 46.49%. Chirabuje grass had highest ADF content (71.96%) followed by Pirlekhar and Nikhari (68.74 and 62.99% respectively and Bichro was noted with least ADF 22.75%).

Table 2: *ADF content of grasses*

S.N	Range of content	Number of sample
1	20-30	7
2	30-40	29
3	40-50	55
4	50-60	36
5	>60	8
Total		135

Lignin

Lignin content of grasses is given in Table 3. Lignin content of grass species ranged between 4.4% for Sihuli to 39.3% for Marbindo species. Lignin content of 49 species was found to range between 1-10%, 69 species have lignin between 10-20% and 21 species have 20-30% lignin.

Table 3: *Lignin content of grasses*

S.N	Range of content	Number of sample
1	1-10	41
2	10-20	69
3	20-30	21
4	>30	4
Total		135

Hemicellulose

Hemicellulose content of grasses is given in Table 4.

Table 4: *Hemicellulose content of grasses*

S.N	Range of content	Number of sample
1	1-10	50
2	10-20	49
3	20-30	36
4	30-40	20
5	>40	1
Total		135

Most of the grass species had hemicellulose content in the range of 1-10% followed by 10-20% and 20-30% in 49 and 36 grass species respectively. It was also noted that hemicellulose content of 20 grass species ranged between 30-40%. There was only one grass, which had hemicellulose content above 40%. On an average, hemicellulose content for different grass species varied from 1.43 to 51.42%, although, average content recorded was 15.76%. Bichro grass had highest hemicellulose content (51.42%) followed by Phurke and Daha (37.57 and 35.71% respectively). Similarly, hemicellulose content of Nilgiri was found to be the least (1.43%).

Cellulose

Cellulose content of grasses is given in Table 5.

Table 5: Cellulose content of grasses

S.N	Range of content	Number of sample
1	1-10	4
2	10-20	14
3	20-30	37
4	30-40	56
5	40-50	20
6	50-60	4
Total		135

Table 5 showed that cellulose content of 56 grass species have cellulose content in the range of 30-40% followed by 20-30% for 37 species and 40-50% for 20 species respectively. Average cellulose content for different grasses were recorded 32.85%, however, it varied from 1.19 to 60.03%. Cellulose content was found to be highest for Chirabhuje (60.03%) and lowest (1.19%) for Marbindo.

Mineral content

Mineral (Ca & P) content of grasses is given in Annex 3.

Calcium (Ca)

Calcium content of grasses is given in Table 6. Calcium content of different grasses was found to vary from 0.19-2.36%.

Table 6: *Calcium content of grasses*

S.N	Range of content	Number of sample
1	0.1-0.3	12
2	0.3-0.5	28
3	0.5-0.7	28
4	0.7-0.9	16
5	>0.9	33
Total		**117**

Highest calcium was found in *Bethe* (2.80%) followed by *Khoragama* and *Kote* (2.58 and 2.36% respectively) while least calcium (0.19%) was found in Boss grass.

Phosphorous (P)

Phosphorous content of grasses is given in Table: 7

Table: 7 Phosphorous content of grasses

S/n	Range of content	Number of sample
1	0.1-0.3	51
2	0.3-0.5	51
3	> 0.5	8
Total		**110**

It was noted that phosphorous content among species was not as variable as was calcium. Phosphorous content was found in the range of 0.1-0.5%, and only 8 species had phosphorous content more than 0.5%. Average phosphorous content for grasses was found to be 0.32%, with a range from 0.11 to 0.65%. Boss grass was noted poor in both calcium and phosphorous (0.11%). Similarly, both Kikyu and Myang myang grass were superior (0.65%) in phosphorous content in comparison to others followed by Kodejhar and Janera (0.57 and 0.56% respectively).

DISCUSSION

Pasture grass plays a significant role in sustaining the livestock farming system in Nepal. There is no tradition of cultivating grass in the private cultivable land for forage production. However, grasses growing naturally in and around the farmland are cut and carried to stall feed the animals. Green forage during the rainy season is not a problem because of abundant growth of local grasses and shrubs in and around the farmland. Shortage of feed to all kinds of animals is the main reason of their low productivity. Increased human population has resulted in reduction of grazing land in mid hills and terai regions. On other hand, increased livestock population in these two regions has caused a massive disappearance of palatable native species of grasses and legumes from communal as well as private lands.

Nepal has 1.7 million ha pastureland in different agro-ecological zones. They are being overgrazed by animals that resulted in deterioration of pastureland despite some attempts to improve or sustain the produtivity of pastureland by introducing different grass and legume species

Because of limited pastureland and increasing livestock population, improvement of pastureland is urgently indeed. The present study showed 39 grass species had more than 15 percent CP but with considerable variation in the CP which may be due to differences in

harvesting time, age of the species, soil type, agro-climatic condition and other environmental factors which were not considered during the sample collection.

CONCLUSION

In Nepal, most of the ruminants are suffering from the lack of nutrition, especially during the dry season. There are some species, which remain green throughout the year and can be cultivated in private land. On the other hand, pastureland is one of the reliable sources, which provide the green grasses to the animals in dry season to some extent. Therefore improvement of pasturelands is needed. Knowledge of the nutritive value of herbage is essential for developing efficient grass production systems. Therefore, it is concluded that, the knowledge of the nutrient content of the different grass species available in Nepal would be useful to formulate ration, which would be cost effective and suitable to increase production performances of animals.

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Annex 1: Dry matter, Organic matter, Crude protein and Total ash content of different grasses (DM basis)

S/n	Grass species	**Scientific Name**	Dry Matter	Organic matter	Total Ash	Crude Protein	Number of observation
1	Anjan	*Cenchrus ciliaris*	NA	84.80	15.20	9.93	4
2	Ankhle	*Crassocephalum crepidioides*	NA	96.0	4.0	8.40	2
3	Arthunge	*Heteropogon contortus*	NA	94.01	5.99	6.40	1
4	Banso	*Digitaria ciliaris*	21.27	85.71	14.28	12.71	6
5	Barpang	*NA*	NA	92.55	4.45	12.09	1
6	Barseem	*Trifolium alexandrinum*	NA	85.30	14.70	21.10	4
7	Bethe	*Chenopodium album*	16.66	77.95	22.05	22.87	6
8	Bichro	*NA*	NA	90.38	9.62	6.10	1
9	Biranchi	*NA*	NA	90.41	9.59	7.15	1
10	Boss	*NA*	NA	96.90	3.10	5.63	1
11	Buki	*Gnaphalium affine*	17.10	89.64	10.36	19.65	6
12	Chilali	*NA*	NA	88.57	11.43	12.27	1
13	Chirabhuje	*NA*	NA	90.73	9.27	6.72	1
14	Chitre banso	*Paspalum flavidum*	NA	91.84	8.16	8.02	6
15	Chor buki	*Anaphalis spp.*	NA	91.49	8.51	16.43	1
16	Chugla	*NA*	NA	95.09	4.91	12.55	1
17	Cocksfoot	*Dactylis glomerata*	NA	88.79	11.21	13.19	11
18	Composite	*NA*	NA	89.23	10.77	18.77	17
19	Daam	*NA*	NA	87.15	12.85	10.02	5
20	Daha	*Sonchus spp.*	NA	93.81	6.19	4.47	1
21	Dami local	*NA*	27.20	77.24	22.75	5.42	4
22	Dande	*Neyraudia reynaudiana*	NA	93.61	6.39	8.51	1
23	Debre grass	*Spatholobus parviflorus*	NA	90.40	9.60	15.88	2
24	Desmenthos	*NA*	NA	90.04	9.96	17.68	1
25	Desmodium	*Desmodium unianatum*	NA	85.37	14.62	18.48	9
26	Dhape	*NA*	NA	92.50	7.50	8.47	2
27	Dhimchi	*Festuca leptopogon*	16.0	83.18	16.82	14.68	4
28	Dhoge	*NA*	NA	92.99	7.01	7.96	1
29	Dolo	*NA*	NA	88.28	11.72	17.22	6
30	Dubo	*Cynodum dactylon*	27.13	83.81	16.19	11.11	20
31	Dush	*NA*	36.67	72.70	27.30	13.76	2
32	Festuka	*NA*	NA	85.06	14.94	10.66	6
33	Gajarpata	*NA*	NA	83.0	17.0	22.03	4
34	Gandhe	*Ageratum conyzoides*	NA	86.05	13.95	20.72	2
35	Ghode banso	*Paspalum distichum*	NA	91.73	8.27	16.78	1
36	Ghode buki	*Anaphalis cortorta*	NA	94.18	5.82	13.61	1
37	Ghode dubo	*Cynodon dactylon*	NA	94.36	5.64	3.80	5
38	Ghortapre	*Centella asiatica*	NA	80.87	19.13	14.0	1
39	Ghote	*NA*	NA	87.60	12.40	13.87	4
40	Hada	*NA*	25.93	86.39	13.61	12.29	10
41	Harkuta	*Carex baccans*	NA	95.0	5.0	16.0	1

42	Heg desmodium	*NA*	25.35	89.52	10.48	20.92	1
43	Hikum	*NA*	17.25	88.11	11.89	15.29	4
44	Janera	*NA*	NA	83.95	16.04	15.43	2
45	Jhome	*NA*	17.30	75.90	24.10	11.10	1
46	Jhus	*NA*	22.70	89.90	10.10	6.90	1
47	Jiswa	*NA*	21.52	75.30	24.70	7.28	5
48	Joint vetch	*Vicia spp.*	NA	88.36	11.64	22.83	1
49	Jyakcha	*NA*	20.33	86.43	13.57	17.51	3
50	Jyalo	*NA*	NA	92.47	7.53	11.49	1
51	Kana	*NA*	22.50	81.81	18.19	12.31	5
52	Kanari	*NA*	NA	82.22	17.78	8.05	4
53	Kanbuchi	*NA*	NA	88.09	11.91	21.63	2
54	Kane	*Floscopa scandens*	22.50	81.81	18.19	12.31	2
55	Kans	*Saccharum spontaneum*	NA	89.79	10.21	8.78	5
56	Karaunte	*Mariscus sumatrenis*	NA	91.73	8.27	7.40	1
57	Karimari	*NA*	24.30	69.80	30.20	8.20	1
58	Kaune banso	*NA*	NA	86.85	13.15	8.71	5
59	Khar	*Andropogon pumilis*	NA	92.99	7.01	7.53	12
60	Kharsami	*NA*	NA	86.22	13.78	9.53	5
61	Kharuki	*Andropogon contortus*	NA	90.76	9.24	7.29	4
62	Khoragama	*NA*	16.0	84.44	15.56	17.85	2
63	Kikyu	*Pennisetum clandestinum*	NA	87.25	12.75	20.32	2
64	Kodejhar	*Paspslum scorbiculatum*	14.0	86.89	13.11	12.74	5
65	Kote	*Medicago falcata*	18.33	90.10	9.90	18.08	9
66	Kudju	*Pueraria thunbergiana*	20.15	85.44	14.56	16.77	3
67	Kukur banso	*Equisetumdebile*	NA	91.61	8.39	5.21	1
68	Kyaramba	*NA*	15.0	85.67	14.33	21.19	4
69	Lab Lab	*Dolichos lablab*	NA	89.77	10.23	19.97	2
70	Ladini clover	*Trifoliu m repens var.*	NA	87.40	12.60	14.88	5
71	Lare	*NA*	NA	93.58	6.42	7.87	1
72	Local	*NA*	NA	87.42	12.58	9.76	40
73	Lucerne	*Medicago sativa*	NA	91.0	9.0	11.70	8
74	Madilo	*NA*	NA	91.53	8.47	8.57	1
75	Maize leaves	*Zea mays*	13.14	90.41	9.59	5.74	1
76	Makara	*NA*	14.50	70.20	29.80	10.25	2
77	Malingo	*Arundinaria aristata*	NA	83.96	16.04	10.87	1
78	Marbindo	*NA*	NA	77.54	22.46	20.10	1
79	Marmindo	*NA*	NA	86.67	13.33	22.04	4
80	Masyam	*NA*	NA	88.85	11.15	11.72	1
81	Mixed	*NA*	26.27	84.83	15.17	13.69	54
82	Molasses	*Melinis multiflora*	21.45	89.73	10.27	8.92	15
83	Motha	*Cerex inanis*	22.90	86.31	13.69	10.87	8
84	Myang myang	*NA*	NA	97.91	2.09	10.73	2
85	Nama	*NA*	20.25	90.98	9.02	14.65	4
86	Nepier	*Pennisetum purpereum*	17.79	80.33	19.67	9.45	5
87	Nikhari	*NA*	NA	94.38	5.62	5.02	1
88	Nilgiri	*NA*	NA	95.46	4.54	11.67	1
89	Numril	*NA*	29.57	92.10	7.90	11.12	1

