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Editorial

Livestock farming is an integral part of hills and mountain agriculture in Nepal. Almost every household maintains mainly cattle, buffaloes, goat and poultry and to a lesser extent sheep and pigs. These livestock provide milk, milk products, meat and eggs for human consumption and dung for manure. Oxen are used as draught power for ploughing and mules are used as pack animals for transporting consumers goods in the mountain. Wool provides even clothing to the family.

The sale of live animals for slaughter and / or draught power together with their surplus products - primarily milk and ghee provides farmers with a major source of cash income.

Livestock farming is closely interrelated with various components such as crop production, forest, pasture, market and household. During the past when human and livestock populations were not competing with arable land, adequate cultivated lands were available for crop production and sufficient greens, fodder and roughages were available for livestock. During that period, these components were supporting each other: livestock providing manure to the farms and the farms providing greens and crop residues to livestock. As the populations of human and livestock started increasing tremendously they began relentlessly destroying the forest and the pastures for survival and brought most of the marginal land under cultivation which consequently resulted in environmental imbalance there by increasing soil erosion, shortage of fodder and fuel wood supply.

Productivity per livestock unit is in continuous decline. The reasons being limited feeding resources, presence of large number of low producing animals, legal protection for cattle and female animals slaughtering, improper breeding and inadequate animal health services.

Animal health services have been effectively improved within last few years in the country establishing livestock service branch office and some service centres in each district. Attempt has been made to establish nine service centres in each district. Veterinarian and animal health technicians have been deputed in the field and everyone is devoted to extend their services. It is expected that the result of their devotion, dedication and contribution will be seen in the near future. Maintaining healthy and productive animals will definitely lead achieving the target of the Basic Needs.

Livestock is our national wealth. Let us save them and make them capable of producing more valuable animal proteins. Further more, livestock can support the national economy and can also contribute fulfilling the Basic Needs of Nepalese people.

Editor-In-Chief

EVALUATION OF AUTOGENOUS TOTAL TENDON GRAFTING IN BOVINE

* P. N. Sahay, L. L. Dass, A. A. Khan and Md. Ehsan

Summary

The study was conducted in twelve male buffalo-calves of about two years age. In nine animals autogenous total tendon grafting was done in severed superficial flexor tendon of hind leg and observations in terms of clinical, morphological and histological changes at 21, 28 and 42 days in these animals were recorded. In three animals, which acted as control, the severed tendon was left unrepaired for noticing spontaneous healing, if any. Severity of adhesion, gliding of the tendon within the sheath, impairment in the gait and weight bearing were reciprocal to the healing period in grafted animals. As the period of healing increased, normalcy returned. Histological study evidenced that graft acts as a template to bridge the gap and provides support for the new growth. In control animals, lameness and inability to bear weight persisted throughout and there was no evidence of union of the severed ends even by 42 days.

Introduction

Subsequent to injury, the functions of the tendons are impaired. As a result of severance a wide gap appears between the two cut ends of the tendons due to high contractibility of the muscles. The tendons of the leg are in a more vulnerable position and trauma to these from sharp objects is a frequent problem (Johnson and Lowe, 1974). In the absence of tendon repair, the animal is incapacitated as spontaneous healing of sheath covered tendon is not accomplished (Whittick, 1974). Hence there is apparent need to bridge the wide gap created in such circumstances.

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Tendon graftings have been extensively studied in canine and equine (Mason and Shearon, 1932; Potenza, 1964; Stromberg and Tufvesson, 1977) but there is paucity of evidenced work in bovine (Saikia and Nigam, 1976). In the present study, autogenous total tendon grafting has been evaluated on the basis of clinical, gross and histological findings in experimentally induced trauma of flexor tendons in buffalo calves. The technique employed would pave the way for its widespread application in field conditions.

Materials and Methods

The study was conducted on twelve clinically healthy buffalo calves of about two years age. The animals were divided in four groups of three animals each. In the animals of groups I, II and III, tendon transplantation was done in superficial flexor tendon and observations of various parameters were done on 21, 28 and 42 days, respectively. The IVth group acted as control, in the animals of which the severed flexor tendon was left unrepaired and observations made.

For creating the grafting bed in the tendon, the superficial flexor tendon was exposed aseptically under epidural anaesthesia by a curved cutaneous incision of 15 cm commencing below the hock on the medial aspect of leg. A longitudinal incision of about 8 cm was given on the sheath of the exposed tendon and the edges of former were separated and retracted. A 5 cm piece was excised from the sheathless tendon to prepare recipient bed. The portion of tendon thus obtained formed the grafting (autogenous) material.

Koch-Mason suture using 3/0 silk thread was applied to the upper and lower stumps of the recipient tendon and thread ends were left longer. Similarly, Koch-Mason suture was also applied on both the ends of the graft tendon which was then placed in the grafting bed. The free ends of the thread were tied at both the ends to secure the graft position. The retracted tendon sheath was then brought over the grafted tendon and its edges were sutured in continuous fashion using 4/0 silk thread. The skin wound was routinely closed. The metatarsal region of the leg was covered with medium plaster cast to achieve partial immobilisation of the grafted zone. During this procedure the limb was slightly flexed to preclude stress on tendon sutures. Streptopenicillin was injected daily for six days.

Clinical manifestations were recorded daily in the grafted as well as control group animals. Plaster cast was removed after 21, 28 and 42 days in groups I, II and III respectively and in one animal each on corresponding day from control group. Gross observations in terms of local reaction, adhesions and gliding function of the tendon were made after exposing the site of grafting. Subsequently, a piece of tendon from the junctional zone was taken and fixed in 10% formaline. Sections were stained with H and E stain for general histology and with Van Gieson's stain for collagen demonstration.

Results and Discussion

Clinico-morphological aspects:

There was rise in temperature, pulse and respiration in all the animals after a day of surgery but it was inconsequential and subsided in two days time. The animals preferred to sit with the extension of the operated limb, but normal sitting posture was observed by 18-20 days of grafting. Though hock and fetlock joints were not included in the plaster cast, this feature could be attributed to the temporary stress on the grafted tendon. As the union of host-graft tendon progressed, normalcy returned. Similarly, limping gait was conspicuous in all the animals for first 10 days and the animals were unable to bear weight on the operated limb. But by 17-21 days the animals of groups I, II and III exhibited normal gait and adequate weight bearing. Contrarily the animals of control group exhibited impaired gait and inability to bear weight till the period of observation. Since primary function of the tendon is to transfer muscle contraction to the skeletal movement (Braden, 1975) the early phase of gait impairment and lack of weight bearing is well explained in the grafted animals. In the control animals, persistence of such symptoms even by 42 days is suggestive that in absence of bridging the tendon gap, restoration of limb function is not feasible.

Application of plaster cast excluding the hock and fetlock joints was primarily aimed to provide partial immobilisation of the grafted zone and prevent suture disruption, at the same time to allow adequate circulation and gentle active movement. The later has been considered necessary (Pulvertaft, 1956) for satisfactory result in

such cases of grafting. Removal of plaster cast exhibited normal external appearance in all the animals except one in group I, where oedema around cutaneous sutures was observed. This could be due to exaggerated pressure of the plaster cast or mild infection.

On exposing the grafting zone, full gliding of the tendon within sheath was discernible in groups II and III. However in group I, it was not adequate. Gliding of the tendon within sheath was assessed by flexion and extension of the hock and fetlock joints and manual manipulation. Similarly, in the animals of group II and III there was no evidence of adhesion of tendon with sheath and of the sheath with surrounding structures. But in the animals of group I mild degree of adhesion persisted at both the locations. This suggested that by 1 days of grafting the gliding function of the tendon is not restored and there is persistence of adhesion. However, with the progression of healing time both these features were clinico-morphologically found satisfactory. The continuity of tendon in all the animals suggested accomplishment of the union.

In control group animals the adhesion of the sheath with cut ends of the tendon was pronounced. An exuberant growth of the fibrous tissue at the site of operation was also noticed. Careful removal of the adhesion revealed thin and small sheet of fibrous tissue extending from the superficial flexor tendon and inserting into the deep flexor tendon. However, such growth was lacking in the distal stump of the severed tendon. Since spontaneous healing of sheath covered tendon is not accomplished (Whittick, 1974) and feasibility of union of severed tendon without surgical aid is precluded.

Histological aspect:

The microscopic examination at 3 weeks (Group I) showed greater proliferation of fibroblast in the host tissue and some of the larger capillaries could also be located near the site of union. The host-graft union was histologically adequate. Proliferation of tendon cells and fibrils from the host tendon and connective tissue from the tendon sheath at both the ends of host-graft junction were apparent. The former had parallel orientation to the long axis while the later were horizontally

migrating at the site of union. In group II animals (4 weeks) the pattern of healing remained almost similar to group I, except diminution in the fibroblast proliferation from host tissue.

In the animals of group III (6 weeks) exaggerated proliferation of tendon cells and fibrills from host tendon were noticed which gave a feathering appearance. The line of union between host and graft was histologically complete and any demarcation was not feasible. Cellular infiltration and congestion of blood vessels were absent and thickening of the tendon sheath was very less.

These features suggested that by third week of autogenous total tendon grafting new tendon was formed from the organization of the scar of the host tendon and as the role of sheath as an uniting structure diminished, it assumed the gliding function. This observation is in corroboration of earlier finding in dog (Mason and Shearon, 1932). In homologous preserved tendon grafting in bovine the healing of tendon has been reported to be accomplished by longitudinal arrangement of developing fibrous tissue from the host tendon, paratendon and sheath (Saikia and Nigam, 1976). On the basis of the observations of this study it can be further postulated that the graft tendon acts as a template to bridge the gap and support for healing process without actively participating in the process of healing. However, revascularization of the graft through the formation of new vessels at the host graft junction for the survival of the graft (Urbaniak and Goldner, 1973) was also evidenced in this study.

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**SOME CLINICAL AND PATHOLOGICAL OBSERVATIONS ON CATTLE
SUFFERING FROM HAEMATURIA IN THE HILLS OF EASTERN NEPAL***

Dr. S. N. Mahato**

Summary

Some clinical and pathological aspects of bovine haematuria commonly encountered in cattle in the hills of eastern Nepal were investigated. The results of the study revealed that the condition was clinically characterised by intermittent chronic haematuria and progressive macrocytic normochromic anaemia. The haematuria was accounted for by haemorrhages from lesions in the mucosa of urinary bladder. The predominant histopathological lesions in the bladders of the 12 cases studied were transitional cell carcinoma in five, hyperplasia and invasiveness of transitional epithelium indicative of early malignancy in six, and degeneration and desquamation of the epithelium with prominent leucocytic infiltration in one. The findings suggested that the condition to be similar to the enzootic bovine haematuria (EBH) a noninfectious disease of cattle associated with prolonged consumption of bracken fern in forage.

Introduction

Red water (passing red urine) has long been recognised as a common problem in cattle in the hills of Nepal. It has previously been thought to be due to infection of *Babesia* species. The initial investigations of the condition carried out by Pakhribas Agricultural Centre, Dhankuta (PAC) revealed that the colouration

* The study was carried out at Pakhribas Agricultural Centre (PAC) as part of the Veterinary Investigation Programme in the Hills of Eastern Nepal funded by the Oversea Development Administration of the United Kingdom.

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of urine was due to haematuria and indicated that *Babesia* infection as aetiological factor to be unreal (Hopkins and Mahato, 1985; Mahato, 1986). The present work was undertaken to study the clinical and pathological changes in cattle suffering from haematuria, in the hills of eastern Nepal. A major difference between this and previous studies being that here the affected animals were kept under close observations until they died.

Materials and Methods

Six male and six female indigenous hill cattle with clinically apparent haematuria were purchased from farmers in Dhankuta, Terhathum and Taplejung districts. A thorough clinical examinations were carried out on arrival of the animals at PAC. Periodically temperature, pulse and respiration were recorded. The clinical history of the animals are presented in Table 1.

Urine samples were collected fortnightly and were examined for colour, pH, microparticles, protein and ketones. Occasionally, the samples collected aseptically from the animals passing pus cells in the urine were subjected to bacteriological examinations. The bacterial isolates were characterised and studied for their sensitivity to the commonly used drugs for urinary tract infections.

At an interval of fifteen days, blood samples were collected from the jugular vein in vacutainers containing disodium edetate as anticoagulant. The haematological examinations included total red blood cell count (RBC), estimation of haemoglobin (Hb) and packed cell volume (PCV), and total and differential leucocyte (WBC) counts. From the above, associated deductions, such as mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated.

When the animals died, post-mortem examinations were carried out. Although all organs were examined, particular attention was paid to the organs of urinary system. Pieces of urinary bladder fixed and preserved in 10 per cent buffered normal saline were processed to paraffin wax, then sectioned and stained with haematoxylin and eosin for histopathological studies.

Table 1. *Clinical History of the Haematuria Cases*

Animal No.	Description*	History as reported by farmers	Period of observation (days)	Approx. duration between onset of the disease and death
1.	Cow (9)	IH since two years (after second calving)	38	2 years
2.	Cow (12)	IH since two years (after third calving)	85	2 1/4 years
3.	Ox (15)	IH since three years	250	3 3/4 years
4.	Ox (10)	IH since two years	45	2 years
5.	Ox (10)	IH since a year	45	1 years
6.	Cow (10)	IH since a year (after second calving)	126	1 1/3 years
7.	Ox (10)	Haematuria since five weeks	16	7 weeks
8.	Ox (8)	IH since one and half years	22	1 1/12 years
9.	Cow (18)	IH since 10 years (after second calving)	24	10 years
10.	Cow (15)	IH since a year (after third calving)	14	1 year
11.	Cow (12)	IH since a years (after second calving)	23	1 year
12.	Ox (17)	IH since seven years	46	8 years

* Figures in parenthesis indicate age of the animal in years at the time of purchase
IH = Intermittent haematuria

Results

Clinical manifestations

The temperature, pulse and respiration were normal in all cases except for the animal No. 7. A moderate febrile reaction was observed in it. Intermittent chronic haematuria was observed as the main clinical manifestation. Intermission of haematuria ranged from five days to 90 days. Most of the animals died within three years of first recalling the symptoms of haematuria by the farmers. However, animal No. 9 and 12 survived for ten and eight years respectively whereas No. 7 survived only for seven weeks.

Changes in urine composition

The urine was alkaline. In the active cases the colour of urine varied from pale pink to bright red. Microscopic examination of such samples revealed presence of unhaemolysed red blood cells. Blood clots were occasionally present in the urine. Microhaematuria was detected in the inactive cases by microscopic examination of the urine sediment smears.

Large epithelial cells were present in the urine of nine animals. In the urine of two cases (Nos. 7 and 9) pus cells were also detected. Proteinuria was observed in almost all cases, the quantity of protein ranging from traces to 3g/l of urine.

Bacteriology of urine

Isolation of *Escherichia coli* and *Corynebacterium renale* was obtained from the urine of animal Nos. 7 and 9 respectively. Both the isolates were highly sensitive to Ampicillin, Strepto-penicillin, Oxytetracycline, Kenamycin and Septrin, and resistant to Urolucocil.

Haematology

The haematological values of individual animals are shown in Table 2.

Progressive macrocytic normochromic anaemia, as evident from high values for MCV and normal values for MCHC with lower values for RBC, Hb and PCV,

were seen. All the animals showed moderate to severe leucopenia, particularly lymphopenia with relative increase in eosinophils and monocytes. Neutrophilia was observed only in animal No. 7.

Gross pathology

The lesions were confined to the urinary bladder in all the animals while in animal No. 9 both kidneys and ureters were also involved showing lesions of suppurative pyelonephritis. The entire wall of urinary bladder was hard and thickened measuring 3 to 14 mm, except in a solitary case (No. 7) in which thickness was within the normal range of 2 to 3 mm. Neoplastic changes were seen in the form of mucosal hyperplasia in animal No. 11 and several red to blackish lentil to pea sized nodular elevations on the bladder mucosa in animal Nos. 1, 3, 4, 6 and 10. Cauliflower type polyps occupying almost the entire vesical cavity (Fig. 1) were encountered in five cases (Nos. 2, 5, 8, 9 and 12). In animal No. 7, the mucosal surface was inflamed and eroded.

Histopathology

Considerable hyperplastic changes in the tissues of the bladder wall with polypoid papillary growth of mucous membrane were present. Marked proliferation and cancerous transformation of transitional epithelium (Fig. 2 & 3) with ballooning of epithelial cells and hyperchromasia of their nuclei were observed in five cases (Nos. 2, 5, 8, 9 and 12). Infiltration of neoplastic epithelial tissues to deeper layers with lymphofollicular and fibroblastic reactions were noted in animal Nos. 1, 3, 4, 6, 10 and 11. Invasion of the transitional epithelium into muscular layers was seen only in animal No. 9 (Fig. 4). In all cases the capillaries in the submucosa were considerably distended, presenting an angiomatous appearance with multiple foci of haemorrhages.

In animal No. 7, no hyperplastic or cancerous changes were detected, instead there was degeneration and desquamation of the epithelium and prominent leucocytic infiltration.

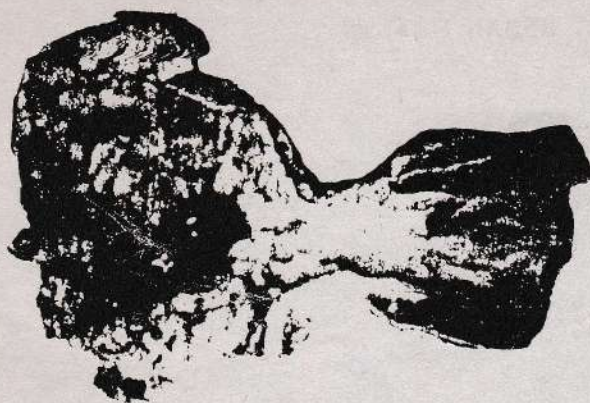


Fig. 1. *Cauliflower like growths on the mucosa of urinary bladder*



Fig. 2. *Section of urinary bladder showing carcinoma in the wall*

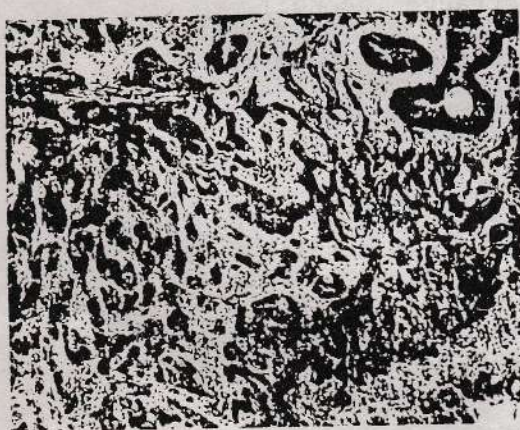


Fig. 3. *Section of urinary bladder showing hyperplasia and tumorous transformation of transitional epithelium cells.*

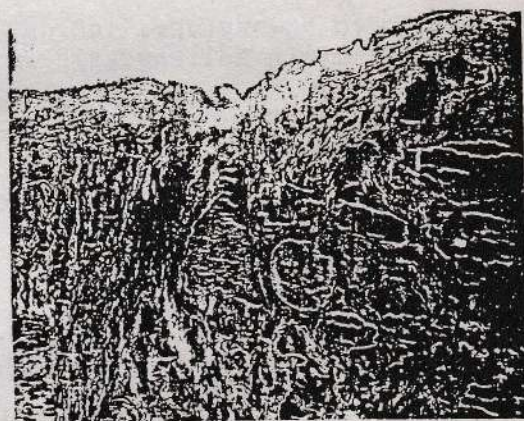


Fig. 4. *Photomicrograph of the haemorrhagic area of urinary bladder showing transitional epithelium infiltrating into muscular layer.*

Table-2 Haematological Results of the Individual Animals.

[illegible]

Figures under A and B indicate initial and final values respectively and in parenthesis indicate mean $\pm$ standard deviation

* The animal was under observation for 14 days only. During this period only one sample was taken.

*** Values estimated in sixty animals (Mahato, 1985)

Discussion

The observations reported in this paper support the previous findings (Hopkins and Mahato, 1985; Mahato, 1986) suggesting that the bovine haematuria, a problematic disease in the hills of eastern Nepal, is predominantly a cancerous condition of the urinary bladder. The clinical, haematological and pathological findings of the condition appear to be similar to those of enzootic bovine haematuria (EBH).

EBH, a chronic, non-infectious disease of cattle, associated with prolonged consumption of bracken fern in forage has extensively been studied and reported from various parts of the world (Dutta, 1953; Pamukcu, 1955; Smith and Beatson, 1970; Mc Kenzie, 1978; Rajendra *et al*, 1979). On the basis of experimental findings, Mc Crea and Head (1981) reported that a haemorrhagic cystitis is seen in the initial stage of the disease, and only later a true bladder neoplasm developed. Animal No. 7 may, therefore have died during the early stage of EBH.

Various types of tumours of urinary bladder, such as transitional cell carcinoma, papilloma, adenoma, adenocarcinoma, squamous cell carcinoma, fibroma, haemangioma and haemangio sarcoma have been reported in cattle affected with EBH (Pamukcu, 1957); Mugeru *et al*, 1969; Pamukcu *et al*, 1976; Rajendran *et al*, 1979; Nandi, 1982). In this study, however, only transitional cell carcinoma was observed which might be due to limited number of cases examined. The hyperplastic changes of the epithelium noted in all cases were characteristic of an early developing tumour. Pamukcu *et al*, (1976) interpreted epithelial hyperplasia as representing an initial change of vesical tumour and postulated the possible alteration from the epithelial hyperplasia into papilloma and further into various types of cancerous proliferation.

Due to limited facilities in Nepal, cattle passing red coloured urine have received little investigation; thus the known cause had been attributed to Babesiosis. However this study provides support for the conclusion that most of the cases frequently attributed to Babesiosis could be EBH.

Acknowledgements

The author is grateful to the staff of Pakhribas Agricultural Centre, Dhankuta, especially Shri P. P. Rai, Shri Kisan Rai, Shri P. B. Thapa, Dr. L. L. Swift and Dr. T. R. Neupane for their invaluable assistance in this study. The help by Dr. B. K. P. Shaha, Central Animal Health Centre, Kathmandu in histopathological study is acknowledged. ODA of UK, the funding agency is also acknowledged.