90	Oat	*Avena sativa*	15.34	93.05	6.95	17.35	23
91	Panaki	*NA*	9.0	83.70	16.30	11.0	1
92	Pang	*NA*	NA	84.39	15.61	13.35	23
93	Pangbuchi	*NA*	15.23	84.15	15.85	18.05	1
94	Panjabi	*NA*	NA	78.24	21.76	7.84	5
95	Para grass	*Panicum purpurascens*	NA	83.33	16.67	6.31	2
96	Paspalum	*Paspalum dilatatum*	19.75	90.02	9.98	17.16	3
97	Phurke	*Arunlinella nepalensis*	NA	93.93	6.07	5.98	2
98	Pirlekhar	*Hydrangia robusta*	NA	96.59	3.41	3.71	1
99	Pongi	*NA*	NA	84.24	15.76	22.0	1
100	Pyauli	*Reinwardtia indica*	NA	89.52	10.48	15.97	4
101	Raigrass	*Lolium multiflorum*	NA	89.88	10.12	9.13	24
102	Raikhursani	*NA*	25.30	85.25	14.75	19.21	8
103	Ramba	*NA*	29.93	92.30	7.70	12.63	5
104	Red clover	*Trifolium pratense*	NA	91.81	8.19	13.55	17
105	Rhodes	*Chloris gayana*	21.85	91.03	8.97	11.03	2
106	Rubina	*NA*	29.66	92.99	7.01	19.80	4
107	Sailo	*NA*	NA	90.89	9.11	13.75	1
108	Salimo	*Eulaliopsis binata*	31.90	89.59	10.41	8.38	6
109	Salingo	*Eulaliopsis binata*	NA	93.0	7.0	8.30	2
110	Saptal	*NA*	NA	88.59	11.41	13.54	1
111	Sentro	*Centrosema pubescens*	23.25	91.22	8.78	20.77	2
112	Setaria	*Setaria anceps*	15.05	91.17	8.83	11.13	7
113	Seto pang	*NA*	28.04	93.34	6.66	9.17	1
114	Shama	*Echinochloa crusgalli*	NA	89.76	10.36	5.48	2
115	Shella	*NA*	15.60	78.10	21.90	8.90	1
116	Signal	*Brachiaria decumbens*	20.30	89.88	10.12	9.54	7
117	Sihuli	*NA*	NA	71.72	28.28	14.06	1
118	Sindur pang	*NA*	NA	93.17	6.83	10.86	2
119	Sinke	*Cheilanthes sp.*	NA	92.31	7.69	3.86	1
120	Siratro	*Macroptilium atropurpureum*	16.95	89.68	10.32	17.26	4
121	Siru	*Imperata cylindrica*	30.64	92.05	7.95	7.24	17
122	Sisnoo	*Utrica dioica*	NA	88.0	12.0	5.10	1
123	Sorbus	*NA*	NA	95.23	4.77	9.36	3
124	Stylo	*Stylosanthes guinansis*	21.45	90.62	9.38	13.84	19
125	Sunbuki	*Gnaphalium affine*	NA	93.95	6.05	12.97	1
126	Sunhemp	*NA*	20.20	89.40	10.60	26.91	1
127	Tall fescue	*Festuca arundinacea*	NA	86.10	13.90	8.41	6
128	Tarmindo	*NA*	NA	88.01	11.99	14.18	1
129	Teosinte	*Euchlaena mexicana*	NA	83.74	16.26	13.73	2
130	Thakaila	*NA*	NA	78.67	21.33	15.28	1
131	Tharthare	*NA*	NA	93.75	6.25	4.97	1
132	Theme	*NA*	NA	94.99	5.01	3.97	1
133	Thulo pang	*NA*	NA	91.14	8.86	14.06	1
134	Vakra	*NA*	NA	93.62	6.38	7.68	1
135	Vetch	*Vicia sativa*	14.90	81.13	18.87	14.16	3
136	White clover	*Trifolium repens*	NA	87.56	12.44	20.49	27
	Average		***21.96***	***87.87***	***12.13***	***12.0***	***686***

Annex 2: Fibre and lignin content of different grasses

S/n	Grass species	Fibre and lignin content				
		NDF	ADF	Lignin	Hemicellulose	Cellulose
1	Aankhle	72.50	53.50	12.90	19.0	40.60
2	Anjan	67.35	58.29	21.06	9.06	37.22
3	Arthunge	77.39	45.13	8.86	32.26	36.28
4	Banso	61.07	43.25	9.14	17.81	29.11
5	Barpang	66.82	50.29	11.22	16.53	39.07
6	Barseem	59.65	51.71	19.11	7.94	32.60
7	Bethe	32.65	29.81	20.45	2.83	9.36
8	Bichro	74.17	22.75	13.36	51.42	9.39
9	Biranchi	75.33	40.41	12.84	4.92	27.47
10	Boss	79.48	46.16	6.15	33.32	40.01
11	Buki	48.04	39.84	19.90	8.18	18.33
12	Chilali	46.27	41.71	14.50	4.56	27.21
13	Chirabhuje	76.28	71.96	11.93	4.32	60.03
14	Chitre banso	73.06	48.05	12.07	25.03	35.97
15	Chor buki	66.02	33.14	6.06	32.88	27.08
16	Chugla	54.46	52.08	17.20	2.38	34.88
17	Cocksfoot	54.09	36.81	8.42	17.23	26.56
18	Composite	49.95	41.62	22.47	8.33	19.15
19	Daha	81.68	45.97	8.46	35.71	37.51
20	Dam	81.04	61.50	15.43	19.54	46.07
21	Dami local	63.67	48.50	13.25	15.16	35.23
22	Dande	72.22	38.17	6.06	34.05	32.11
23	Debre grass	59.09	41.10	17.70	17.99	23.40
24	Desmenthos	57.94	47.96	21.24	9.98	26.72
25	Desmodium	54.76	39.58	10.17	15.17	29.41
26	Dhape	72.40	58.90	10.71	13.49	48.19
27	Dhimchi	60.17	50.91	23.0	9.33	27.91
28	Dhoge	76.48	41.12	5.84	35.35	35.29
29	Dolo	44.69	40.35	28.78	4.34	11.56
30	Dubo	67.46	42.72	12.82	23.73	31.02
31	Dus	54.65	47.62	21.43	7.04	26.19
32	Festuka	60.0	39.05	9.70	20.95	29.35
33	Gajarpata	48.15	43.24	31.86	4.92	11.37
34	Gandhe	49.70	44.05	12.37	5.65	31.68
35	Ghode banso	70.14	37.06	6.82	33.08	30.24
36	Ghode buki	60.56	54.33	13.21	6.23	41.13
37	Ghode dubo	74.40	51.04	14.56	25.54	36.16
38	Ghortapre	34.77	30.41	9.39	4.36	21.02
39	Ghote	77.87	60.47	18.17	17.45	42.25
40	Hada	72.48	54.93	15.31	17.56	37.60
41	Harkuta	76.90	48.70	16.60	28.20	32.10
42	Heg desmodium	47.96	43.14	19.05	4.83	24.09
43	Hikum	48.23	39.99	10.09	8.24	29.90
44	Janera	58.36	38.84	7.91	19.51	30.93
45	Jhome	63.60	51.30	29.70	12.30	21.70
46	Jiswa	64.46	53.98	23.78	10.48	30.24
47	Joint vetch	40.17	31.37	7.05	8.80	24.32
48	Jyakcha	54.16	43.52	15.73	10.64	27.79
49	Jyalo	58.43	52.96	12.22	5.47	40.74

50	Kana	57.77	43.78	20.49	13.99	23.29
51	Kana	57.77	43.78	20.49	13.99	23.29
52	Kanari	61.40	38.85	8.45	22.60	30.40
53	Kanbuchi	31.77	28.50	18.35	3.27	10.14
54	Kans	72.57	48.56	8.49	24.0	40.07
55	Karaunte	66.34	46.83	7.53	19.51	39.31
56	Karimari	72.10	59.90	30.90	12.20	29.10
57	Kaune banso	70.20	47.0	9.18	23.33	37.62
58	Khar	74.09	51.40	12.01	22.68	39.39
59	Kharsami	75.96	61.86	12.56	14.09	49.30
60	Kharuki	72.17	49.56	10.15	22.61	39.64
61	Khoragama	32.35	29.11	11.10	2.44	18.81
62	Kikyu	57.42	37.38	8.29	20.04	29.09
63	Kodejhar	62.44	51.88	9.13	10.54	42.75
64	Kote	58.0	49.65	13.75	8.55	35.90
65	Kudju	46.02	38.61	10.46	7.40	28.09
66	Kukur banso	73.91	42.58	6.52	31.33	36.06
67	Kyaramba	39.49	33.25	20.42	6.23	12.83
68	Lab lab	51.54	40.30	13.48	11.24	26.82
69	Ladini clover	51.55	39.90	10.55	11.60	29.40
70	Lare	68.12	47.58	8.18	20.54	39.40
71	Local	62.61	42.56	12.82	20.04	29.06
72	Lucerne	56.45	49.05	13.05	7.45	35.95
73	Madilo	72.11	42.81	7.59	29.30	35.22
74	Maize leaves	64.56	50.23	6.41	14.33	43.82
75	Makara	31.90	29.30	13.10	2.50	16.20
76	Malingo	62.28	48.42	27.15	13.86	21.28
77	Marbindo	48.46	40.51	39.32	7.96	1.19
78	Marmindo	44.62	37.33	23.32	7.29	11.51
79	Masyam	55.64	40.58	8.96	15.06	31.61
80	Mixed grass	59.55	50.57	20.29	8.98	30.52
81	Molassess	72.41	52.83	8.82	14.18	44.0
82	Motha	61.71	44.21	13.88	17.49	30.31
83	Myang myang	53.70	39.70	12.27	14.0	27.44
84	Nama	63.28	47.65	11.24	15.63	36.38
85	Nepier	58.52	41.62	16.41	16.90	24.15
86	Nikhari	73.87	62.99	9.80	10.87	53.19
87	Nilgiri	60.68	59.25	22.42	1.43	36.83
89	Numril	69.16	57.29	13.16	11.87	42.13
90	Oat	57.16	43.27	8.41	13.88	34.85
91	Panaki	69.60	57.50	30.10	12.10	27.40
92	Pang	65.39	52.65	19.01	12.73	33.64
93	Pangbuchi	35.27	31.49	10.10	3.78	21.49
94	Panjabi	63.74	50.42	22.88	13.32	27.54
95	Para	67.37	41.75	4.97	25.61	36.78
96	Paspalum	65.53	50.73	15.67	14.80	35.06
97	Phurke	80.25	42.68	5.0	37.57	37.68
98	Pirlekhar	75.40	68.74	18.11	6.66	50.63
99	Pongi	47.30	33.0	12.82	14.30	20.18
100	Pyauli	50.23	40.53	13.13	9.73	27.40
101	Raigrass	63.99	39.64	7.79	24.36	31.84
102	Raikhursani	40.54	33.62	22.11	6.92	11.50
103	Ramba	65.49	54.61	15.86	10.87	38.77

104	Red clover	56.20	48.80	18.10	7.35	30.75
105	Rhodes	70.89	58.97	21.39	11.91	37.59
106	Rubina	38.77	34.93	19.03	3.83	15.90
107	Sailo	60.17	42.42	10.69	17.76	31.73
108	Salimo	72.68	52.55	13.03	20.12	40.79
109	Salingo	78.70	60.0	11.90	18.70	48.10
110	Saptal	60.90	49.82	14.01	11.08	35.81
111	Sentro	54.03	45.35	17.74	8.68	27.61
112	Setaria	72.96	60.13	14.21	13.69	43.87
113	Seto pang	71.44	55.01	11.30	16.43	43.71
114	Shama	67.31	41.38	5.52	25.93	35.86
115	Shella	63.40	49.50	20.10	13.90	29.40
116	Siatro	54.28	45.25	12.59	9.03	32.66
117	Signal	71.17	52.09	10.94	17.65	42.58
118	Sihuli	51.10	23.83	4.39	27.27	19.44
119	Sindur pang	72.17	39.21	7.0	32.96	32.21
120	Sinke	70.38	61.11	8.61	9.27	52.51
121	Sisnoo	74.30	56.20	18.10	18.10	38.10
122	Siru	74.69	54.67	10.0	20.22	44.67
123	Stylo	56.11	47.55	10.81	9.14	36.15
124	Sun buki	61.09	55.47	12.95	5.62	42.53
125	Sunhemp	30.99	27.63	9.18	3.36	18.45
126	Tall fescue	60.70	37.25	6.40	23.45	30.90
127	Tarmindo	40.85	33.15	28.23	7.71	4.92
128	Teosinte	64.30	41.24	5.81	23.06	35.43
129	Thakaila	38.41	31.96	12.56	6.45	19.40
130	Tharthare	73.40	43.09	8.84	30.35	34.22
131	Theme	58.43	52.96	12.22	5.47	40.74
132	Thulo pang	66.21	38.82	7.38	27.38	31.44
133	Vakra	47.12	38.01	8.03	9.11	29.98
134	Vetch	60.64	54.55	20.42	6.09	34.13
135	White clover	41.49	35.61	9.40	7.18	24.22
	Average	***62.53***	***46.49***	***13.55***	***15.76***	***32.85***

Annex 3: Mineral content of different grasses

S/n	Grass species	Mineral Content	
		Calcium	Phosphorous
1	Anjan	0.32	0.40
2	Arthunge	0.46	0.24
3	Banso	0.72	NA
4	Barpang	0.25	NA
5	Barseem	1.64	0.40
6	Bethe	2.80	0.35
7	Boss	0.19	0.11
8	Buki	0.40	0.18
9	Chilali	1.47	0.25
10	Chitre banso	0.95	0.35
11	Chor buki	1.43	0.34
12	Coksfoot	0.52	0.28
13	Composite	0.49	0.29
14	Daha	0.28	0.18
15	Dam	0.41	NA
16	Dami local	0.76	0.23
17	Dande	0.82	0.51
18	Debre grass	0.77	0.26
19	Desmenthos	1.95	0.31
20	Desmodium	0.90	0.29
21	Dhape	0.42	0.26
22	Dhimchi	0.51	0.39
23	Dhoge	0.34	0.25
24	Dolo	0.69	0.27
25	Dubo	0.60	0.31
26	Duss	1.38	0.42
27	Furke	0.24	0.29
28	Gagarpata	0.82	0.52
29	Ghode banso	0.80	0.27
30	Ghode buki	0.61	0.26
31	Ghode dubo	0.42	0.15
32	Ghortapre	1.29	0.34
33	Ghote	0.57	0.34
34	Hada	0.51	0.31
35	Heg desmodium	2.06	0.40
36	Hikum	0.77	0.34
37	Janera	0.55	0.56
38	Jhome	0.21	0.37
39	Jhus	0.58	0.38
40	Jiswa	0.58	0.34
41	Joint vetch	1.39	0.26
42	Jyakcha	0.79	0.24
43	Jyalo	0.58	0.20
44	Kana	1.28	0.29
45	Kanbuchi	0.42	0.34
46	Kans	0.49	0.30
47	Karaunte	0.34	0.15
48	Karimari	0.56	0.30
49	Kaune banso	0.30	0.35

50	Khar	0.39	0.20
51	Kharsami	0.62	NA
52	Kharuki	0.58	0.25
53	Khoragama	2.58	0.40
54	Kikyu	0.48	0.65
55	Kodejhar	0.99	0.57
56	Kote	2.36	0.27
57	Kudju	1.60	0.31
58	Kukur banso	0.20	0.19
59	Kyaramba	1.02	0.26
60	Lab lab	1.67	0.37
61	Lare	0.49	0.20
62	Local	0.81	0.35
63	Madilo	0.29	0.37
64	Maize leaves	0.57	0.31
65	Makara	0.46	0.34
66	Malingo	0.65	0.13
67	Marbindo	0.65	0.48
68	Marmindo	0.55	0.33
69	Masyam	1.10	0.33
70	Mixed	0.82	0.22
71	Molassess	0.89	0.49
72	Motha	0.69	0.33
73	Myang myang	0.34	0.65
74	Nama	0.67	0.21
75	Nepier	0.89	0.47
76	Numril	0.33	NA
77	Oat	0.40	0.31
78	Panaki	0.59	0.29
79	Pang	0.42	0.26
80	Pangbuuchi	0.49	NA
81	Panjabi	0.30	0.49
82	Para	0.48	0.41
83	Paspalum	0.53	0.42
84	Pirlekhar	0.32	0.32
85	Pongi	0.23	0.39
86	Pyauli	1.51	0.33
87	Raigrass	1.08	0.33
88	Raikhursani	1.28	0.27
89	Ramba	0.98	0.18
90	Rhodes	0.47	0.54
91	Rubina	2.01	0.19
92	Sailo	1.36	0.20
93	Salomo	0.54	0.37
94	Saptal	2.04	0.26
95	Sentro	1.11	0.33
96	Setaria	0.40	0.43
97	Seto pang	0.26	NA
98	Shama	0.50	0.38
99	Shella	0.28	0.26
100	Signal	0.48	0.26
101	Sihuli	0.60	0.22
102	Sindur pang	0.76	0.27

103	Sinke	0.33	0.19
104	Siratro	1.29	0.28
105	Siru	0.47	0.23
106	Stylo	1.28	0.28
107	Sunbuki	0.64	0.19
108	Sunhemp	1.33	0.43
109	Tarmindo	0.89	0.35
110	Teosinte	0.52	0.53
111	Thakaila	0.34	0.40
112	Tharthare	0.59	0.40
113	Theeme	0.52	0.14
114	Thulo pang	0.92	0.37
115	Vakra	1.51	0.36
116	Vetch	1.04	0.20
117	White clover	1.79	0.46
	Average	***0.78***	***0.32***

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Nepalese Vet. J. 28: 95-102
Nepal Veterinary Association

Review article

CHITIN AND CHITOSAN: VERSATILE BIOPOLYMERS OF THE 21st CENTURY

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ABSTRACT

Chitin, a second most abundantly available polysaccharide next to cellulose has wide applications in agriculture, skin and wound management besides other biomedical applications. Chitosan, a deacetylated chitin has its widespread use in wound healing, fracture repair and in the management of degenerative joint diseases.

INTRODUCTION

Chitin, the most abundantly available natural polysaccharide next to cellulose in the world, is a unique biopolymer consisting of un-branched chain of β-(1-4)-2-acetamido-2-deoxy-D-glucose or N-acetyl D-glucosamine (Fig.1). Chitin is the principal polysaccharide found in the exoskeletons of crustaceans (crabs, shrimps, lobsters, etc.), mollusks and insects where it occurs in combination with proteins and minerals. Certain types of fungi, algae and yeasts also possess chitin in their cell walls.

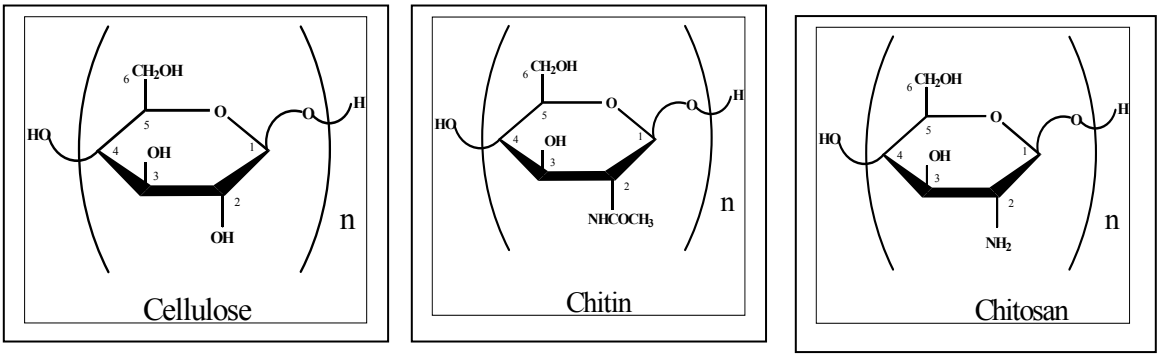

Figure 1. Chemical structure of naturally occurring polysaccharides

Chitosan is a deacetylated chitin with strong positive charge imparted from its amino group at Carbon-2 position. Chitin and chitosan biopolymers have begun to enter

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diverse market segments ranging from wastewater treatment to health care. Agriculture, cosmetics, toiletries, food and beverages, and product separations are other market segments. Chitosan containing cookies and noodles are also present in the Japanese market.

There is no clear-cut demarcation between chitin and chitosan. Chitin is extensively acetylated while chitosan is largely deacetylated. Unlike in most polysaccharides, presence of many amino groups in chitosan imparts a positive charge. Chitin is insoluble in water and acid while chitosan dissolves in acidic solutions but not in most of the organic solvents and water at neutral pH. The structure and percent chitin content vary depending on the sources. Most forms of chitin have an alpha structure, in which the polymer chains are tightly bonded. But cuttle fish (squid pen) chitin has a beta type crystalline structure, in which polymer chains are arranged instead in parallel to one another. The squid pen chitin has a lower degree of crystallinity than alpha chitin, is easily dispersed in water, and is more easily degradable by lysozyme and chitinase than colloidal chitin.

Currently, chitin and chitosan are produced mainly from the wastes of seafood processing industries. Of late, fungal mycelia of antibiotic fermentation plants are drawing much attention due to superior quality chitin-chitosan production for medical uses.

Production methods

Many countries that are surrounded by oceans are extracting chitin and chitosan from the seafood wastes while some other countries have just started to extract chitin-chitosan from fungal sources. Chitin and chitosan are commercially manufactured using a well-established chemical method, deproteination followed by demineralization (Hirano, 1996). Manufacturing steps differ from one company to another and some adopt demineralization first followed by deproteination later. Production of chitin and chitosan by enzymatic method is a novel approach to avoid chemical pollution but this method is still confined to laboratory scale.

Biological properties

Chitin and chitosan are naturally occurring rare muco-polysaccharides that are biodegradable on the earth and biocompatible within the mammalian tissues and plants. Chitin and cellulose when implanted in the mammalian body, the former is soon biodegraded and disappeared completely in due course of time, whereas the latter is not biodegraded and will remain *in situ* for a long period of time despite their small difference in the chemical structure. Chitin and their derivatives appear to be accepted by the body with

no or less side effects. That might be the reason why some *Majhi* people (tribal people dwelling in the river banks of Central Nepal) were reportedly using crude preparation of crushed powder of sun-dried, fresh water crab shells in wounds since the date unknown as a traditional practice.

There are many novel applications of chitin and chitosan and their derivatives and some of their biomedical applications are listed below:

Wound healing

Japanese researchers have pioneered the application of chitin biomaterial for wound healing in human and animals (Kifune, 1992 and Minami *et al*., 1992, 1993). Undocumented reports reveal that tribal people (*Majhi*) dwelling in and around big rivers of Central and Eastern Nepal have been using crude preparation of crushed powder of sun dried, fresh water crab shells in treating wounds since the time immemorial based on their indigenous traditional knowledge (ITK). Chitin has advantages over most wound dressings in its capacity to accelerate healing, reduce pain and scarring, take up wound exudates, and reduce or eliminate dressing changes. It has been found that chitin is better for human application, whereas chitosan for animal wounds. Since animal wounds are dirtier than human wounds and needs stronger tissue reaction, chitosan is better than chitin.

The coastal areas of Japan sea side are very rich in seafood processing and chitin and chitosan production. Researchers (Minami et al., 1992, 1993 & 1999; Okmaoto et al., 1992 & 1993; Shigemasa et al., 1998) championed veterinary practice with chitin and chitosan and these investigators have demonstrated dramatic effects of chitin and chitosan on wound healing in small, large and zoo animals. Same Japanese Companies have commercialized a composite material prepared from fine particles of chitosan and lactose called Chitofine© since January 2001 for the management of wound. The optimal dose suggested is 0.1 mg per square cm of wound site and can be used in the form of suspension for wound lavage. One caution to be taken while using chitosan is that dogs are sensitive than other animals and if chitosan injected in dogs is more than 150 mg/kg body weight, it will end up with fatal pneumonia as shown by Minami *et al* (1997).

Management of fracture and joint diseases

Chitosan and its derivatives have exhibited osteogenic activity subsequent to their application on artificially made bone defects in animal models (Borah *et al*., 1992; Muzzarelli *et al*., 1993). Khanal *et al* (1997) has found that chitosan and its 5-methyl pyrrolidinone derivative were highly biocompatible inside the osseous tissues and are

able to speed up the healing process of broken bone in clinical cases of limb bone fracture in dogs presumably by accelerating the growth of bone forming cells, and thereby filling the gaps created by loss of bone tissue. The most convincing findings in their study were the speedy recovery of bone fracture in 5-6 weeks as compared to 7 weeks or more in control, and the superiority of cuttle fish chitosan over shrimp shell chitosan. The reason for exudation from the site of skin sutures in one animal (16.7%) treated with chitosan was not clearly known. It was found that dose of intra-osseous application of chitosan powder after fracture correction differed with sources and the degree of deacetylation of chitosan, 5-10 mg/kg for beta chitosan and 10-30 mg/kg for alpha form of partially deacetylated chitosan.

As of now, there have been no ideal treatment regimens available for synovitis leading to osteoarthritis both in human and animal patients. Long-term usage of traditional drugs (cortico-steroids and non steroidal anti-inflammatory drugs) produce undesirable side effects and can have deleterious effects on chondrocyte and matrix homeostasis. Attention recently has shifted to new alternative methods of treatment for medical management of osteoarthritis that focus on slowing the process of cartilage degradation and promoting synthesis of cartilage matrix. Research has led to marketing of orally administered disease-modifying agents such as glucosamine hydrochloride and chondroitin sulphate that may directly influence chondrocyte metabolism in a beneficial manner. These disease-modifying drugs reportedly counter the destructive inflammatory process and encourage normalization of synovial fluid and cartilage matrix (Canapp *et al*., 1999).