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A REPORT ON CLINICAL CASE OF TRYPANOSOMIASIS IN DOG

Dr. S.P. Gautam and Dr. N.P. Ghimire*

A two years old locally bred male Tibetan Mastiff dog weighing about 27 kg. belonging to Fr. Watrin of Thamel, Kathmandu was admitted to Kathmandu Veterinary Clinic, Maharajgunj on 5 September 1988 with a history of gradual loss of appetite and health.

Clinical examination revealed dullness, depression, unwilling to move and stand, lethargic, and lying on the ground resting the head in between the knees. Rough hair coat with patchy alopecia, dry and scaly skin, corneal opacity of both eyes, oedema of dependent parts mainly scrotum, legs, ventral abdominal areas were the prominent symptoms observed (Fig 1). Severe anaemia was indicated by bleached and pale oral and ocular mucosae. Auscultation revealed tachycardia with thumping heartbeat, deep and rapid respiration. The animal was hyperthermic (104°F).

Faecal sample and blood sample were collected for further laboratory investigation. The dog was treated symptomatically for anaemia using Imferon 2 ml I/m and a course of Ampicillin 500 mg and Livogen 2 ml I/m was instituted immediately to check the secondary infection and anorexia.

Laboratory examination of faecal sample shown absence of any parasitic eggs or larvae or segments. Direct examination of wet thick and thin blood smears collected from radial vein which was very thin and watery revealed heavy jerking and swirling movement on 10x and 45 x power of microscope indicating presence of some haemoprotezoan

* Veterinary Officers; Biological Products Division, Veterinary Complex, Tripureswor, Kathmandu.



Fig. 1. *Trypanosoma* infected dog before treatment.

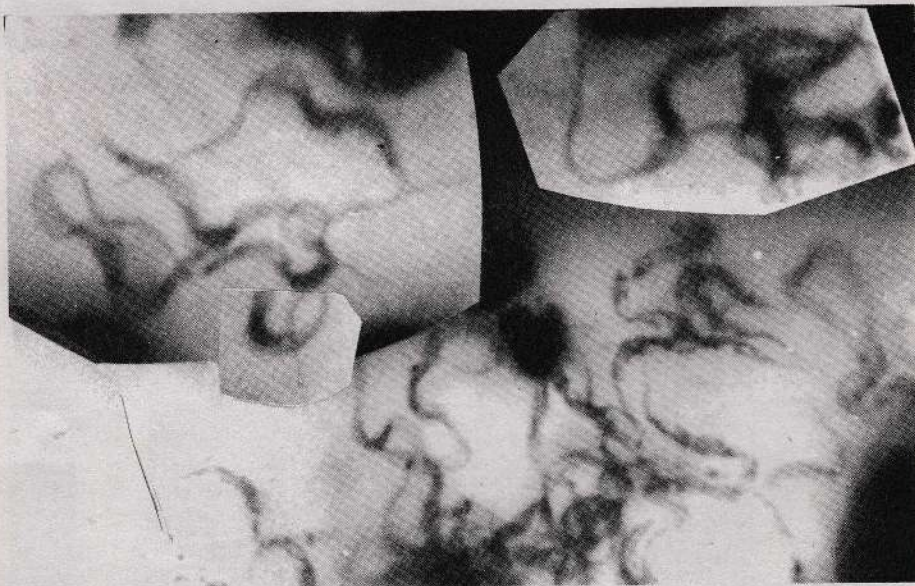


Fig. 2. *Trypanosoma evansi* in groups, photomicrography of Giemsa stained blood smear.

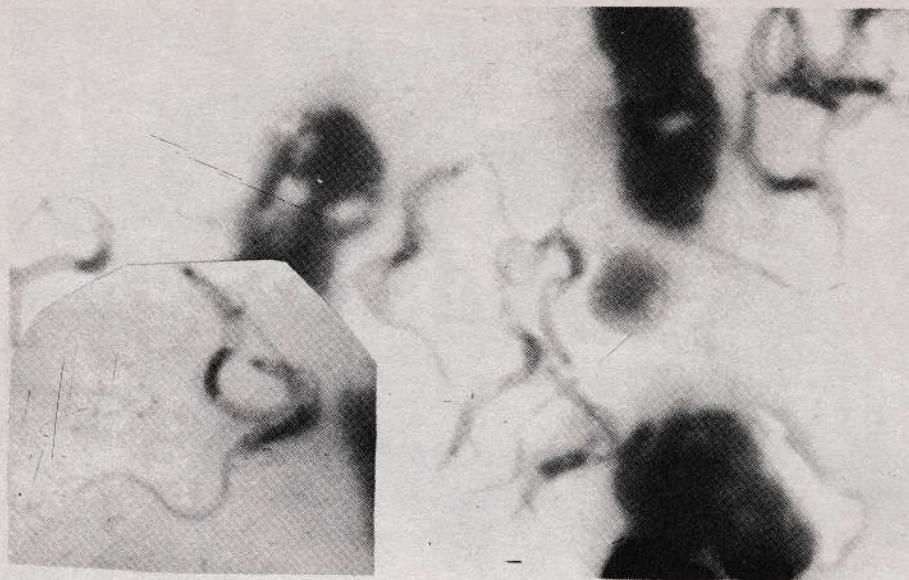


Fig. 3. *Trypanosoma evansi* showing undulating membrane kinoplast and flagella on photomicroscopic view.



Fig. 4. *Trypanosoma* injected dog after treatment and recovery.

infection. Further routine haematological examination of blood sample collected on EDTA revealed severe anaemia (Haemoglobin 4 mg/ml), high ESR value, total erythrocyte count - 2 million/cumm, total leucocyte count - 4 thousands/cumm.

The microscopic examination of blood smear stained with Giemsa's stain showed high eosinophilic count and bunch of polymorphic, slender, cresentically elongated parasites. The parasite, possessed pointed terminal ends, well developed undulating membrane, terminal kinetoplast, centrally located nucleus and a long free flagellum (Fig. 2 & 3). Thus, morphologically the parasite resembled to *Trypanosoma evansi* as described by different authors (Soulsby. 1983; Merchant and Barner, 1973.)

After the laboratory confirmation of *Trypanosoma* infection the dog was treated with Berenil (Diminazene aceturate) at the rate of 3.5 mg/kg. body weight by deep I/M route. As a supportive treatment for anaemia and anorexia, Imferon 2 ml I/M, Livogen 2 ml I/M and oral glucose therapy were continued for four days. Blood samples were examined for 3 consecutive days but no parasite was detected after 24 hours of Berenil therapy. The animal recovered gradually and photograph taken after fourth day showed the animal bright and lively (Fig 4.)

Discussion

Trypanosomiasis, caused by *Trypanosoma evansi*, a haemoprotozoan parasitic infection of almost all domesticated animals especially fatal to horse, camels and dogs, is prevalent in Indian subcontinent. It is characterised by intermittent fever, anaemia, oedema and urticarial rashes. In more acute form of disease in canines, parasitemia develops within 7 - 10 days with marked oedema, corneal opacity, rapid ematiation, hoarse voice due to laryngeal oedema sometimes confused with rabies and death within 2 - 4 weeks of infection (Soulsby. 1983). In buffalo, cattle and pigs it is normally nonpathogenic and often form reservoir of infection (Siegmund *et. al.*, 1973; Merchant & Barner 1973). The disease can be transmitted mechanically by intermittent feeding of blood sucking flies and mosquitoes and is more common during and following monsoon season. Carnivores can also be infected by eating the flesh from infected animals (Siegmund *et. al.*, 1973).

In Nepal, the disease has been observed among elephant at Gokarna National Park during the year 1983, in horses of Balaju purchased from India (Mishra, 1988), in Holstein crossbred cattle of Chabahil area immediately after their arrival from Punjab, India during July 1988, (Basnyat *et. al*; 1988) and in dogs at different occasions and places.

In Kathmandu Veterinary Clinic, Maharajgunj, Kathmandu 100 blood samples were collected from different breed of dogs, out of which 10 cases were found positive for *Trypanosoma* infection. It is interesting to note that all of the positive cases were from Tibetan Mastiff breed alone. Since not much work has been done in this field, it is suggested to carryout the further detailed epidemiological study of the disease in this country.

Acknowledgement

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THE INCIDENCE OF ETHMOID CANCER - A REVIEW

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Summary

Carcinoma of ethmoid is an emerging neoplastic condition affecting the domestic animals and captive wild animals. Though first reported from Scandinavian countries, now it is prevalent in South India in an endemic form. Affected animals first show nasal discharge which gradually develop dyspnoea, snoring, exophthalmos and bulging of the forehead. Aetiology is not yet known. Among various methods so far employed, exfoliative cytology technique was found best to detect the early cases. Histologically the tumour was epithelial in origin. The affected animals are immunologically deficient in advanced stages of the tumour growth. The neoplastic cells could neither be grown in tissue culture nor the condition could be reproduced in the experimental animals. Endoxan Asta was found best effective chemotherapeutic agent. There is still a long way to understand the various important aspects like aetiology, behaviour of the neoplastic cells and proper control measures.

Introduction

Tumours and cancers are known since antiquity. In the domesticated animals, three tumour conditions affect the head and neck regions, i. e. horn cancer, eye cancer and the ethmoid cancer. Of these, the former two have been studied in their various aspects. The clinicopathological features, symptomatology and aetiological factors have more or less been established. The immunological status of the affected animals have been monitored as a result the incidence has been minimised.

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Tumour arising from the mucosa of the ethmoturbinate region is an important neoplastic condition affecting different species of the domestic animals. It was first reported by Moussu (1906) from Sweden; followed by Stenstrom (1915). Since then, the incidence of this tumour has been reported from different parts of the world, although, it is no longer encountered in Scandinavian countries. Similar conditions have been reported by Pospischil *et al.* (1969, 1982) from Germany. It was first reported in India by Muthappa in 1930 in Madras State. Subsequently the prevalence of this tumour has been reported from Kerala, Tamilnadu, Andhra Pradesh, Karnataka and Orissa. Recently the incidence has increased considerably. Cross-bred animals at the peak of production are found more affected. Therefore this neoplastic condition is of considerable economic importance for the livestock owners.

Epidemiology

Carcinoma of the mucosa of the ethmoid has been reported in cattle, buffaloes, goats, pigs, monkeys, and captive wild animals. Among domesticated animals, high yielding cross-bred animals at the stage of their second or third trimester of pregnancy or at the peak of lactation were found more affected, though non-descript animals were also found affected. There is no breed specificity or species barrier for this tumour. The highest incidence was found in the age group of 6-9 years followed by above 9 years and lastly 3-6 years. Season has no direct aetiology on the incidence of this tumour. There is no significant difference in the area wise distribution.

Symptomatology

Initial clinical features manifested by the tumour bearing animals are profuse nasal discharge which is mucus in nature at first and mixed with blood later. In certain instances there may be intermittent epistaxis. In the advanced stages of tumour growth the animals show dyspnoea manifested by mouth breathing, snoring and abdominal type of respiration. Finally unilateral or bilateral exophthalmos develops. The ailing animals manifest circling movements, cachexia, bulging of the forehead and rarefaction of the frontal bone. Swelling of the parotid, submaxillary and retropharyngeal lymph nodes is often observed.