Glucosamine hydrochloride is an amino-monosaccharide nutrient and precursor of the disaccharide unit of glycosaminoglycans (GAG), which is the building block of the ground substance of articular cartilage (proteoglycans). Chondroitin sulfate is predominant GAG found in the articular cartilage and a natural component of several other body tissues (tendons, bones, vertebral discs, and cardiac and corneal tissues). Chondroitin sulfate competitively inhibits the enzymes that degrade proteoglycans in cartilage and synovium, substantially increases intrinsic viscosity and the concentration and hydrodynamic size of hyaluronate. Exogenous glucosamine stimulates synthesis of proteoglycans, exerts trophic action on joint cartilages and favours sulfate ester formation with chondroitin sulfate. Such activities counterbalance degeneration of cartilages and results in beneficial activities in patients with degenerative joint diseases (DJD) (Muzzarelli, 1993). Simultaneous administration of D-glucosamine and collagen peptides was effective not only for repair of damaged cartilage, but also for increasing normal cartilage proteoglycan and glycosaminoglycan content in a controlled experiment (Minami *et al.*, 2002). These disease-modifying agents promote synthesis of cartilage matrix and slowing of cartilage degradation irrespective of their routes of administration.

With more understanding and the advancement of modern day chemistry and biology, once costly D-glucosamine is being produced at a cheaper rate from chitin by hydrolysis and deacetylation by some companies. It is hoped that D-glucosamine as a disease modifying agent and nutraceutical will replace traditional approaches and steroids for the management of degenerative joint diseases once the cost becomes more affordable.

Application in production medicine

Mastitis and infection of reproductive tract like metritis in dairy cows are posing a big problem in dairy industry in Nepal and throughout the world, as well. Chitosan powders suspended in sterile saline solution can also be infused in the mastitic udder for the management of mastitis in combination with parenteral antibiotic therapy, which is called as Chitosan Antibiotic Combination Therapy (CACT). This therapy is reported to exhibit synergistic activity in combating mastitis and reproductive tract diseases; therefore it could be a boon to Nepalese farmers for treating their infertile cows.

Miscellaneous biomedical usages

Sulfated derivative of chitin (S-chitin) was found to prevent tumor metastases (Tokura *et al*., 1992) and phosphated derivative of chitin (P-chitin) as pneumo-protective (Khanal *et al*.; 2001 & 2002). Chitosan's ability to bind with fat has led to marketing of chitosan capsules for obesity control in the department stores of countries like Japan, Korea, Taiwan and Thailand. Research has indicated that chitosan can be used as a natural hypocholesterolemic agent. Chitin-chitosan could be used as an effective medium for drug delivery, including controlled release of drugs. Chitosan's activity as a hemostatic agent, causing coagulation of red blood cells independent of classical coagulation pathways, has a lot of uses in surgeries. Chitosan inhibits the formation of scar tissue by preventing the formation of fibrin strands in wounds. Because of these properties, chitosan along with chitin can be used to form sutures, dressing bandages and sponges with properties not found in other competing products.

Uses in cosmetics and toiletries

Well-known companies in Germany and Japan have patented many cosmetics containing chitin and chitosan. Some others have patented carboxymethyl chitosan into skin care products and oral health products. Chitosan derivatives protect against microbial infections on skins and activate skin cells, resulting in the prevention of the skin aging. Chitosan, Carboxyl methyl chitin have a moisturizing function on skins, a protecting function of mechanical hair damages, and an anti-electrostatic function on hairs.

Application in food and feed additives

Knowingly or unknowingly, we consume chitin/chitosan while eating mushrooms, bread (bakers' yeasts) and soft shrimps. In Japan, chitin and chitosan are considered as natural products and two companies are already marketing chitosan containing cookies and noodles. Chitosan is used as a food additive for the improvement of food qualities in some commercial foods. Chitosan inhibits the growth of harmful bacteria and molds in foods even at low levels of NaCl, and salted fresh pickles and soy sauces.

CONCLUSIONS

This paper has reviewed about multifaceted applications of chitin and chitosan especially in veterinary practices. Nepal has yet to start from the beginning to exploit the full potential of these eco-friendly, versatile biopolymers on a more scientific way despite the possession of indigenous traditional knowledge about magic wound healing property of chitin by some tribal people.

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Nepalese Vet. J. 28:103-108
Nepal Veterinary Association

Review article

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

J. N. B. Shrestha[1]

ABSTRACT

The conservation of domestic animal diversity consisting of an array of breeds, populations and landraces with identifiable morphological characteristics and production performance has been considered as and important issue in animal agriculture. Some of the domestic animals have demonstrated considerable promise for the genetic improvement of efficiency in the commercial production of meat, milk and fiber from the livestock and poultry species. Despite the availability of an incredible amount of animal genetic resources in the world and the knowledge and skills the increase productivity, the animal agriculture in Nepal has failed to realize possible benefits from scientific advances in genetics, nutrition and husbandry, to the same extent as other developing countries. There is opportunity for increased productivity from crossbreeding and formation of composite populations based on exotic breeds that have demonstrated potential genetic merit in performance for economic traits in combination with indigenous animals known for adaptability to the local environment without jeopardizing the health of livestock and poultry in Nepal. One must rely on the veterinary profession to ensure that genetic improvement of efficiency can be achieved without compromising the animal health status of the domestic animals in the country. At the same time, veterinarians while continuing to service and protect domestic animals have enjoyed a close relationship with the producers that raise them. This association not only provides access to farm animals but also facilitates the identification of indigenous animals with unique traits and an opportunity for a commitment towards the conservation of domestic animal genetic resources.

INTRODUCTION

Animal agriculture has been a dependable source of food, fiber, power, fertilizer, emplyoment, property and companionship to humankid for cinguries. This is even more obvious in Nepal as over 90% of the small-holder farms of sedentary, nomadic and semi-nomadic origin supplement their income from the sale of animal and animal products amounting to 14% of the gross domestic product.

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Besides domestic animals are idolized and sacrificed in nearly every religious ritual in the country. Thus, the significant economical and cultural importance of domestic animal genetic resources to the Nepalese society deserves special meaning in nation building.

Many inhabitants including those of the farming community will recall the indigenous animal populations that were once common in the mountains and valleys of Nepal, which are now absent from the landscape. This can be attributed to the loss of grazing land and traditional dietary sources, rise in the incidence of disease and parasite infestation, urban encroachment, shifts in the relative economic importance of production traits that has changed the fundamentals of farming practices and regressive policies of the government. At the same time, the highly diversified owner-operated small-holder farms that raised indigenous breed populations have been declining at an alarming rate and are under increasing threat of facing extinction, While Nepal has followed the world wide trend towards concentration on a few breeds, lines or strain for commercial production of livestock and poultry, the mainstream agriculture producers and the general public are not fully aware of the consequence resulting from the failure to prevent the erosion of domestic animal diversity.

Major commercial companies, which own breeding populations of livestock and poultry, have achieved considerable success from breeding technology based on crossbreeding, formation of composite populations and selection to improve efficiency of commercial production. Concurrently, the general public has benefited from plentiful supply of wholesome food and variety of merchandise, all at a reasonable price. In another development, nearly all poultry and pig breeding stocks that provide the world with a safe and secure food supply are now in the hands of only a few multinational corporations. The access to domestic animal genetic resources that were once readily available is going to become more difficult in the future, compromising the ability of breeding methodologies employed for the genetic improvement of livestock and poultry species. The technology to recreate the array of genotypes represented in domestic animals under threat of extinction is not presently available and may not be developed for centuries. Because of these facts, the conservation of domestic animal genetic resources has thus become a preoccupation in many countries (Shrestha, 1990; Gerrits and Shrestha, 1995; Shrestha, 1998). The veterinarians having achieved success in securing the nations' food supply by providing service and protection to domestic animals, now need to diversity and participate in solving conservation issues that are critical to the future of the country.

Domestic animal diversity

The wide variation in the topography and climate in Nepal has led to the evolution of indigenous livestock and poultry populations with diverse morphological characteristics and production performance. These include the Achhami, Khailla, Pahari and Siri cattle, Gaddi, Lime, Parakote and Terai buffaloes, Chyangra, Khari, Singhal and Terai goats, Baruwal Bhyanglung, Dhorel, Jumli, Kage and Lampuchhre sheep, Bamel, Chwanche, Hurra and Sanu Banel pigs, Yak and Chauri, Jumli horse, Bhote pony, mules and donkeys, Sakini poultry and other animals that remain unclassified but are indigenous to the country (Shrestha *et al.,* 1998).

Historically, domestic animals with considerable promise were investigated for their inherent potential often lacking in the indigenous livestock and poultry species. The imported animals and their crosses not only excel in productivity and ability to adapt in the local environment but were well accepted by the community-at-large. In the last few decades, indigenous animals with genetic merit for adaptability to local environments and their crosses to augment the Nation's food supply for the growing human population. Consequently, the transformation of animal populations from indigenous to crossbreed continues to be widespread across every region of the country, but at high input costs. Despite achievements in terms of increased productivity, the rise in crossbreeding activity, unless regulated through legislation, must be considered as the most serious threat to the erosion of domestic animal diversity.

The change in farming practice has resulted in increased mechanization and the use of chemical fertilizers questioning the real advantage of raising large numbers of indigenous animals as a source of power and manure. At the same time, there has been serious concern over the detrimental influence of climate change, rise in inbreeding and the disruption of the natural surroundings on the structure and management of animal agriculture. It is, therefore, important that indigenous genetic resources be identified, characterized protected and preserved in order to secure a sustainable source of safe food supply, further Nepal's competitive advantage in the marketing of animal and animal products and alleviate poverty in the countryside. As of today, legislation to establish animal breeding policy does not exist in the country. However, general guidelines on crossbreeding have been adopted by the Department of Livestock Services.

There is agreement that the Siri cattle at extinct, however small numbers of crossbreds have been located; the Sanu Banel pig population are approaching extinction; the Parkote and Lime buffaloes are endangered; Lulu and Achhame cattle, Lampuchhre and Kage sheep and Chwanche and Hurrah pigs are under threat due to low numbers and the Yak population continues to decline. It has been proposed that

these populations should be maintained in their habitat, e.g. the Parkote and Lime buffaloes in Kaski and Palpa districts; Lulu and Achhame cattle in Mustang and Achham districts, respectively; Yak in Solukhumbu and Mustang districts; Bhyanglung and Lampuchhre sheep in the Mustang and Banke districts, respectively and Chwanche and Hurrah pigs in the Kaski and Palpa districts and Banke district, respectively. There is agreement among breeders that no exotic breeds should be crossed with indigenous populations under threat, endangered or at risk of extinction. Nevertheless, breeding policy for the conservation with utilization of indigenous animals need to be developed. This can be achieved with group breeding schemes and prompt exchange of sires among smallholder farms (Shrestha *et al.,* 2000). Again, the concept of Farm Parks for indigenous animals has been under discussion.

Nepal has no dependable policy on the quarantine of domestic animals necessary to safeguard the animal health of the livestock and poultry species in the country from the introduction of communicable diseases that have been responsible for destitution and incredible monetary loss in many countries. Despite these problems, importation of animals is important if one has to introduce genetic merit that is not inherent among the populations established or indigenous to the country. Quarantine of domestic animals can be achieved by developing proper breeding policy and Livestock Services Act without jeopardizing the health status of the domestic animal populations in the country. It is therefore pertinent that the roles of the veterinarian need to be enhanced by actively participating in developing policy consistent with their concern over protection of the animal health in the country.

Emerging technologies

There are wide variations known to exist in genetic resources, both among and within animal populations (Simon, 1990; Scherf, 2000). This great potential might be exploited to achieve biological limits in domestic animals while providing for the changing consumer preferences and environment, contributing to the efficiency lf production. Efforts to determine genetic distance among animal populations based on highly polymorphic microsatellite markers, markers, mitochondrial DNA D-Loop sequences and fingerprinting have helped locate populations that are distant on the evolutionary scale, thereby increasing the possibility of identifying a large number of segregating genes for performance in the diversified populations. The microsatellite markers from the divergent genetic populations with distinct features have helped develop maps for the livestock and poultry species. Although knowledge in this area is rapidly expanding, it could take some time before the average mammalian genome thought to consist of 50,000 to 100,000 genes can be sequenced and correlated with performance.

A number of markers that have already been identified could be used as tools to rapidly screen breeding populations for specific characteristics. This technology may also be used in combination with quantitative genetic methodologies to improve the accuracy of predicting the genetic merit of farm animals.

There is a need to maintain breeding populations with a large amount of variability or those that have specific attributes. These populations can act as a reservoir of specific nucleotide sequences related to performance traits of economic importance in domestic animals. In the future, veterinarians having acquired the knowledge and technical skills could gain from emerging technology and augment the health and productivity of the livestock and poultry species.

Transgenic animals created from novel reproductive technology perhaps may one day demonstrate resistance to specific disease. In this process, specific genes from DNA of indigenous animals and species relatively close from and evolutionary point of view could be incorporated into the genome of the transgenic animals. At the same time, it may be possible to create new products and pharmaceuticals of exceptional value. The potential benefit through the savings in handling, vaccination and the use of anti-parasite and antibacterial drugs could result in significant saving to the producer and provide a safer food supply to the consumer. A number of veterinarians in the developed countries have been in the forefront of gene transfer technology, possibly those in Nepal can follow.

Future consideration

A major problem in the conservation of domestic animals is the lack of funding. Additional budgetary allocations arte needed to increase research effort in animal agriculture including the conservation and the use of domestic animal genetic resources. The inability to obtain additional funds may seriously compromise the competitiveness of animal agriculture.

In this past decade, there have been a number of initiatives proposed by the governmental and non-government agencies (Agriculture Perspective Plan, Livestock Master Plan, National Conservation Strategy and Nepal Environmental Policy and Action Plan in the National Biodiversity Action Plan) that have not materialized in active conservation. The failure to influence the conservation of animal genetic resources over the last decade has been transparent. One could rely on the veterinarian profession to identify domestic animals with unique morphological characteristics and production performance as they are responsible for safeguarding the health status of domestic animals and will always be an integral part of conservation activities.

CONCLUSION

Domestic animals are of great economical importance and represent an important sector of agriculture. As of today, the technology, human resources and mechanisms to effect the long-term identification and conservation of unique, valuable domestic animals does not exist in Nepal. Therefore, the veterinarian should be encouraged to play a significant role in the identification, characterization and conservation through utilization of domestic animals to ensure availability of sufficient genetic resources for future improvement of livestock and poultry species.

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Nepalese Vet. J. 28: 109-113
Nepal Veterinary Association

Short communication

EMERGENCE OF NEW FMD VIRUS STRAINS IN NEPAL

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ABSTRACT

Among the seven different serotypes of Foot and Mouth Disease (FMD) virus, serotypes O, A, C and Asia 1 have been detected in Nepal. FMD outbreak due to serotypes O has been found to be the most predominant type (71%) followed by Asia 1 (16 %), A (10%) and C (3%). However, outbreak of FMD serotype C has not been recorded in the country since 1996. Different topotypes and genotypes (strains) of serotype O have also been isolated by World Reference Laboratory (WRL), Pirbright from the samples sent from different countries. Pan Asia was a new and virulent strain of serotype O that belonged to the Near East-South Asia (NE-SA) topotype and was identified from India in 1990. Among the 159 samples collected during 2003 for serotyping, six samples were sent to WRL, Pirbright for laboratory confirmation and antigenic characterization. The test at WRL confirmed them to be of serotype O. On further characterization, Pan Asia strain was isolated from the sample taken from Kathmandu valley, which belonged to the Middle East- South Asia (ME-SA) Topotype. A different strain IND 2001 (further phylogenitic analysis yet to confirm this strain) was also isolated from the sample taken from Sunsari, which belonged to the same Topotype. Hence, emergence of such new strains of FMD virus has necessiated for a laboratory based detailed epidemiological study, which will be helpful to understand the epidemiology, develop the vaccine and vaccination strategy for the country.

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INTRODUCTION

Foot and Mouth Disease (FMD) is one among the major Transboundary Animal Diseases (TAD), infecting all cloven-hoofed animals such as cattle, buffalo, sheep, goat and pigs. The causative agent of the disease is the virus belonging to Picornaviridae family. The disease is reported throughout the country irrespective of seasons and altitudes. Among the seven serotypes: O, A, C, Asia1, SAT1, SAT2, SAT3, identified for FMD, O, A, C and Asia-1 serotypes had so far been reported from Nepal, however, serotypes C has not been reported since 1996.

Outbreaks due to Serotype "O" are most predominant in Nepal, with several topotypes and genotypes/strains present under serotype "O" including the strain recorded in 2001, Pan Asia strain of the virus belonging to Near East-South Asia (NE-SA) topotype was responsible for the epizootic in the United Kingdom. Pan Asia lineage of the NE-SA Topotype of FMDV-O, originated in India in 1990, spread to Greece, Bulgaria in 1996 and then to Taiwan, China, Korea, Mongolia, Russia and Japan in 1997, South Africa in year 2000 and then to U.K., France and Netherlands in 2001 (FAO, 2003).

In Nepal, outbreak of FMD with less prominent clinical signs and high adult mortality were reported from different parts of the country in 2003 (during the months of April, May and June) along with reports of FMD outbreaks even in the vaccinated animals.This outbreak was investigated and representative samples from the outbreak were sent to WRL, Pirbright for laboratory confirmation, virus isolation and antigenic characterization. The present paper reports the findings of the outbreak investigation.

MATERIALS AND METHODS

A total of 159 samples were collected in 50% buffered glycerine from different parts of the country. These samples were transported to the laboratory in ice maintaining cold chain. Samples were prepared aseptically for serotyping of FMD virus by Indirect Sandwich ELISA Test. From among the collected the samples, six were sent to World Reference Laboratory, Pirbright, England for further confirmation, and antigenic characterization.

RESULTS AND DISCUSSION

Distribution of FMD virus serotypes present in Nepal is presented as figure 1, and the result of antigenic chatacterization of Nepal FMD virus is presented as Table 1.

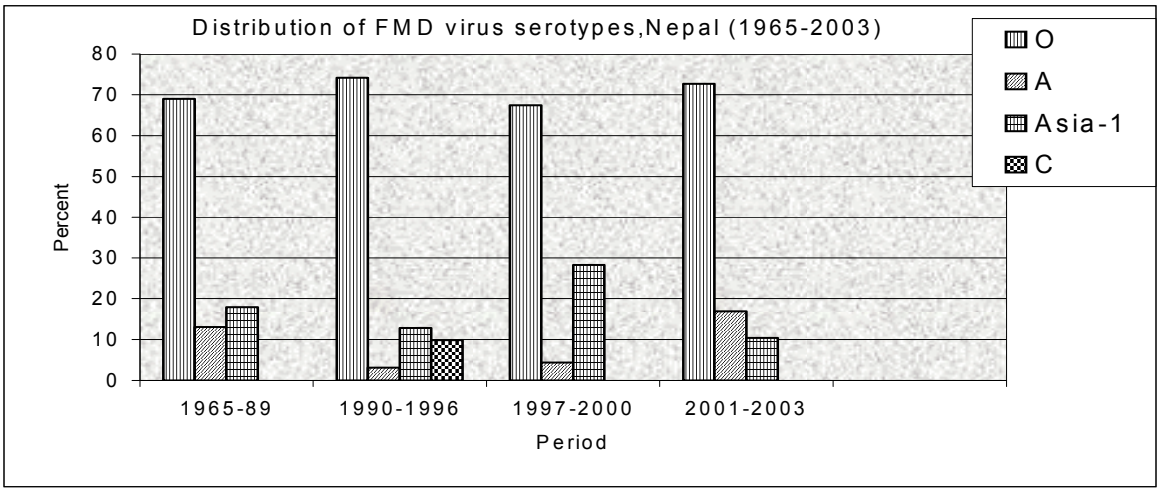

Figure 1: Distribution of FMD Virus Serotypes, Nepal 1965-2003, July).

Table 1: Antigenic characterization of Nepal FMD virus

Reference	WRL Reference	Description of Sample	Serotyping Results by ELISA	Remarks
Sunsari	NEP 1/2003	Bovine mouth lesion	O	Direct antigen ELISA positive, Positive after passage in BTY * and IB-RS-2 ** cells
Kathmandu	NEP 2/2003	Bovine mouth lesion	O	Direct antigen ELISA positive, Positive after passage in BTY * and IB-RS-2 ** cells
Kathmandu	NEP 3/2003	Bovine mouth lesion	NVD***	Direct antigen ELISA positive, Positive after passage in BTY * and IB-RS-2 ** cells
Lalitpur	NEP 4/2003	Bovine mouth lesion	O	Direct antigen ELISA positive, Positive after passage in BTY * and IB-RS-2 ** cells
Kathmandu	NEP 5/2003	Bovine mouth lesion	O	Direct antigen ELISA positive, Positive after passage in BTY * and IB-RS-2 ** cells
Kathmandu	NEP 6/2003	Bovine mouth lesion	O	Direct antigen ELISA positive, Positive after passage in BTY * and IB-RS-2 ** cells

NDV-No virus detected, BTY-Primary Bovine Thyroid cells, IB-RS-2-Continuous Pig kidney cell line

In Nepal the percentage of FMD serotype recorded so far from from 1965 – 2003 has been as follows:

Serotype O	71 %
Serotype A	16 %
Serotype C	3 %
Serotype Asia1	10 %

However, serotype C, reported during the period 1990-1996, has not appeared since then.

The characterization result of FMD virus sent to WRL Pirbright UK is presented as Table 2.

Table 2: Characterization of FMD virus from Nepal.

Serotype : O	**Date: 21/08/2003**
Virus Isolate: NEP/06/2003	
Material Used: 10% Epithelium Suspension	
Region Sequenced: VP1	Seq.filename: NEP 03-06
RT-PCR Primer set: O1C-272F/NK61	
Topotype: ME-SA	No. of ambiguities: 0
Genotype/strain: **Pan Asia**	Date seq. last updated: 16/07/2003
No. of nt determined/no. in gene: 639/639	Total no. of comparisions made: 1300

Ten most closely related viruses

Pos	**Virus name**	**File name**	**No. nt** compared	**No.nt** matched	**No. of** ambiguities	**%** Identity	**%** Difference
1	O/LEB/14/98	LEB98-14	614	588	3	95.77	4.23
2	O/IRN/12/98	IRN98-12	418	400	26	95.69	4.31
3	O/LEB/6/98	LEB98-06	560	535	0	95.54	4.46
4	O/LEB/9/98	LEB98-09	582	556	17	95.53	4.47
5	O/LEB/17/98	LEB98-17	624	596	3	95.51	4.49
6	O/SYR/1/98	SYR98-01	624	596	9	95.51	4.49
7	O/BAR/8/98	BAR98-08	639	610	0	95.46	4.54
8	O/IRQ/26/2000	IRQ00-26	637	608	2	95.45	4.55
9	O/TAW/3/99	TAW99-03	454	433	11	95.37	4.63
10	O/BAR/1/99	BAR99-01	323	308	0	95.36	4.64

Pos	Virus name	File name	No. nt compared	No.nt matched	No. of ambiguities	% Identity	% Difference
1	O/TAW/2/99	TAW99-02	639	609	0	95.31	4.6
2	O1/Manisa/TUR/69	TUR69-E	639	572	0	89.51	10.49
3	O/IND/R2/75*	IND75-A	639	570	0	89.2	10.8
4	O/IND/53/79	IND79A-53	639	566	0	88.58	11.42
5	O/MOR/1//91	MOR91-01	193	168	5	87.05	12.95
6	O/TAI/189/87*	TAI87-B	527	456	3	86.53	13.47
7	O/ISR/1/88	ISR88-01	207	179	3	86.47	13.53
8	O/HKN/6/63	HKN83-06	516	417	6	80.81	19.19
9	01/BFS/1860/UK/67	UKG67--A	637	514	2	80.69	19.31
10	O/PHI/5/95	PHI95-05	639	507	0	79.34	20.66

nt: nucleotides

*** not a WRLFMD reference number**

Table 1 and 2 are the Test results of FMD Samples sent to WRL, Pirbright in 2003. The samples were positive for serotype "O".This virus isolate NEP/06/2003 was further classified into the Pan Asia strain belonging to the Middle East-South Asia (ME-SA) topotype.This has clearly indicated the emergence of Pan Asia virus topptype in Nepal.This isolate when compared with the 10 most closely related viruses, was nearest to O/LEB/14/98.Similarly, when compared with the Reference strains, the one which is closest to this isolate is O/TAW/2/99. This finding opens the area for updating the present vaccine with the new strains of serotype"O", Pan Asia strain which would confer higher level of protective immunity to the animals.

Though the sample size for the characterization was small it has indicated the emergence of a new strain of FMD virus in the country. Outbreak of FMD could be due to this strain also in other parts of the country.Hence, a detailed epidemiological study including isolation, identification and characterization of FMD Virus from the field sample is required to justify the incorporation of the new strain in the existing vaccine.

ACKNOWLEDGEMENTS

We would like to extend our sincere thanks to Dr. Nigel Ferris and the concerned staff of WRL Pirbright, for conducting laboratory tests and antigenic characterization of the samples.We also extend our sincere thanks to Dr. S. N.Mahato (Director General, DLS) and Dr. D. R. Ratala) Programme Director, AHD) who encouraged us for laboratory investigations.We also thank to the field staff who helped us to collect the samples from the outbreak areas.

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Nepalese Vet.J. 28: 114-117
Nepal Veterinary Association

Short communication

TRADITIONAL CHINESE VETERINARY MEDICINE, CONCEPT AND PRACTICES

B. Sharma[1] and B. N. Chaulagai[2]

ABSTRACT

Traditional Chinese Veterinary Medicine (TCVM) is a scientific summary of the rich experience of the Chinese struggle against disease since 4000 years back. Zang organs consist of heart, lung, liver, spleen and kidney. Fu organs consist of gall bladder, stomach, small intestine, large intestine and urinary bladder. Yin-Yang and five-element theory are two fundamental principles of TCVM. Yin and Yang are 2 aspects of the unity of opposite things. The five elements include the most basic elements in nature such as wood, fire, earth, metal and water. TCVM holds that the animal body maintains a relative dynamic balance among viscera and tissues and between the body and its external environment in which contradictions may cause disease. The etiology may be six exo-pathic factors such as wind, cold, summer heat, dampness, dryness and fire. Pathogenesis may include the struggle between the vital and evil, imbalance of yin and yang and disorders of ascending and descending qi. Treatment is based on wholesome by using Chinese herbs, herbs, acupuncture and moxibustion.