Aetiology

An understanding of the aetiological factor is essential for the effective therapy. During the last several years studies have been carried out by various workers to

locate the possible agents associated with this tumour. Possibility of virus association was attributed by Sulochana (1980). Serum of the tumour bearing animals was found containing antibodies against New Castle Disease Virus (NDV). Chicks reared with tumour bearing and challenged by NDV were found resistant. Nair (1980) observed virus like particles in the ultrastructures of the neoplastic cells. Attempts to reproduce the condition with tumour extract failed in all experimental animals. These observations ruled out the possibility of virus alone as a causative factor. Studies on genetic predisposition and environmental factors were also not fruitful. Rajan *et al.* (1972) attributed mycotoxin as a promoting factor for the ethmoid tumour but this also could not be proved.

Clinico-pathology

Anaemia, moderate leucocytosis and eosinophilia with occasional neutrophilia have been observed in all the cases of ethmoid carcinoma (Nair 1973, and Sreekumaran 1980). Decrease in serum phosphorus with low calcium level are frequently observed in animals with rarefaction of the frontal bone (Sreekumaran and Rajan 1982). Increased serum LDH activity in advanced cases is significant. CSF reveals slight lymphocytic pleocytosis.

Diagnostic Tests

Exfoliative cytology: This method is best suitable for early screening of the suspected animals. The exfoliated cells in the nasal discharge show hyperchromatic nucleus with clumping of the chromatin, anisokaryocytosis and anisocytosis. Cytoplasm is occasionally vacuolated. Vijayan and Rajan (1982) perfected this technique and formulated the criteria for early diagnosis of the neoplasm.

Ehrlich test: Rajan and Vijayan (1981) employed this test. When the Ehrlich's reagent is added to the plasma of the animals to be tested, immediately a dense white precipitate is formed. During incubation in water bath, the colour of the suspension becomes purple blue and more darker colour is observed in cancer positive animals.

Mucus block technique: Vijayan and Rajan (1982) employed this technique. Mucus was collected by scoup and by aspiration to prepare paraffin blocks. Hematoxylin and Eosin

stained sections were examined under microscope. The squamous and columnar epithelial cells suggesting adenocarcinoma and squamous cell carcinoma were seen. In some cases pseudoalveolar pattern was evident. Occasionally cells in mitosis were also seen. Infiltrating lymphocytes and macrophages were frequent.

Radiography: X-ray plates of the head of the tumour bearing animals at the lateral view were found best to detect the tumour growth.

Pathological Features

Gross Pathology: The growth was seen originating from ethmoid mucosa. This extends and fills the frontal, sphenopalatine and maxillary sinuses. In some cases it extends upto orbital cavity, destroys lamina cribrosa and enters the cranial cavity. The tumour mass is fleshy, cauliflower like firm and has focal areas of necrosis, suppuration and cystic degeneration. There is rarefaction of the frontal bone. Metastasis are common in the regional lymph nodes and occasionally in the liver. In the later stages, atrophy of the spleen may be observed.

Histopathology: Histologically the tumours are epithelial in origin. The most common histological types encountered in bovines are adenocarcinoma, squamous cell carcinoma and undifferentiated carcinoma. In some cases transitional cell carcinoma, osteoma, myxosarcoma and rarely fibro-myxo-chondro osteoma, myxo-chondro-osteosarcoma, histiocytic tumour, malignant lymphoma, reticulum cell sarcoma and fibro sarcoma were reported.

Ultrastructurally the cells were found either well differentiated secretory structures or undifferentiated or differentiated squamous epithelium. There was great variation in the size and shape of the nucleus. The nucleus had irregular membrane and had more than one nucleoli. The mitochondria were swollen. The rough endoplasmic reticulum was dilated and contained flocculent electron dense material. Structures were very much similar to the virus particles seen in the neoplastic cell.

Immunological Findings

It has been found that the animals which are in advanced stages of tumour growth are immunologically deficient (Reddy and Rajan 1982, Sulochana *et al.* 1982, Sreekumaran and Rajan 1982, and Reddy and Rajan 1983a, 1983b), and show reduced

CMI response. The lymph nodes revealed stimulation of T-cell dependent areas in response to tumour antigen. BCG was found better immunostimulant than the tumour antigen. Higher rate of peripheral blood lymphocyte blast transformation was observed with tumour antigen and better immunostimulation with BCG in the vaccinated animals. Macrophage activity was much less in tumour bearing animals than those of the controls.

Propagation and Transmission

Several attempts to cultivate the cells of ethmoid carcinoma in the laboratory have produced not much satisfactory results. All the methods and media, employed for culture did not help the cells to grow. However, Sulochana (1980) and Karki (1985) could grow the cells in monolayer for ten days. Subsequently clumping and rounding of the cells started and the cells died out. Features of cytopathic effect (CPE) appeared in the monolayer.

The tumour condition could not be reproduced in the laboratory animals even several routes and methods were employed. Autologous, homologous, and heterologous animals either with or without immunomodulation did not show any tumour growth. However, in homologous transplantation the animals developed growth upto three weeks. Histologically the growth was a granulomatous reaction showing proliferating neoplastic cells in the tissue section.

Treatment

The survival period of the tumour bearing animals was significantly increased when vaccinated with tumour extract during the early stages. The chemotherapeutic effect with ENDOXAN-ASTA* (Cyclophosphamide) was found satisfactory when given in early stages.

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SEASONAL VARIATION OF CERTAIN MILK CONSTITUENTS OF HARYANA COWS AND MURRAH BUFFALOES AT IAAS LIVESTOCK FARM, RAMPUR

Dr. Nagendra P. Sah*

Summary

An experiment was conducted to evaluate the seasonal variation in specific gravity, fat, SNF, protein and TS contents of Haryana cows and Murrah buffaloes milk at the IAAS livestock farm, Rampur (Chitwan), during 1983-84. The monthly values of these constituents were determined for a period of nine months. The average values of specific gravity, percentages of fat, SNF, protein and TS were 1.0294, 4.95, 8.34, 3.56, and 13.88 respectively for Haryana cows milk and 1.0295, 7.19, 8.72, 3.60 and 16.06 respectively for Murrah buffalo milk.

Introduction

The composition of milk varies throughout the year. These variations have become increasingly important to the producers. The increased emphasis now being placed on the solid-not-fat (SNF) and total solid (TS) portions of milk which make desirable to know more about factors that cause variations in these constituents in the milk of individual cows. Determination of specific gravity, percentage of fat, SNF, protein and TS contents are essential to know nutritional and qualitative standard of milk.

Several workers (Bartlett, 1934; Das and Sharma, 1977; Davis and Harland, 1974; Johnson, 1961; Nickerson, 1960 and Reder, 1938) have indicated that various environmental factors have influenced on the composition of milk. Nutrients content of the feed has

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also influenced the composition and seasonal effect on the fat and SNF percentage of milk have been reported by Johnson (1957) and Specht (1956). They have found the percentage of fat and SNF in milk to be lowest in summer months and highest during winter months. Johnson (1957) reported the percentage of fat and SNF of 152 Holsteins to be 3.34% and 8.58% respectively. Again Johnson (1961) indicated that breed and stage of lactation have significant effect on variation of fat, SNF and TS contents of cows milk.

Studies on protein content of milk in cross breeds have been reported by Srivastava *et al.* (1978). They reported the protein content of Haryana x Holstein Friesian, Haryana x Brown Swiss and Haryana x Jersey to be 2.85 ± 0.03 , 2.95 ± 0.04 and 3.00 ± 0.04 respectively.

Peterson (1950) reported fat, SNF and TS content of Jersey cow to be 5.36%, 9.40% and 14.80% respectively.

Basu *et al.* (1962) estimated the constituents of milk of tropical cows to be 4.7% fat, 8.7% SNF and 13.6% TS. Mahanta (1974) studied various constituents of milk of European (*Bos taurus*) and Zebu (*Bos indicus*) cattle and reported the percentage of fat as 3.37 and 4.97; SNF as 8.90 and 8.46 and TS as 12.65 and 13.45 respectively.

Baruah (1972), as reported by Das and Sharma (1977), estimated the specific gravity, percentage of fat, SNF and TS to be 1.0304, 4.7, 9.10 and 13.79 respectively in indigenous cows of Assam and 1.0270, 4.5, 8.55 and 13.20 respectively in Jersey x Indigenous F_1 cows.

Variation in quantity and composition of milk as affected by climatic factors are of much importance in choosing the breed suitable for a region. Literatures on the performance of Haryana cows and Murrah buffaloes are limited in Nepal so far the milk constituents are concerned. Because of this fact variation in milk constituents of Haryana cows and Murrah buffaloes was chosen for the present study.

Materials and Methods

This study is concerned with the variation in the constituents of milk from individual pure breed Haryana cows and Murrah buffaloes at Rampur, Chitwan. Milk

samples from seven pure breed Haryana cows and nine pure breed Murrah buffaloes were collected for the present investigation. The numbers of experimental Haryana cows and Murrah buffaloes were identified and same lactating animals were used throughout the study. The age of the cows varied from their first to fourth lactations whereas buffaloes were all from third lactation. These experimental animals were maintained under similar feeding and managerial conditions. Samples of milk were taken from each cow on the first day and from buffaloes on the second day of each Nepali month for nine months of lactation period.

Specific gravity of milk samples were determined by using specific gravity bottles (Lactometer), the percentage of fat by Gerber's method and percentage of SNF and TS by using Richmond's formula as described by Rai (1980) and total nitrogen was determined by Kjeldahl method. The total nitrogen percentage was multiplied by the factor 6.25 to obtain the percentage of equivalent protein.

During the entire period of investigation the daily air temperature and relative humidity were recorded. The average air temperature was lowest in Poush and Magh and highest in Baisakh and Jestha. The average temperature in Poush, Magh, Baisakh, and Jestha were 16.66°, 14.67°, 27.81°, 28.76°C and the relative humidities were 90.8, 94.25, 43.97 and 75.42 respectively.

Results and Discussion

The average monthly recordings of milk yield, specific gravity, percentage of fat, SNF, TS and protein composition in milk of Haryana cows and Murrah buffaloes are presented in Table - 1. The data show that the most widely variable constituent of milk was butterfat, followed in order by TS, SNF and protein. In general, it was noted that when the butterfat content increased, there was a corresponding rise in TS, SNF and protein contents. Again, in most cases an inverse relationship was noted between milk production and TS, SNF and butterfat percentages of milk.

There is no report on variation of these characteristics in Haryana cows and Murrah buffaloes to compare with the present findings. However, these findings support the results of previous investigators who conducted experiments on indigenous cattle and their crossbreds. The mean values of fat, SNF and TS in indigenous cattle reported by Das

Table - 1. Average Values for Milk Constituents of Haryana Cows and Murrah Buffaloes.