INTRODUCTION

Traditional Chinese Veterinary Medicine (TCVM) is a scientific summary of the rich experience of the Chinese struggle against disease since 4000 years back. Four great classics of TCVM are as follows (Annon, 2003). The Yellow Emperor's Internal Canon of Medicine was one of the oldest texts of TCVM, which had been used for guiding medicinal practices. Treatise on Exogenous Febrile and Miscellaneous Disease was also important tool of TCVM. It was based on signs and symptoms and treatment was based on wholism. Shennong's Herbal classic described 365 kinds of herbs and these herbs were recorded in their book. These medicines had been used for treating human and animals. *Materia Medica* was one of the most comprehensive books of TCVM. The book recorded 1892 herbs with more than 1000 prescriptions. Therefore, it gave new vision for different diseases and treatment methods (Annon,2003).

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[2] Animal Health Directorate, Tripureshwor, Kathmandu, Nepal

Basic features of TCVM

Concept of wholism

Wholeness means unity and integrity. It gives due importance for interrelation between animal body and nature. There is unity between interior and exterior conditions. The basic features are:

Animal body acts as an organic whole:

Zang organs consist of heart, lung, liver, spleen and kidney. Fu organs consist of gall bladder, stomach, triple warmer (upper, middle and lower), small intestine, large intestine and urinary bladder. The heart corresponds with the small intestine, governs the skin and hair and opens in the nose. The spleen corresponds with the stomach, governs the muscles, and limbs, and opens in the mouth. The liver corresponds with gall bladder, governs the tendons, and opens to the eyes. The kidney corresponds with the bladder governs the bone, and opens to the ears.

Relationship between animals and nature:

In spring season, there are predominant warm diseases. In summer, and autumn season, there are more heat stroke cases. The winter season favours cold disease. Therefore change in the nature directly influences the physiological and pathological conditions in animal body.

Bian Zheng Lunzhi (Syndrome differentiation and treatment)

Palpation, olfaction, observation, interrogation to the livestock owners may reveal signs and symptoms. These are correlated with exo-pathogenic factors vital energy, evil energy and different course of disease. The disease is grouped based on similar signs and symptoms. Similar disease will be treated in the same fashion.

Basic theory of TCVM

- Ying and Yang: Ying and Yang are two fundamental principles or forces in the universe, opposing and supplementing each other. e.g. Heaven and earth, outside and inside, movement and stability.

- Yang: All hyper-functional exited, hot, moving, strong, bright, invisible, light and clear, up and outward and all that have active specific characteristics belong to yang.
- Yin: All that are waning, restricted, cold, weak, dark, visible, heavy and turbid, down and downwards, in and inwards, and all that have inactive specific characteristics belong to yin.
- Opposite of yin and yang.
- Interdependence of yin and yang.
- Waxing and waning of Ying and Yang.
- Transformation between Ying and Yang.

Theory of five elements

1. Order of generation: Wood to fire to earth to metal to water to wood. Wood is the precursor of fire and fire is the precursor of earth etc.
2. Order of restriction: Wood to earth to water to fire to metal to wood.
3. Theories of qi, blood, and body fluid:
   Qi is the most basic substance of which the world is comprised. Everything in the universe results from the movement and changes of qi.
4. Function of qi: Driving, warming, defending, consolidating and governing action.
5. Diagnosis of Disease: Palpation, olfaction, observation, interrogation to the livestock owners are some of the primary tools of disease diagnosis in TCVM.

Differentiation of Diagnosis:

External causes

Six exogenous pathological causes like climatic factor: wind, cold, summer-heat, dampness, dryness and fire.

Internal causes

Disturbance of Zang and the fu organs may cause disease.
Improper feeding and malnutrition are some of the etiology of disease.
Overstrain; stresses or lack of physical exercises may lead to sickness.

Other factors

The conditions, which cause surgical injury or fracture of bone, and bleeding etc.

Treatment

1. Herbal preparation.
2. Acupuncture: finding out different acupoint in different livestock.
3. Moxibustion: Application of heat in certain area and stimulation could be done for relieving pain or treatment of chronic disease.
4. Magnetic therapy: It is helpful for removing iron objects, nails from stomach.

RECOMMENDATIONS

Attempts should be made to transfer the TCVM technology in our traditional method livestock treatment and synchronize it according to time (Annon, 1992), because traditional Veterinary Medicine is economic and eco-friendly, does not have side effects hence, it can be utilized as a sustainable technology as it utilizes the local herbs for treatment.

ACKNOWLEDGMENT

We would like to thank Rene Chonyang, Director of North Hebei campus for his kind support in the TCVM programme.

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Nepalese Vet. J. 28: 118-120
Nepal Veterinary Association

Short communication

AUTOGENOUS VACCINATION FOR ORAL PAPILLOMATOSIS IN DOGS

Raja Ram Shrestha[1]

ABSTRACT

Study conducted at central veterinay hospital for the treatment of oral papillomatosis with simplified autogenous wart vaccination by autogenous vaccine in ten dogs suffering from oral papillomatosis inoculating showed complete recovery within a period of 20-28 days. Port inoculation indicated that autogenous vaccines could be useful for treating oral papillomatosis in dogs. The findings are discussed in this paper.

INTRODUCTION

Oral papillomatosis is a disease state characterized by the development of multiple papillomas in oral region. Canine viral papillomatosis is characterized by multiple papillomas, occurring most commonly on the oral mucosa and lips of young dogs (Blood and Studdert, 1988). The warts generally develop on the lips and spread to the buccal mucosa, tongue, palate and pharynx. The papilloma may be single, or confluent cauliflower-shaped masses with a rough surface (Vegad, 1995) and are usually whitish gray, varying from 3mm to several centimeters in diameter (Hine, 1988). The papillomas may be horn-like (*cornu cutaneum*) and being benign growth outwards, away from the basement membrane do not burrow underneath (Rao, 1983). They are slow in growth (benign), not adherent to overlaying skin and usually do not ulcerate (Venugopalan, 1994).

The tumors may persist for several months, occasionally longer, but spontaneous regression is usual (Blood and Studdert, 1988). They may spread by direct contact with infected animals usually through the abraded skin. They are generally of little significance, but they may be cosmetically undesirable or sufficiently extensive to be harmful (Hine, 1988). Dysphagia, dysorexia, hypersalivation and hemorrhage may be present in severe papillomatosis.

The infectious papillomas of dogs (*verruca vulgaris*) and those of cattle are caused by virus (Rao, 1983) and canine papilloma virus induces warts in mouth of dogs (Vegad,1995). Occurring primarily in young animals, viral papillomas are caused by one of various strains of papovavirus and are common in dogs, cattle and horses (Hine, 1988).

[1]Central Veterinary Hospital, Tripureshwor

Casual virus of oral papillomatosis among dogs is attributed to species-specific strains of Papillomavirus, a genus of Papovavirus, and is transmissible by intradermal injection (Blood and Studdert, 1988).

MATERIALS AND METHOD

A total of ten cases of dogs of both sexes and different age groups brought to the central veterinary hospital were the subjects for the treatment. During the study, the following methodology was used for the diagnosis and treatment.

Tentative diagnosis was based on the history given by the owners. Confirmatory diagnosis was made with the help of visual and clinical examination of the wart. The nature of papilloma was observed with primary consideration to the evidence of bleeding and any evidence of malagnancy.

Use of electric surgery is advocated for the removal of warts. However, in this study, they were grasped with thumb forceps and papillectomy was performed by surgical blades and scissors along with adequate provision for hemostasis. Local and general anesthesia were also used as per the need.

Few warts were collected from the affected dog and ground in mortar and pastel. After boiling the warts in distilled water, the mixture was allowed to settle for sedimentation. Five ml supernatant fluid was collected and the sediment was then discarded. One ml of the fluid was drawn in syringe and injected subcutaneously to the same dog.

RESULTS

Eight out of ten cases were treated successfully after the autogenous vaccination. Regular visit to the hospital was requested to the owners for the close observation of the recovery. Eight owners showed up with their dogs to the hospital and complete recovery was noted in 20 to 28 days. One of the treated dogs showed hypersensitivity reaction and symptomatic treatment was provided. One of the owners did not visit the hospital after the injection; hence the response could not be monitored.

DISCUSSION

The infectious nature of canine oral papillomatosis was described in 1898 and in 1930. These tumorous growths were transmitted by injecting wart material that had been passed through a berkfeild filter. Various treatment measures have been advocated by various authors apart from the faith in spontaneous recovery. The warts are usually benign and disappear spontaneously after several months. Dogs recovered from infection develop

immunity to reinfection (Vegad, 1995) and in young animals prognosis is not favorable due to chances of recurrence (Venugopalan, 1994). Almost all authors agree in surgical removal of the warts and autoimmunization for the effective treatment. The treatment in this study is however, different and simpler than recommended by Venugopalan (1994), who has recommended rather a complicated procedure. Further, single vaccination was made in this study which also differs from Venugopalan (1994). Similarly, the one ml subcutaneous injection in this study is also far lower than 5-10ml recommended by Venugopalan, (1994).

CONCLUSION

Autoimmunization has long been recognized for the treatment of oral papillomatosis. Medical therapy as Sodium bismuth tartarate, podophylin, anthiomaline and bismuth often fail to respond and surgical removal is indicated. Reoccurrence of papillomatosis was not noted in the study subjects. Hence autoimmunisation is recommended as a suitable, handy, easy, fast and economic method for the treatment of oral papilloma in dogs in developing countries like Nepal.

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Nepalese Vet. J. 28: 121 - 123
Nepal Veterinary Association

Short Communication

ANTHELMINTIC TREATMENT OF SHEEP AND GOATS IN KARNALI REGION FOR PRODUCTION IMPROVEMENT

G.P. Khakural[1], D.N. Dhungana[2], S.P. Shrestha[1], Y.K. Shrestha[3]

ABSTRACT

Livestock farming specially sheep and goat husbandry is very potential agricultural enterprise in Karnali region of Nepal; however, the overall productivity is low. There are multidimensional problems, complex in nature, associated with declining sheep and goat population and their productivity. These problems are diseases, shortage of feed stuffs, indiscriminate breeding, shepherding problem, predation and plant poisoning, trapping and stealing. The study (n=167, sheep=88, goat=79) was undertaken on control of internal parasites (gastrointestinal nematodes) by drenching with (Fenbendazole @ 5 mg/kg body weight) and its effect on growth of sheep and goats. Before drenching prevalence of parasites in sheep was 87.5% and 45.5% in goats. Sheeps were more infested than goats because sheep graze and goats browse the vegetation. The parasitic burden was reduced after drenching but the drenching response was not remarkable as the weight gain was low (0.6 kg) despite drenching, possibly because, this season (winter) is the feed scarcity season of the year.

INTRODUCTION

Karnali region is in northern part of Mid Western region of Nepal. It is one of the remote areas of Nepal. The topography of this region is naturally difficult and unsuitable for crop production. The productivity of land available for crop cultivation is also very low. In most of the places, climatic condition allow single crop production per annum. Therefore, overall socioeconomic status of general people is relatively very poor. Livestock farming, especially sheep and goat husbandry is very potential however, the productivity is low. Sheep and goats are an important means for pack use: carrying grain, pulses, common salt, sugar and other household consumables in addition to wool and meat production. People sacrifice them in religious ceremonies. Their indirect use is in contribution to replenishing soil fertility through manuring of agriculture fields. Wool and pashmina (fine wool) production for making various woolen items for self use and for sale in local market was also cited as one of the reasons for keeping sheep and goat.

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[2] District Livestock Service Office, Jumla
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Sheep and goat meat are valued as a source of main animal protein for ethenic group of population. There is also a tradition of providing sheep and goat as gift (*Pewa*) to their sister and daughter. There are multidimensional problems, complex in nature due to which productivity and production of sheep and goats declined over time. The migratory system of sheep and goat rearing is declining. The overall flock productivity is low due to many factors associated with health and management. Few studies indicate high flock mortality, between 15-52% as main problem in other parts of the country, (Oli and Gatenby, 1990, Karki and Dhaubadel, 1991) which might be the case in this region also. The major cause of mortality has been reported to be diseases and parasites, followed by predation. This study was under-taken to assess the effect of drenching (control of internal parasites) on growth of sheep and goats.

MATERIALS AND METHODS

The present work focused on the study of gastrointestinal nematodes by anthelmintic drenching. The following methods were applied for this study.

For the study, four VDCs were selected from *Jumla* districts of *Karnali* Region (*Guthichaur*, *Patrasi, Kalikakhetu* and *Sinja*). The faecal samples were collected from the animals and monitoring of parasite burden was carried out by evaluating eggs per gram (epg) faecal samples collected pre and post drenching. The anthelmintic used was Panacur (Fenbendazole @ 5 mg/kg body weight). Body weight was recorded before drenching and one month post treatment.

RESULTS AND DISCUSSIONS

The prevalence of gastrointestinal parasites in sheep and goats is presented in Table 1:

Table 1: Prevalence of internal parasites in sheep and goats of Karnali region.

S. N	Age Group	Sheep			Goat			Total (Sheep & Goat)		
		No of sample examined	No of sample positive	%	No of sample examined	No of sample positive	%	No of sample examined	No of sample positive	%
1	<6 months	2	0	0	9	6	66.6	11	6	54.54
2	7-12 months	26	21	80.67	18	6	33.33	44	27	61.36
3	13 to 24 months.	30	30	100	21	11	52.38	51	41	80.39
4	>2 years	30	26	86.66	31	13	41.93	61	39	63.93
Total		88	77	87.5	79	36	45.5	167	113	61.67

Prevalence of internal parasites was more in sheep (87.5%) than in goats (45.5%) probably because the sheep graze from the ground directly but the goats browse. Sheep between 1 to 2 years of age were more infested (100%) because they may have taken more parasitic egg while grazing. The kids were more infested (66.66%), reason for which is unclear. The effect of drenching in body weight gain of sheep and goats is presents in Table 2

Table 2: Effect of drenching on body weight gain in sheep and goats of Karnali.

S. N	Age group	n	EPG		Avg. Body wt.(kg)		Wt. Diff. kg
			Pre	Post	Pre	Post	
1	< 6 months	11	100	-	17.9	18.59	0.69
2	1 to 2 months	44	300	-	21.75	22.27	0.42
3	12 to 24 months	51	300	233.3	31.4	31.4	0
4	> 2 year	61	190	-	33.7	33.9	0.2

Note: n = 167 (Sheep = 88, Goat =79), Time interval = One month

Parasitic burden reduced after drenching except in some cases and weight gain also increased except in1 to 2-year age group. The weight gain (0.6 to 0.77 kg) was not as expected, possibly because this season (winter) had scarcity of feed stuffs (natural vegetation) and animals could not get enough feed during this period.

ACKNOWLEDGEMENTS

We would like to thank Dr. A. Pradhan for official help, Dr. K.B. Bohara for site selection, all collaborators with their staffs and farmers of Jumla and SGRP, Guthichaur staff for their help in conducting the study.

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Nepalese Vet. J. 28: 124-127
Nepal Veterinary Association

Short Communication

SEROLOGICAL EVIDENCES FOR THE ABSENCE OF AVIAN INFLUENZA A VIRUS IN POULTRY IN NEPAL

G. R. Pant[1]

ABSTRACT

Highly pathogenic avian influenza has not been reported in Nepal to date. A serological survey for antibodies to avian influenza viruses was carried out in different farms of 5 districts of Nepal. A total of 64 serum sample were collected and these sera were tested by C-ELISA at Australian Animal Health Laboratory, Geelong, Australia for antibodies against influenza A viruses. None of the tested sera were found positive, thus giving scientific evidence for the absence of influenza A viruses in domestic poultry of Nepal.

INTRODUCTION

Avian influenza viruses are members of the *Orthomyxoviridae* family, which is divided into types A, B and C. Only influenza type A, causes disease in birds. *Influenza A viruses* have 15 haemagglutinin and 9 neuraminidase subtypes on the basis of antigens present on the surface of virus. All highly pathogenic avian influenza (HPAI) viruses are H5 or H7 subtypes but not all H5 and H7 viruses are highly pathogenic. Influenza viruses are inactivated by heating at 60°C for half an hour and also by acid pH. This virus is killed by oxidising agents, formalin and iodine compounds. Virus can survive for long periods in tissue and in water, remain infective in fecal material for as long as 30 days at 4°C and for 7 days at 20°C (Webster and Kawaoka, 1998).

Wild aquatic birds are natural reservoir of *influenza A viruses* and migratory birds play important role for the transmission of disease. Avian influenza viruses are highly contagious and are transmitted by direct contact with secretion from infected birds, especially from faeces, contaminated feed, water, equipment and clothing. Broken contaminated eggs may infect chicks in incubator. Apathogenic and mild pathogenic influenza A viruses occur in water birds worldwide.

[1] Central Veterinary Laboratory, Tripureshwor, Kathmandu

Clinical signs of HPAI in poultry are severe depression, inappetence, drastic decline in egg production, reduction in feed and water consumption, facial edema with swollen and cyanotic combs and wattles, petechial hemorrhage on internal membrane surface, redness of leg skin and sudden death with mortality up to100%. Severe congestion and hemorrhage of musculature as well as internal organ are prominent lesion observed on postmortem examination (Jordan and Pattison, 1998) Confirmatory laboratory diagnosis is essential to differentiate HPAI from Acute fowl cholera, New Castle Disease and Infectious laryngotracheitis. While virus isolation is the gold standard diagnostic test for HPAI, Agar gel immunodiffusion (AGID), Haemagglutination inhibition tests and C-ELISA are used for serological diagnosis and surveillance of this disease (World Organization of Animal Health, 2002).

Outbreaks of HPAI were reported in Thailand, Vietnam, Indonesia, Japan, Laos, South Korea, Cambodia and China in 2004, with over two hundred million of birds killed to control the infection in these countries. Thirty-one people have died due to bird flu in Vietnam and Thailand to date. Avian influenza has not been reported in Nepal and this country has maintained a HPAI disease free status till to date. The Department of Livestock Services of His Majesty's Government of Nepal has leading role to prevent the entry of HPAI by implementing prompt and strict preventive action when this disease appeared in several nearby countries. The approval of National Contingency Plan for Prevention and Control of Avian Influenza, 2060 (2004) is one of the major steps adopted by His Majesty's Government of Nepal. Laboratory facilities were also developed at Central Veterinary Laboratory (CVL) for the confirmatory diagnosis of HPAI and international linkage was established with OIE reference laboratories. Samples were collected from different districts and tested at laboratory to rule out the presence of any sero type of influenza A virus present in Nepal. This article is a report on the result of laboratory examination of the serum samples from Nepalese domestic poultry conducted at Australian Animal Health Laboratory (AAHL), Australia.

METHODOLOGY

Sixty-four serum samples were collected from chicken and duck farms located in Nuwakot, Banke, Lalitpur, Bhaktapur and Kapilbastu districts of Nepal. Out of 64 samples 10 were collected from ducks, 10 from layers and 44 from broilers. These sera were inactivated for 30 minutes at 56°C and sent to AAHL, Australia.

Table.2 Description of serum samples

District	Species/Type	Age	Date of collection	Number
Nuwakot	Poultry/Broiler	8 weeks	29/12/2003	30
Banke	Duck	2 years	30/10/2003	6
Lalitpur	Poultry/Layers	32 months	06/01/2004	10
Bhaktapur	Poultyr/Broiler	40 days	07/01/2004	14
Kapalbastu	Duck	9 months	30/10/2003	4
Total				64

Sera were tested in July 2004 by conducting C-ELISA at AAHL, an OIE reference laboratory to detect antibodies against influenza A viruses. Briefly, Nunc polystyrene, 96-well microplates are coated with purified influenza A virus. Test sera are diluted 1:10 and added to antigen-coated wells, immediately followed by a monoclonal antibody against influenza A virus nucleoprotein. The binding of the monoclonal antibody was detected by an anti-mouse horseradish peroxidase conjugate followed by tetra methyl benzidine as the chromogen. A serum was considered positive if the percentage of inhibition of monoclonal antibody binding was more than 50.

RESULTS AND DISCUSSION

The percentage of inhibition of all tested sera was less than 40, thus providing evidence of freedom from influenza A virus in domestic poultry in Nepal. As all influenza A viruses have antigenically similar nucleocapsid and matrix antigens, the competitive enzyme-linked immonosorbent assay (C-ELISA) can detect antibodies to all subtype of influenza A viruses. Although the number of tested sera was small it covered two species of birds from various geographical locations. This study provided similar results with our previous study conducted at CVL in February 2004 to detect antibodies against influenza A virus in 38 poultry sera. All of the 38 tested sera were negative in Heamagglutination Inhibition (HI) test using Avian Flu antigen and Avian Flu antiserum supplied by Central Veterinary Laboratory, England.

Testing was also done at the CVL in September 2004 to detect Influenza A virus using Avian Influenza Virus Antigen Test kit supplied by SD BIOLINE, Korea. Of the 29 cloacal swabs 20 were collected from Lalitpur, 8 from Taplejung and 1 from Kathmandu. All these tested samples were found negative, providing additional evidence for the absence of circulating influenza viruses in domestic poultry in Nepal.

HPAI was not observed in any farm in Nepal during active surveillance at the beginning of 2004 when 2 million birds on 670 organized poultry farms were inspected. The clinical evidence of this disease was also not seen in 100,000 of birds closely monitored in 41 districts at high risk. These districts were considered at high risk because of dense population of poultry and geographical location as they are very close to Wild National Park and bordered with India and China.

Poultry sector contributes 4% to Agriculture Gross Domestic Products in Nepal. Protection of growing poultry industry from HPAI is essential. Though HPAI has not been reported in Nepal however threat of this disease should not be neglected because of various risk factors. Evidence of HPAI outbreaks in Asian countries, free movement of migratory bird, live poultry market, mixed farming system, inadequate bio-security, evolution of influenza A virus and complex epidemiology are important risk factor to be considered for the control of HPAI in Nepal. Therefore testing of required number of samples from all ecological zones and risk areas for avian influenza is necessary to confirm the absence of avian influenza from Nepal and to identify which subtypes of influenza A viruses are present in Nepal.

ACKNOWLEDGEMENT

Authors would like to thank to staff of Central Veterinary Laboratory, Tripureshwor, Kathmandu, Nepal and Australian Animal Health Laboratory, Geelong, Australia for providing help and support for this study.

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Nepalese Vet. J. 28: 128-134
Nepal Veterinary Association

Short communication

OUTBREAK OF OTORRHOEA IN MID-EASTERN REGION OF NEPAL

S. Singh[1] & N. K.Yadav[2]

ABSTRACT

This study was conducted during outbreak of otorrhoea, reported around, Janakpur area during September-November, 2000, to determine the prevalence rate, microbiological involvement and response to different antiseptics and antibiotic therapy. The result of this study showed that only buffaloes were infected with this epidemic. Out of 622 buffaloes examined, 136 (21.86%) buffaloes were infected. Bacteriological examination revealed, Streptococcus sp. as most prevalent (42. 85%) followed by mixed infection (28.57%) of Staphylococcus sp. (21.42%) and Corynebacterium sp. (7.14%). The recovery time from treatment with different antiseptic and antibiotics revealed insignificant difference. Both treated and untreated animals recovered completely within 5-18 days. The economic losses, which occurred, was about 20-25% decrease in milk production and cost involved in treatment. Present paper reports the epidemiological status of otorrhoea, its symptoms, economic loss and response to different treatment regime.

INTRODUCTION

Buffalo farming is the integral part of Nepalese livestock industry. Buffaloes are multipurpose and economically the most important livestock species raised under the farming system of Nepal. They are the provider of milk, milk products such as Ghee (clarified butter oil) dung (manure and Fuel), meat and hides, draft power and also the reserve capital for farm households. Otitis externa, inflammation of external ear, occurs rarely in adult cattle but is frequent in adult buffaloes. Animals with otitis externa show the clinical signs which include otorrhea and frequent shaking and intermittent rotation of the head towards the affected side with apparent inflammation of external ear canal (Tyagi and Singh, 1993). Both ears are affected in most cases and there is a stinking, blood-stained discharged with patch alopecia below the ear. Perusal of the available literature didn't reveal any report on outbreak of otorrhea, its etiology and treatments in buffaloes from Nepal. Therefore, the present paper, reports the findings of an outbreak of otorrhea, recorded in the eastern region of Nepal.

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MATERIALS & METHODS

Study population

In this study, 1253 domestic animal (mainly ruminants) cases of different age groups were examined at district livestock service office (DLSO) from Dhanusha and Lohana village development committee of Dhanusha district. To evaluate the prevalence rate of otorrhea in buffaloes, secondary data from neighboring DLSOs like Mahottari and Sirha, were alos used.

Data collection

1253 animals of different age groups (622 buffaloes and rest others), which were admitted to the DLSO, Dhanusha, were examined thoroughly. Also with help of questionnaires, farmers, veterinarians and field practitioners were interviewed.

Bacteriological examination

The odorous discharges were collected aseptically from the ears of 14 infected buffaloes in sterile bottles, randomly. The collected materials were cultured at regional veterinary laboratory, Janakpur on appropriate media and microorganisms were identified on the basis of colony characteristics, growth pattern and Gram's reaction.

Treatment methodology

A total of 136 buffaloes found with otorrhoea were grouped on the basis of different treatments adopted.

- Group: A =Antiseptic washing of infected ear by 2% H_2O_2 solution daily for 10 days
- Group: B= Antiseptic washing of infected ear by 2% H_2O_2 solution daily once & local used of Mercurochrome 2% solution daily for seven days.
- Group: C = Local use of liquid Himax solution twice daily for seven days in infected ear
- Group: D = Local use of Mercurochrome 2% solution daily for seven days and parental use of antihistaminic (Zeet Inj n)@ 10ml I/M daily for 3 days.
- Group: E=Local use of Mercurochrome 2% solution daily for seven days and parental use of antibiotics, like
  * Bacipen (Ampicillin) Inj (2gm.), I/M daily for 3 days
  * Inclox (Ampicillin + Cloxacillin) Inj (2 gm.), I/M daily for 3 days

- Group: F=Local ear wash with Neem leaf extract twice daily for 10 days
- Group: G=Nine buffaloes were observed without giving any treatment, as controls.