	Month	Milk Yield (L/day)	Sp. gr.	Fat %	SNF %	TS %	Prot. %
H A R Y A N A C O W S	Ashwin (Sep-Oct)	1.46	1.0296	6.56	8.86	3.27	15.42
	Kartik (Oct-Nov)	1.67	1.0299	4.03	6.86	3.78	12.57
	Mansir (Nov-Dec)	2.38	1.0290	5.27	8.45	3.73	13.73
	Poush (Dec-Jan)	3.80	1.0237	5.92	8.82	3.70	14.74
	Magh (Jan-Feb)	4.00	1.0308	5.60	9.31	3.34	14.91
	Falgun (Feb-Mar)	3.83	1.0311	5.25	8.97	3.72	14.22
	Chaitra (Mar-Apr)	3.03	1.0311	3.50	8.73	3.20	13.01
	Baisakh (Apr-May)	2.30	1.0296	4.10	8.49	3.44	13.33
	Jestha (May-Jun)	2.23	1.0298	4.35	8.53	3.86	13.02
	Ranges	1.46 to 4.00	1.0237 to 1.0311	3.50 to 6.56	6.86 to 9.31	3.20 to 3.86	12.57 to 15.42
	Over all average	2.74	1.0294	4.95	8.34	3.56	13.88
M U R R A H B U F F A L O E S	Ashwin (Sep-Oct)	2.51	1.0299	6.48	8.99	3.46	15.47
	Kartik (Oct-Nov)	2.40	1.030	6.83	9.01	3.58	15.84
	Mansir (Nov-Dec)	3.00	1.0278	6.37	8.53	3.35	14.90
	Poush (Dec-Jan)	2.16	1.0285	8.80	8.97	3.56	17.88
	Magh (Jan-Feb)	1.99	1.0292	8.85	8.89	0.59	17.74
	Falgun (Feb-Mar)	2.00	1.0277	6.96	8.46	3.50	15.41
	Chaitra (Mar-Apr)	1.61	1.0321	7.15	9.59	3.59	16.74
	Baisakh (Apr-May)	2.15	1.0283	6.37	8.54	3.91	14.91
	Jestha (May-Jun)	2.30	1.0320	6.86	8.53	3.82	15.69
	Ranges	1.61 to 3.00	1.0277 to 1.0321	6.37 to 8.85	8.85 to 9.59	8.53 to 9.01	14.90 to 17.88
	Over all average	2.34	1.0295	7.19	8.77	3.59	16.06

and Sharma (1977) were 4.91, 8.83 and 13.19 respectively which are in accordance with the present findings. Similarly the percentage of fat, SNF and TS in Zebu cattle reported by Mahanta (1974) were 4.97, 8.4 and 14.45 respectively, also support the present investigation. Again the present findings are also comparable with Basu *et al.* (1962) who have conducted experiments on tropical cows. Table - 1 further indicates that the value for SNF and fat percentage in the milk of Haryana cows and Murrah buffaloes is higher than the reconstituted milk which has 8.0 per cent SNF and 3.0 per cent fat in Nepal.

The specific gravity of milk of indigenous cows of Assam reported by Baruah (1972) to be 1.0304, which is in agreement with the specific gravity of milk of Haryana cows. The specific gravity of Haryana cows milk (1.0294) was found to be slightly higher than Jersey (1.0290) and Red Dane cows (1.0291) as reported by Baruah (1972).

Haryana cows milk showed higher protein content (3.56%) than that of cross breeds - Haryana x Holstein Friesian (2.85%), Haryana x Brown Swiss (2.95%) and Haryana Jersey (3.00%) as reported by Srivastava *et al.* (1978).

Table-1 also shows the monthly average percentage of fat in both the cases was higher in Poush and lower in Baisakh and Jestha. The effect of feed and environmental temperatures on the percentage of fat in cow's milk has been reported by numerous investigators (Birtlett, 1934; Davis and Harland, 1947 and Johnson *et al.*; 1961). The data reported herein indicate that the higher temperature in summer is responsible for the lower fat content in the milk of Haryana cows and Murrah buffaloes.

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USE OF BLOOD GASTRIN IN THE DIAGNOSIS OF OVINE HAEMONCHIASIS*

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D.E. Jacobs and D.R. Adikari**

Introduction

Infection with the abomasal nematode, *Haemonchus contortus*, is an important cause of impaired weight gain in sheep maintained under tropical and subtropical conditions. Experimental studies have indicated that this loss in production is associated with the blood-sucking activities of the parasite and an impairment in the efficiency of abomasal digestion caused by a drop in hydrochloric acid production. The later results in a marked elevation in stomach pH and an accumulation of non-activated pepsinogen. There is also an increase in permeability of the abomasal mucosa which allows pepsinogen to pass from the abomasal lumen into circulation. Blood levels are significantly elevated within a week of infection making pepsinogen a useful tool in the diagnosis of abomasal parasitism. However, the methods currently employed in measuring pepsinogen levels require considerable experience and are both time-consuming and relatively inaccurate compared with other potential assay methods.

Recent work at The Royal Veterinary College has demonstrated a marked increase in circulation levels of the gut hormone, gastrin, in association with the abomasal nematode, *Ostertagia ostertagi*, in cattle (Fox et al, 1987). The aims of this investiga-

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tion were (i) to monitor changes in pepsinogen and gastrin values in lambs exposed to *Haemonchus* infection; and (ii) to evaluate the use of blood gastrin as a tool in the diagnosis of ovine haemonchiasis.

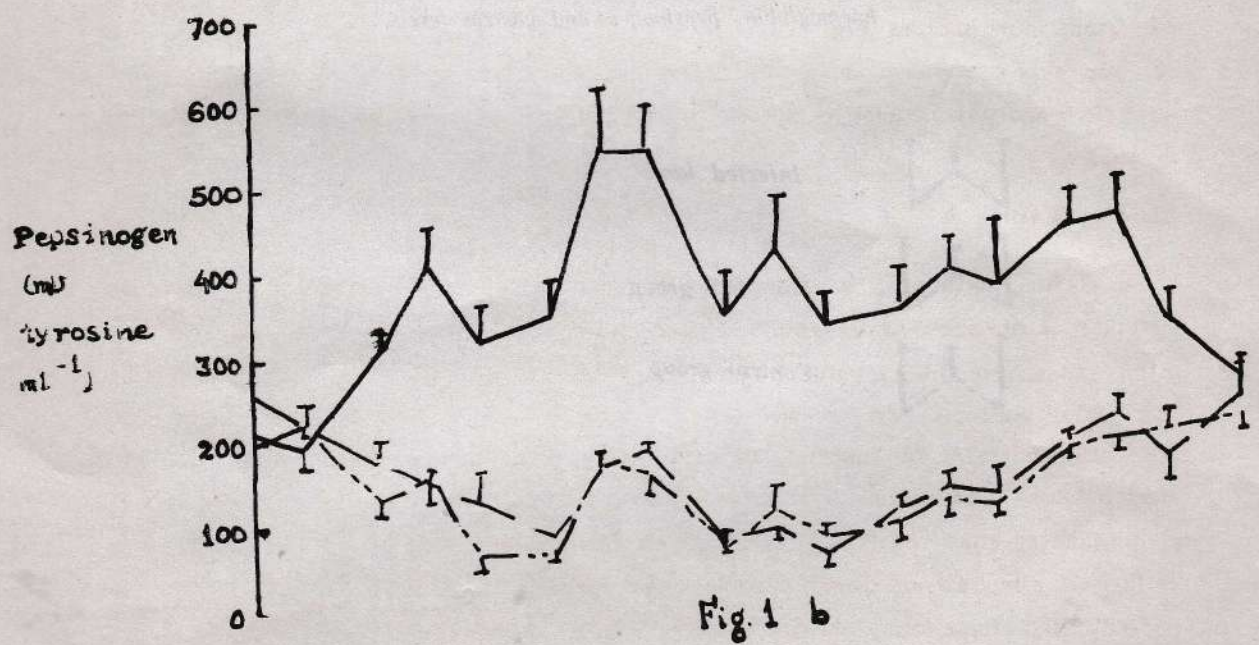
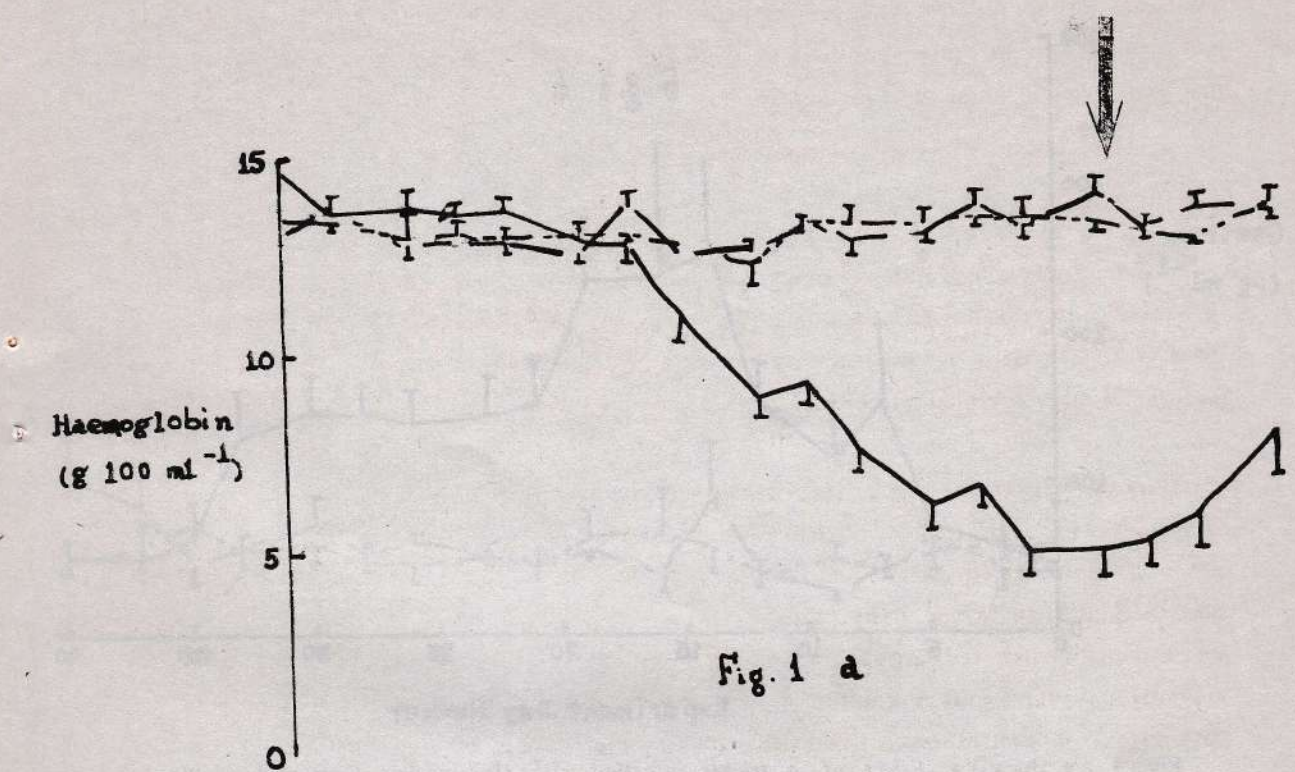
Materials and Methods

Experimental Infection

The diagnostic value of blood gastrin was assessed by direct comparison with the results of pepsinogen assay on aliquots of the same blood samples collected from lambs exposed to experimental infection. Twelve, three-month old, Suffolk-cross ram lambs reared under conditions designed to minimise the risk of parasitic infection, were housed and maintained in individual pens throughout the study. After an initial "settling in" period, the lambs were ranked by weight into replicates of three and within each replicate, allocated to experimental groups by random. Animals were assigned to either (i) an infected, *ad lib*-fed group (receiving the equivalent of 2,000 *Haemonchus contortus* infective larvae daily, given thrice weekly, from day 0 to day 33, inclusive; (ii) a control, pair-fed group (restricted to the feed intake of their infected 'pair' the previous day) and (iii) a control, *ad lib*-fed group. This ensured that differences in pepsinogen or gastrin levels between groups due solely to a reduction in appetite could be distinguished from those due to parasite-induced changes in digestive function. All lambs were treated with fenbendazole "Panacur", Hoechst UK Ltd. at the manufacturer's recommended dose rate before the study and also on day 33. Blood samples were collected three times per week during the investigation and stored at -30°C prior to analysis.