RESULTS

Among the 1253 domestic animals examined, only buffaloes were found to be infected to this type of otorrhea outbreak. None of the other species like cattle, sheep, goat, dog, and swine were affected by this disease. Month wise prevalence of otorrhea at DLSO Dhanusha reveals the following figure :(Table 1).

Table 1: Prevalence of otorrhea from Sept-Nov. 2000, recorded at DLSO Dhunusha.

Month	No. of buffaloes examined	Cases of otorrhea	% of otorrhea
September	256	2	0.78%
October	216	74	34.25%
November	150	60	40.0%
Total	622	136	21.86%

Among the total buffaloes examined (622 buffaloes), 136 buffaloes (21.86%) were found with otorrhea especially occurring during Sept-Nov months.

CASES HISTORIES AND CLINICAL OBSERVATIONS

Mainly adult buffaloes were infected by this seasonal infection. The infection was transmitted from one buffaloes to other very quickly within 1-3 days. Infected buffaloes show following signs of dullness and depression with frequent shaking of head and ear. Bad stinking smell was prominent with many flies present inside and around the infected ear.

The treatment record in three neighboring districts also showed the high prevalence of this disease during the month of October Table 2.

Table 2: Prevalence of otorrhea in buffaloes in Terai districts.

Districts		Months		
		Sept.	Oct	Nov
Dhanusha	No.of buffaloes examined	216	209	215
	% Positive for otorrhea	6.01 (13)	38.7 (81)	18.1 (39)
Mahottari	No.of buffaloes examined	107	158	136
	% Positive for otorrhea	3.7% (4)	27.2% (43)	15.4% (21)
Siraha	No.of buffaloes examined	134	135	122
	% Positive for otorrhea	2.2 (3)	24.8 (38)	19.7 (24)

(Figures in parenthesis indicate number of cases)

Generally left ear was more infected but some time both ears were found affected. Firstly, serous discharge oozes out from the infected ear which later progress to form muco-purulent discharge. In few cases, bloody discharge or clot was found inside the ear. Vesicles appeared around the base of external ear after 3-5 days of bad smell and after 2-3 days of serous discharge. Vesicles were also observed in other anterior parts like neck, head and forelimb of the body. Temperature, respiration and pulse rate of the animals was normal. Animals were in appetence and milk production was reduced by 20-25%.

Microbiological findings

Relative frequency of different types of bacteria associated with otorrhea resulted from culture examination (Table 3).

Table-3: Bacteriological examination of otorrhea in buffaloes

Bacteria isolated	Positive number	%
Streptococcus sp.	6	42.85%
Staphylococcus sp.	3	21.42%
Corynebacterium sp.	1	7.14%
Mixed infection (Staph+Strepto)	4	28.57%
Total	14	100%

Streptococcus was the most frequently isolated organism (42.85%) followed by *staphylococcus* sp. (21.42%) and mixed infection of *Staph*. + *Strepto*. in majority of cases (28.57%) only 7.14% of the cases were infected with *Corynebacterium sp*.

Treatment response of otorrhea:

Treatment response to otorrhea was as follows (Table 4)

Table 4: Treatment response to otorrhea

Groups	Treatment adopted	No. of treated animals	Recovery time
A	Antiseptic washing of infected ear by 2% H_2O_2 solution daily for 10 days	15	10-12days
B	Antiseptic washing of infected ear by 2% H_2O_2 solution daily once & local used of Mercurochrome 2% solution daily for seven days	32	8-12 days
C	Local use of liquid Himax solution twice daily for seven days in infected ear	11	10-12 days
D	Local use of Mercurochrome 2% solution daily for seven days and parental use of antihistaminic (Zeet Inj^n)@ 10ml I/M daily for 3 days.	31	7-10 days

E	Local use of Mercurochrome 2% solution daily for seven days and parental use ofantibiotics, like *Bacipen (Ampicillin) Inj^{n}- (2gm.),I/M daily for 3 days *Inclox (Ampicillin+ Cloxacillin) Inj^{n} (-2gm.), I/M daily for 3 days	21	5-7 days
F	Local wash of ear with Neem leaf extract twice daily for 10 days	17	12-15 days
G	Buffaloes were observed without giving any treatment.	9	10-18 days
	Total	**136**	

Above table reveals that the antibiotic therapy leads to quick recovery (5-7 days) of otorrhea than other treatments. Also, both treated and untreated animals recovered within 5-18 days.

Epidemiological observations:

Otorrhea outbreak was mostly in hotter days of Sept-Oct. The outbreak of this disease seems to be supported by hot and humid climate. Mainly adult buffaloes werr Only buffaloes were susceptible to this disease and were infected. The disease caused no mortality but was of moderate morbidity.

DISCUSSION

Pseudomonas sp. is the etiology for otorrhea (Mouli, 1990). Also Proteus mirabilis, Pseudomonas aerogenosa, Corynebacteriom pyogens and E.coli are the causative factor for otorrhea (Dharesar and Pathak 1981). In the present study, the isolated organisms were Streptococcus sp. (42.85%), Staphylococcus sp. (21.42%), Corynebacterium sp. (7.14%) and mixed infection (28.57%) indicating the causative organisms responsible for outbreak of this disease. Regarding the treatment measures, the effective treatment of otorrhea was the use of antibiotics and local application of antiseptics, which led to recovery of the infection in a week suggesting it to be the best course of treatment. Also there was no significant difference among treated and untreated group, which lead to suspect that the etiology may also be ear mites, fungi or virus and gradual recovery may be due to development of immunity. So further study regarding etiology is needed. There may be the chance of developing to chronic infected leading to the infection of otitis media.

The reason behind the occurrence of otorrhea only in buffaloes might be due to their wallowing nature. Wallowing habit of buffaloes in pond and river is expected to increase the relative humidity inside the ear and thus a predisposing factor for high bacterial proliferation (Tyagi and Singh, 1993). This also supports the susceptibility of this otorrheal outbreak, during the hot and humid condition of Sept.-Nov. months in terai districts.

CONCLUSION

Otorrhea is only prevalent in buffaloes at hot and humid month of terai. Buffaloes have major role in farming system of Nepal.This disease lead to economic loss by decreasing milk production by about 20-25%. Thus the disease should be controlled and treated with antiseptics as well as antibiotics to save economic losses to the farmers and earlier recovery of infected animals.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. R. Ray (chief, DLSO.Dhunusha) Dr. U. P. Shah (Asst. vet.officer) for their assistance in this investigation work. The authors also extend their gratitude to Dr. I. P.Dhakal (Reader & Hospital superintendent, Vet. teaching hospital, Rampur), Dr. B. N. Devkota (Lecturer) and other teachers of IAAS for their help and co-operation, and appreciable gratitude goes to Dr. D. K. Singh (Lecturer) for his kind help, suggestion and criticism in preparation of this report.

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Nepalese Vet .J. 28: 135-137
Nepal Veterinary Association

Short communication

SARCOSPORIDIOSIS IN BUFFALO MEAT-A CASE REPORT

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ABSTRACT

Body muscles of male buffalo were received in the central veterinary laboratory from Hetauda revealed a large number of gray boiled rice like elongated cysts on physical examination containing over 15 cysts in 50 gm muscle sample. These cysts were tentatively diagnosed as Sarcosporidia. Histopathological study of the sample revealed the microscopic form of the cystic parasites diagnosed as Sarcocystis.

INTRODUCTION

Sarcosporidiosis is a lesion encountered during meat inspection. It is caused by an intracellular protozoan parasite, the *Sarcocystis*, a coccidian parasite. It occurs in both macroscopic as well as in microscopic form. The occurrence of either form depends on animal species in which infestation occurs. The parasite is cosmopolitan in distribution. It infects a wide range of hosts including mammals, reptiles and birds. It can rarely be found in human skeletal and cardiac muscle when humans are the intermediate or accidental host. Humans can also serve as the definitive host for this parasite after ingesting the cysts through raw or undercooked beef or pork. They cause lesions in all the tissues or organs but especially in the skeletal muscle and nervous tissue. These parasites can resist freezing but they are susceptible to drying. The parasites are also destroyed on adequate cooking.

In all countries where there have been surveys the infection prevalence is lower in cattle, sheep and horses but higher in swine. The major economic loss occurs with those Sarcocystis, which produce macroscopic cyst and meat condemnation. However, clinical disease associated with Sarcocystosis is getting more recognition. It is also known that infection depresses the growth rate. The species from dogs are very pathogenic in experimental work but rarely cause disease naturally. However, Dalmeny disease is now thought to be due to Sarcocystis infection.

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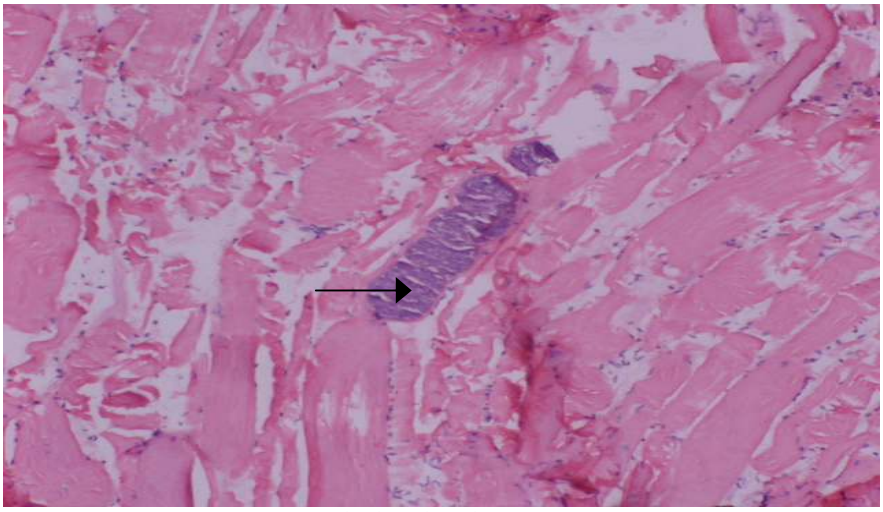
CASE HISTORY

As per information provided by supplier, the male buffalo was quite healthy on ante mortem inspection. But the meat procured from the buffalo was found to be heavily infected with Sarcosporidiosis during post mortem inspection of the carcass. Similarly, most of the buffaloes kept at the slaughterhouse were of weak and emaciated and meat procured from them did not show such an infection. The parasite had been found to be present frequently in the thigh, abdominal, neck muscles. The average size of the macroscopic form of the cysts studied was 6.3mm-1.46cm. They were found to be white or gray in color. The shapes of the cysts were recorded to be straight cylindrical to crescent.

Histopathological Examination

The thigh, abdominal, shoulder and the neck muscles were preserved in 10% neutral buffered formalin. These samples, after fixation, were trimmed off for desirable size, and treated in ascending grades of alcohols following overnight treatment in 50%, two hours in 70%, one hour each in 90% and 95% and absolute alcohol and two treatments in Propanol. The tissues were then kept in chloroform for clearing overnight and then impregnated in two wax baths having 60ºC temperature each for 1.5 hrs. After making the blocks through embedding with wax, they were cut into the section of 5µm thickness. Then the tissue sections were made into permanent slides through staining and mounting with DPX and examined after cooling under the microscope.



Sarcosporidiosis in buffalo meat

Fig 1: Sarcosporidiosis in the skeletal muscle of male buffalo. The solid arrow shows the presence of *Sarcocystis* in the muscle.

Microscopic study of the tissues (Muscles) revealed purple to blue colored cysts located intracellularly in the muscle fibers. The cysts were found to be round, oval, ovoid and elongated. The cysts were partitioned with incomplete connective tissue septa within which spherical spores were present in large number. The external covering of the cysts was thick and uniform. No inflammatory myositis was seen in the muscular tissues, rather myofibrils affected with Sarcocystis were found to be ruptured, probably due to the complete growth of these cysts inside the muscle cells.

DISCUSSION

The morphological features along with histopathological findings showed the cysts to be present in both macroscopic and microscopic forms and resembles to Sarcosporidiosis caused by Sarcocystis as described by Jubb and Kennedy (1963); Soulsby (1973); Radostitis et al (1994); and Herenda et al (2000). Further study based on questionnaire and personal communication to the butchers as well as with retailers reveal that the similar type of lesions in buffalo meat is not uncommon indicating the existence of source of infection, however, human cases of Sarcosporidiosis in Nepal has not yet been reported, probably due to inadequate reporting and recording system. Presence of sarcocysts in the examined meat sample warrants further and detailed epidemiological investigation on the subject to evaluate its prevalence and its risk to human population in Nepal.

ACKNOWLEDGEMENTS

I would like to thank Dr. Anjani Kumar Mishra, field veterinarian from Hetauda who provided me with the meat samples.

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