Blood Analysis

Plasma pepsinogen levels were determined using a modification of the colorimetric method described by Mylrea and Hotson (1969). Blood gastrin assays were performed using a commercially available human radio-immunoassay kit (Serono Diagnostics Ltd; 21 Woking Business Park, Albert Drive, Woking, Surrey GU21 5JY; kit code 10994) previously validated for use in ruminants using procedures outlined by Bolton (1982). Haemoglobin concentration was measured using a colorimetric assay (Coulter Haemoglobinometer) after conversion to cyano-methaemoglobin. Red blood cell counts were performed using an electronic particle counter (Model ZF Coulter Electronics) and packed cell volume using the microhaematocrit centrifuge method.



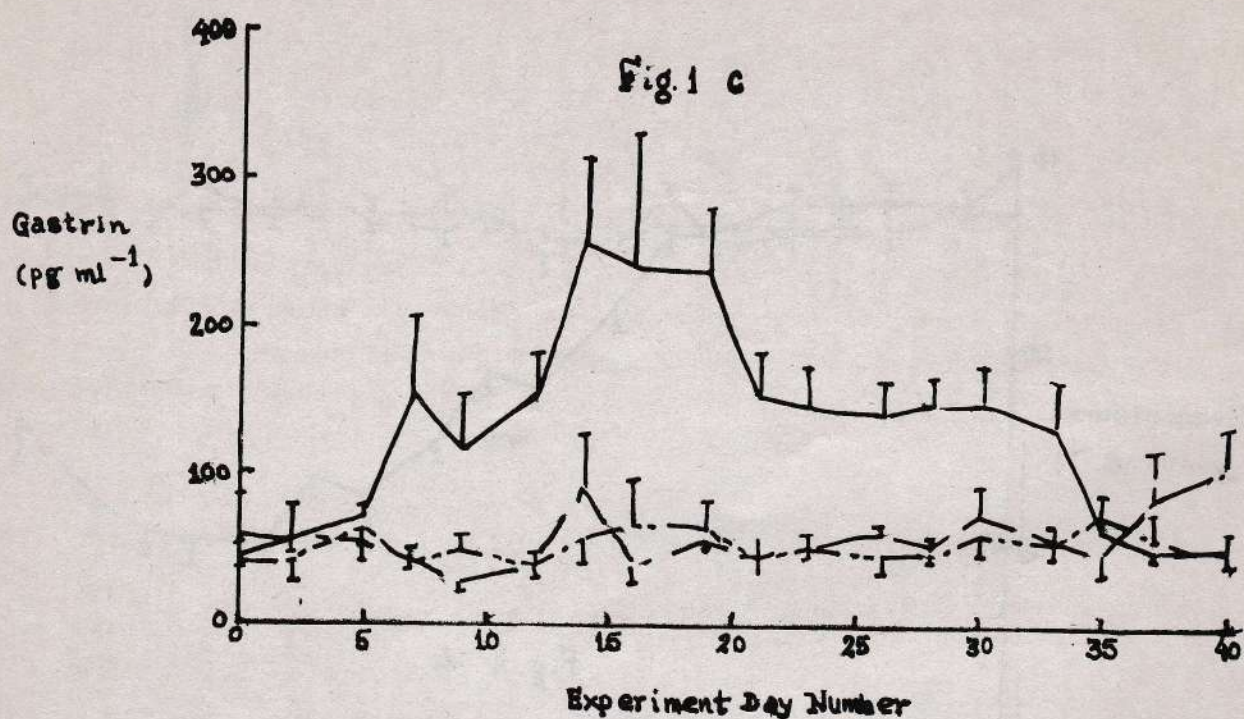
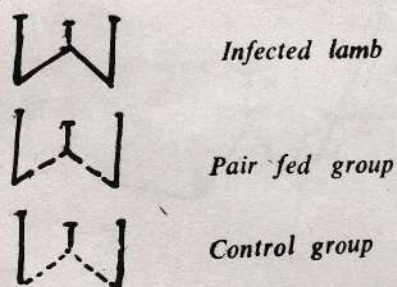


Fig. 1 (a, b, c) : Effect of a trickle infection with *Haemonchus Contortus* on blood haemoglobin, pepsinogen and gastrin levels.



Results and Discussion

There was no significant difference in any of the blood values measured between the *ad lib*- and pair-fed control groups. (Fig 1 a, b, c) Differences between the infected and control animals were therefore due solely to the effects of the parasite burden and not to differences in feed intake.

Marked changes in erythropoiesis were associated with the development and maturation of *Haemonchus contortus*. Infected lambs demonstrated a significant depression ($P \leq 0.01$) in haemoglobin levels (Fig 1 a), red blood cell count and packed cell volume from day 19 post infection. This was related to the feeding activities of the larvae and adult worms.

Changes in digestive function were recorded much earlier during the course of infection than the haematological changes reported above. Infected lambs exhibited a significant increase in pepsinogen levels ($P \leq 0.05$), Fig 1 b, from day 5 followed by a hypergastrinaemia ($P \leq 0.05$) Fig 1c, two days later. The elevated gastrin levels were probably related to the concurrent increase in abomasal pH. This would stimulate hormone secretion by the gastrin cells in the pyloric antrum and, in turn, increase hydrochloric acid production by the parietal cells in the gastric glands.

Comparison of pepsinogen and gastrin data from the infected group revealed a highly significant correlation ($r \geq 0.70$, $p \leq 0.001$) during the study. Peak pepsinogen and gastrin levels were recorded between days 14 and 19 when there was a 3.2 and 3.9 fold difference between infected and control group values, respectively. Both blood values then declined from around day 20 though remained significantly elevated above control until after treatment on day 33 when they returned to preinfection levels.

It should be noted that the gastrin data presented here are taken from just three of the infected lambs. Data from the fourth animal have been excluded since hormone levels were abnormally high (peak level 1400 pg/ml, day 19) and tended to distort data from the remainder of the group.

In conclusion, the magnitude of the gastrin response combined with its close correlation with changes in pepsinogen levels indicates that the hormone is of value in the diagnosis of *Haemonchus contortus* infections in susceptible lambs.

Acknowledgements

Financial support was provided by the the British Council, the Agricultural Research Council and the Central Research Fund, University of London. The authors also wish to thank the Department of Parasitology, Moredun Institue, Edinburgh for providing the *Haemonchus* larvae and the Department of Medicine for performing the haematological estimations.

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EXPERIMENTAL STUDY OF KARABIR (*Nerium indicum*) POISONING IN MICE AND BUFFALO CALF

Dr. N. P. Ghimire and Dr. S. P. Gautam*

Summary

Experimental study on Karabir poisoning in mice revealed that 0.1 to 0.2 ml. of the extract of green leaves was found lethal at intraperitoneal and subcutaneous routes. The mouse LD₅₀ was found between 0.08 to 0.1 ml by intraperitoneal route. In buffalo calf 25 grams of the green leaves was found lethal. The symptomatology and gross pathology of poisoning in mice and buffalo calf was also studied.

Introduction

Karabir (*Nerium oleander*, *Nerium indicum*), commonly known as Bahramase (Shrestha, 1984), is an ever green shrub with long branches attaining a height of 5-20 ft., loaded with double salmon-pink blooms to a size of 1½-2" with distinctive fragrance. The narrow dark green leaves are lanceolate, coriaceous and verticillate with smooth dark green polish in the dorsal part and prominently veined beneath (Annon, 1983).

The plant as a whole and especially the leaves are highly poisonous - 30 to 60 grams being fatal to horse, cattle and sheep, due to the presence of nerioside, oleandroside and other poisonous glycosides similar to digitoxin (Clarke, 1981; Graf, 1981). Horses have been found died eating few leaves at their forage, and cattle have died after drinking water in which Karabir leaves were floating (Clarke, loc. cit.). This decorative shrub is very poisonous and there are many records of poisoning due to

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feeding pruned leaves to the animals or self plucking by untighten animals in the garden (Blood *et. al*; 1983). In Bangladesh, poisoning usually resulted in cattle by accidental ingestion of the leaves when supplied with other green grasses (Samad, 1986; Sobhan, 1980). In India, three out of four goats intoxicated by eating pruned leaves of Karabir in garden were found dead within 24 hours of poisoning (Thakur *et. al*; 1988). According to Kingsburry, 1964 (cited by Sobhan, loc. cit.), Karabir leaves at as little as 0.005 per cent and 0.015 per cent of horse and sheep body weight respectively and at a single to human being has been considered potentially lethal.

Among the two species of Karabir found in Nepal, Rakta Karabir (*Nerium indicum*) and Pahelo Karabir (*Thevatia peruviana*), the former is considered more important because of its toxicity and availability. In Nepal, Rakta Karabir is planted as decorative plant at the garden and its flower is used to worship the god - especially the Sun, by some Hindu females. Most of the people are unaware of its poisoning. A case of Karabir poisoning in Jersey cross bred cow has been observed by the authors on 12 December, 1987 in Kalankisthan, Kathmandu. Since no research work so far has been conducted on Karabir poisoning in Nepal, the authors realised the importance and conducted an experimental study of poisoning in laboratory mice and buffalo calf.

Materials and Methods

Five green leaves weighing 15g of *Nerium indicum* collected from Bansbari area was grinded in a cleaned mortar and pestle and extracted 3.4 ml. of the extract through double folded muslin cloth by manual pressure. Penicillin (10000 i. u.) and Streptomycin (50 mg) was added to the extract to inhibit the bacterial contamination.

Adult healthy white swiss mice weighing about 25 gm. received from Laboratory Animal Unit of Biological Products Division were randomly grouped into 9 groups. These mice were administered with the extract in different dose rates through various routes except the control group which were administered with the same amount of distilled water as their respective group. The symptoms and observation were recorded. (Table - 1)

Furthermore, the poisoning effect of *Nerium indicum* was studied in a buffalo calf weighing 150 kg. as well. The symptoms and gross pathology of poisoning in the buffalo calf feeding 25 gm. of *Nerium indicum* green leaves cut into pieces and mixed with the cooked ration of mice were also recorded.

Table - 1 Toxicity of *Nerium indicum* in Mice and Buffalo Calf.

Group No.	No. of Mice Tested	Markings Coloration	Dose rate of extract given	Route of administration	Time			No of Mice Dead/ Recovery	Percentage Mortality
					Inoculation	Symptoms Initiation	Peak		
1	2	Blue back	0.2 ml.	I/cardial	11.50 a.m.	Instant	Instant	2/0	100
2	2	Blue back & head	0.1 ml.	I/cardial	11.51 a.m.	Instant	Instant	2/0	100
3	2	Blue head	0.2 ml.	I/peritoneal	12.00 a.m.	1 min.	5 min	2/0	100
4	2	Red tail	0.1 ml.	I/peritoneal	12.05 p.m.	2 min.	30 min.	2/0	100
5	6	Red side	0.08 ml.	I/peritoneal	12.15 p.m.	5 min.	40 min.	4/2	66.6
6	6	Red ear	0.05 ml.	I/peritoneal	12.30 p.m.	5 min.	45 min.	2/0	0
7	2	Blue side	0.2 ml.	Subcut.	12.35 p.m.	7 min.	10 min.	2/0	100
8	6	Blue tail	0.1 ml.	Subcut.	12.35 p.m.	7 min.	10 min.	4/2	66.6
9	4	Blue leg	0.025 ml.	Oral	12.50 p.m.	15 min.	60 min.	0/2	0
10	Buffalo	About 150 kg.	25 gm.	Oral	3.00 p.m.	4 hrs.	18 hrs.	Dead	100

Results

The extract administered through intra-cardial route resulted instant death in mice without showing any symptoms. The extract given through other routes caused initially depression, dullness followed by restlessness, dyspnoea, hyperpnoea, palpitation, tachycardia, incoordination, convulsion, dorsal recumbancy, and finally either death or very slow recovery. These symptoms were recorded within fourth day of the poisoning.

While studying the toxicity of the extract it has been noticed that 0.2 ml. was lethal to all the mice administered through every parenteral routes used. Again, 0.1 ml of the extract was found lethal to all the mice given through intra-cardiac and intra-peritoneal routes where as the same amount was 66.6 per cent lethal when administered through subcutaneous route. Similarly, 0.05 ml of the extract caused cent per cent morbidity but no mortality when administered through all routes except intra cardiac. Two drops of extract given orally also produced the symptoms of poisoning but in very milder form. None of the mice of control group produced any symptoms of poisoning.

The experiment conducted in a buffalo calf feeding ten green leaves (approximately 25 gm.) orally resulted into death after 55 hours of poisoning. The major symptoms observed were complete anorexia, dullness, depression, shrunken eyes, rough coat, ruminal stasis, dry muzzle, tachycardia, tachypnoea, cardiac arrhythmia, dyspnoea, uneasiness, tremor, colic, very much weak, lethargic, reluctant to move, restlessness, cessation of rumination, constipation followed by dehydration and death. The intensity of symptom was gradually increasing from a simple depression to those of acute poisoning and finally death was accompanied with violent struggling.

Post mortem examination revealed the presence of sores at the skin of head, limbs and back. Frothy discharge mixed with ruminal content was also found in oral and nasal cavity and upper part of trachea. Highly congested visceral organs, muscular haemorrhage at brisket region, generalized petechia at subcutaneous tissues, gastrointestinal mucosae, heart muscle, liver, kidneys, lungs, etc. along with significant hepatomegaly with rounded edge, yellowish, discoloration, congestion and distended gall bladder were observed.

Discussion

The symptoms and necropsy findings in buffalo calf resembled to that of acute poisoning of Karabir which were also in accordance with the findings of earlier workers (Blood et al, 1983; Clarke, 1970; Sobhan, 1980). Complete anorexia, excessive debility and cardiac arrhythmia were observed as the characteristic signs of Karabir poisoning in buffalo calf. The presence of vomitus into the nasal and oral cavity was indicative to vomition which is yet a rare phenomenon but usually occurring in case of *Nerium* and *Rhododendron* poisoning (Clarke, 1970). Death was probably associated with the ventricular fibrillation (Blood et.al., 1983) accompanied by asphyxiation due to the inhalation of vomitus. Necropsy findings further indicated that death occurred before the initiation of diarrhoea. Severe diarrhoea with the other symptoms as above was observed by authors in cow poisoned with *Nerium* leaves. Hence it could be concluded that Karabir may cause simple gastro-enteritis to per acute death in ruminants depending upon the quantity of plant ingested.

Karabir is not normally eaten by the animals but if supplied as an admixture with some other grasses, the ruminants, because of their voracious nature, may consume the unpalatable plant too. Hence, it could be recommended that care must be taken during collection of grasses especially from the garden or where Karabir is grown. This poisoning usually occurs during the winter season when there is a shortage of green grasses and the Karabir leaves are dropping down to the ground.

Since there has not been any specific treatment or antidote of *Nerium* poisoning so far practiced or recommended, treatment is not usually possible (Blood et.al., 1983). However, in case of poisoning, supportive treatment with fluid therapy, rice gruel and astringent mixture (Sobhan, 1980) and atropine sulphate in conjunction with propranolol (Clarke, 1970; Samad, 1986) has been recommended. According to Thakur and Sinha (1988) Karabir poisoning in goat was successfully treated with magnesium sulphate, chlorpromazine, atropine sulphate, vitamin B-complex and dextrose saline and was found considerably improved by fourth day of the treatment.

It could be concluded that Karabir poisoning may be a livestock as well as public health hazard in Nepal due to accidental ingestion. The incidence of poisoning could be reduced by increasing the public awareness towards this dreadful plant.

Acknowledgement

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A REPORT ON BRUCELLOSIS IN A DAIRY HERD

Dr. Md. Kalimuddin* and Dr. K. G. Narayan**

Brucellosis is important because it is such a zoonosis which simultaneously causes economic loss to the animal industry (WHO, 1971). Present paper records the incidence of brucellosis in a dairy farm having the history of retained placenta including a case of abortion.

A total of 76 serum samples collected in two different occasions over a period of two years were subjected to standard tube agglutination test (STAT) for brucellosis following the method adopted by Alton *et al.*, 1975. *Brucella abortus* plain antigen (strain 19) was obtained from the Biological Products Division, Indian Veterinary Research Institute, Izatnagar. The samples were taken from (i) apparently healthy cows, (ii) cows with history of retained placenta and (iii) an aborted heifer. Sera showing 80 I.U. of agglutinins and above were considered positive for *Brucella* infection as suggested by Sharma *et al.*, 1969.

Distribution of *Brucella* positive animals according to STAT titre in 1981, is presented in table-1. During October, 1981, 24 cows were tested for brucellosis among which three were having the history of retained placenta. Two of these three having history of retained placenta had titre of 160 I.U. and 80 I.U., respectively. During November, 1981 five additional cases of retained placenta were reported. All these seven animals were tested. All except the previous two cases, were, negative. The agglutinin level had reduced to 80 I.U. in both cows. The test was repeated after

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ten days and titre was 40 I.U. in one cow and the another cow which had titre of 80 I.U. died. There was no report of retained placenta and abortion during the year 1982. The farm may therefore, be considered free from brucellosis during this year.

Table - 1 *Distribution of Brucella Positive Animals According to STAT Titre in 1981.*

Date of animals tested	Number of animals tested	No. of animals having history of retained placenta	Titre of positive animals	
			Cow No. I	Cow No. II
21.10.81	24	3	1:160	1:80
18.11.81	7	5	1:80	1:80
28.11.81	1	1	1:40	Died

Table - 2 *Distribution of Brucella Positive Animals According to STAT Titre in 1983.*

Month	Number of animals tested	Titre in International Units (I. U.)			No. of positive animals
		80	160	320	
January, 1983	Heifer (1)	-	1	-	1
February, 1983	Heifer (1)	-	-	1	1
May, 1983	40	8	6	3	17

Table - 3 *Distribution of Brucella Positive Animals According to Their Breeding Performance.*

STAT titre	Total	Repeat Breeders*	Non Repeaters
1:320	3	2	1
1:160	6	5**	1
1:80	8	3	5

* Animals requiring more than three consecutive services.

** Includes one abortion.

Distribution of *Brucella* positive animals after STAT titre in 1983 is presented in table-2. In January, 1983 one of the heifers aborted in the fifth month of pregnancy. The placenta was expelled late and animal developed high fever (103°F) after two days. After five days of abortion it had a titre of 160 I.U. The same animal tested after a month had a titre of 320 I.U. Subsequently in May 1983, 40 animals including this heifer were tested and 17 (42.5%) were found positive. The titres ranged between 80 I.U. to 320 I.U.

Rahman *et al.* (1983) observed 60.0 per cent seropositivity in apparently healthy cows and 78.61 and 60.0 per cent in cows with history of abortion and with retained placenta, respectively. In comparison to this, 41.02 per cent of apparently healthy animals were seropositive. The sero-positivity among cases of retained placenta ranged between 66.7 per cent in October 1981 to 28.6 per cent in November 1981. Prevalence of brucellosis may vary from herd to herd and time to time.

Brucella positive animals according to the breeding performance is presented in table-3. Out of 17 positive animals ten were repeat breeders and seven non-repeaters. Of the ten repeat breeders, seven cows (70.0 per cent) exhibited agglutinin titres of 160 to 320 I.U. whereas 28.6 per cent non repeaters had titres at this range. Similarly, 30.0 per cent repeaters had 80 I.U. while 71.4 per cent non-repeaters had this titre level. A definite relationship between agglutinin titre and breeding performance is indicated that most of the repeaters had high titre and it confirms the finding of Miller and Graves (1932) who reported that *Brucella* positive cows require an average of 2.8 services per conception following normal calvings and 3.6 services per conception in aborted cows.

Acknowledgement

The authors are thankful to Dr. H.R. Mishra, Dean, Ranchi Veterinary College, Birsa Agricultural University, Ranchi, Bihar for providing necessary facilities to carry out this study.

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PROCESSED CHEESE FOODS : A REVIEW

Mr. Tek Bahadur Thapa*

Introduction

Processed cheese foods are the new invention of 1920's (Price and Bush, 1974). Whey disposal was a problem. The whey proteins from whey were precipitated and used in processed cheese manufacture. The resulting cheese was soft in body, pleasing in flavour, and was first offered under the name cheesette and was soon marketed widely in the United States.

Processed cheese food is a product requiring the same computations, selection, trimming, grinding, heating and emulsification principles used for processed cheese (Kosikowski, 1982). But the product contains more moisture and less fat, the cooking temperatures are higher, and pH is lower. Also, optional ingredients like skim milk, milk, cream, cheese whey, cheese albumin, skim milk cheese and whey protein concentrates are used which are not permitted for processed cheeses. Higher heat and lower pH provide greater protection against most bacterial spoilage, which is accelerated in the presence of more water. The final product resembles process cheese, but has a softer body and milder flavour.

Cooking temperature for process cheese food normally is about 82° C (Kosikowski, 1982). Following fat and protein emulsification by alkali salts which leads to a smooth, hot mass, organic acids, such as citric, lactic, acetic or phosphoric are introduced

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carefully to decrease the pH without adversely affecting the texture. The pH range of most processed cheese foods (PCF) ranges between 5.4 to 5.6, and even may show a lower limit of 5.2. Since this is a new product, no standards have been formulated even in India till date, and U.S. Federal Standards specify that PCF should not contain more than 44 per cent moisture and the milk fat content should not be less than 23 per cent of dry matter (Kosikowski, 1982).

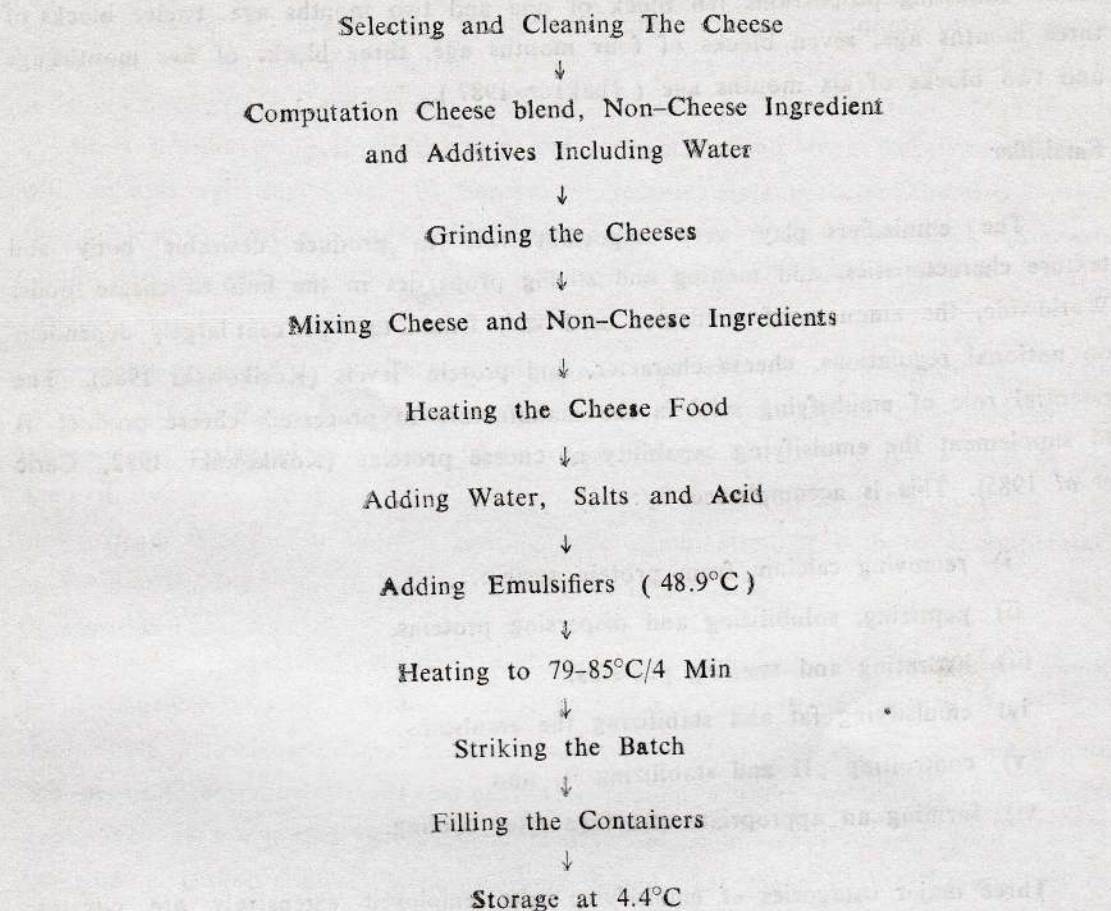
There are reports of processed cheese food manufacture by using fresh cheese whey (Tsvetkova *et al.* 1975), concentrated cheese whey (Kosikowski 1982, Shilovskaya 1979), whey powder (Kosikowski 1982, Tsvetkova *et al.* 1975, Meyer 1973), dried skim milk (Leung *et al.* 1976) and high calcium co-precipitate (Grishin *et al.* 1982). But the addition of whey and whey products in larger amounts imparted defects like sandiness (Olson and Price 1961), Sourness (Kosikowski 1982), salty flavour (Meyer 1973) and textural defects (Kosikowski 1982).

Keeping these defects in mind some workers utilized ultrafiltered whey in the form of WPC to eliminate lactose oriented defects in processed cheese food manufacture (Georgakis 1975, Gupta and Reuter 1987, Thapa and Gupta 1988)

Manufacture of Processed Cheese Food

Figure-I illustrates the flow diagram of processed cheese food manufacture. Natural cheeses, mild to sharp in flavour, after proper selection and cleaning by persons experienced in the profession, are shredded and mixed with non-cheese ingredients and heated at high temperature in either steam jacketed kettles or stainless steel cookers (Kosikowski 1982). The melted cheese in the cooker initially separates into a fat and serum phase until the introduction of suitable emulsifying salts. An upwards pH shift solubilizes protein, resurfaces the fat and gives a smooth homogenous mass. Heating emulsification and unloading of the molten cheese requires only a few minutes. If the process is delayed, oiling off, body thickening, browning and other undesirable features may result. At proper time, the blended smooth textured hot mass is ejected from the cooker and packaged suitably.

Fig-1. Flow Diagram of Processed Cheese Food Manufacture (Kosikowski 1982)



Selection of Cheese Ingredients

Kosikowski (1982) reported the selection of lots of mild, medium and sharp flavour in processed cheese food manufacture. Cheese lot size and number are determined by the final flavour desired in the cheese food. In the mild flavour lots, no more than 15 per cent of the cheese should be of acid or green character. Cheddar and Swiss were used for processing when industries were set up (Price and Bush 1974). The best flavour and texture was found in process products made from Cheddar cheese with an average age of 2.5 months old cheese (Mann 1981). In Amul, the biggest processed cheese plant in India, processed cheese products are

prepared mostly by blending 60 per cent young and 40 per cent old Cheddar cheeses in the following proportions: ten block of one and two months age, twelve blocks of three months age, seven blocks of four months age, three blocks of five months age and two blocks of six months age (Thakkar 1987).

Emulsifier

The emulsifiers play very important role to produce desirable body and texture characteristics, and melting and slicing properties in the finished cheese foods. Worldwide, the amounts of emulsifier used vary from 2 to 4 per cent largely dependent on national regulations, cheese character, and protein levels (Kosikowski 1982). The essential role of emulsifying salts in the manufacture of processed cheese product is to supplement the emulsifying capability of cheese proteins (Kosikowski 1982, Caric *et al* 1985). This is accomplished by:

- i) removing calcium from protein system,
- ii) peptizing, solubilizing and dispersing proteins,
- iii) hydrating and swelling proteins,
- iv) emulsifying fat and stabilizing the emulsions,
- v) controlling pH and stabilizing it, and
- vi) forming an appropriate structure after cooling.

Three major catagories of emulsifying salts employed extensively are citrates, monophosphates and polyphosphates (Kosikowski 1982, Shimp 1985). Each group gives to the final processed cheese product its basic characteristics of firmness, spreadability, smonthness and buffering power. To attain the desired characteristics for a given finished cheese, individual salts from each of the three groups are selected for their key attributes and combined. Gupta and Reuter (1987) reported the use of 2 per cent Joha salts, S-8 and S-10 in a proportion of 2:1 and 1:1 mixture of sodium hydrogen phosphate and tri-sodium citrate in processed cheese food manufacture. These emulsifiers imparted sandiness, mealiness and chalkiness in the finished products. Tri-sodium citrate alone gave smooth body and texture to the cheese foods. Thapa and Gupta (1988) also made similar observation using tri-sodium citrate emulsifier at 2.5 per cent in processed cheese food manufacture in India.

Processing

Processing of cheese food involves the operations like addition of non-cheese ingredients, water, salt and heating, stirring, unloading and packaging of product when it is hot (Kosikowski 1982). Cheese foods are heat processed in two ways namely by direct heating using culinary steam and indirect heating using jacketed vessel fitted with suitable agitating device. In Europe, where spreadable processed cheese is popular, cheese blend is heat processed using direct culinary steam, but for block processed cheese products indirect heating to a temperature range of 80° to 85°C is preferred (Meyer 1973).

Kosikowski (1982) and Caric *et al* (1985) suggested melting temperature in the range of 79° to 85° c for processed cheese foods. Thapa and Gupta (1988) also reported the heat processing of processed cheese foods between 82° to 83°C for 3 minutes. In Amul dairy, processed cheese products are heat processed in a batch of 25 kg using direct steam injection or indirect heating or a combination of both to a temperature of 80° to 85°C for 7 to 10 minutes (Thakkar 1987).

Compositional Aspect

There are limited reports on compositional quality of processed cheese foods (Kosikowski 1982, Gupta *et al* 1984, Gupta and Reuter 1987, Thapa and Gupta 1988). Kosikowski 1982 reported moisture 43.2 per cent, fat 24 per cent, protein 19.8 per cent, carbohydrate 7.1 per cent and calcium 570 mg per cent in cheese foods. Gupta *et al* (1984) reported the chemical analysis of commercial cheese food sample marketed in United States. These workers found that the moisture, fat and pH in cheese foods varied widely from 41.4 to 45.2 per cent, 22 to 27 per cent and 5.42 to 5.86 respectively. At National Dairy Research Institute, Karnal, Thapa (1988) analysed cheese food samples prepared by replacing 20 per cent cheese solids with whey protein concentrates and reported the following composition on dry matter basis: fat 42.19 to 42.34 per cent; protein 44.77 to 44.9 per cent; total ash 8.195 to 8.242 per cent; calcium 608.39 to 611.40 mg per cent and pH 5.50 to 5.53.

Defects

Acceptability of food products is mostly guided by their sensory characteristics. A processed cheese food of good quality has a softer body and milder flavour (Kosikowski 1982) and shows bright, shiny surface reflecting light well. The grain is smooth without sandiness and its slices break evenly and melt easily and uniformly. Major technical problems which plague manufacturers of processed cheese products are

grainy texture, thick glue-like bodies, soapy and tart flavours, gas blowing, pink to brown discolourations and crystal formations. Common defects in processed cheese foods, as reported by Kosikowski (1982): are flavour acid, moldy; body and texture - short, heavy, firm, leaky, sticky and sandy and appearance - brown, gassy and crystal.

Body and Texture

Kosikowski (1982) described that grainy texture in processed cheese food arose from too much young cheese in the blend, an abnormal pH below 5.5 and excess emulsifier. Processed cheese and foods thicken perceptibly under condition of cheese blends being held too long in the cooker, insufficient water present, use of sensitive emulsifiers, and pH well above 5.8 or the addition of too much rework cheese. Sandiness was reported in cheese foods with excess whey addition (Olson and Price 1961) and was due to the insolubility of lactose at higher concentrations - 16.9%. These workers further reported that the excessive moisture, low fat and lack of acidity were associated with defect in body and keeping quality in cheese foods.

Appearance

A number of workers (Thomas 1969, Csok 1978 and Bley et al 1985) reported browning in processed cheese products due to heating, and melts at 95° c. Processed cheese food sometimes, early acquires pinkish brown colour (Price and Bush 1974, and Kosikowski 1982) defect which progressively becomes more intense. Excessive high or prolonged heating of the cheese, insufficient cleaning of cooker walls, presence of colour and lactose all contribute to it. For this reason any blend containing lactose, even in moderate quantities, should not be heated above 85° c. An unnatural pink defect was often attributed to the use of acid cheese (Price and Bush 1974) in processed cheese food manufacture.

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**A REPORT ON SHEEP MORTALITY AT LIVESTOCK DEVELOPMENT
FARM, LAMPATAN, POKHARA**

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Introduction

There is a declining trend in sheep population in Nepal (Rajbhandary and Shah, 1981). The mortality of sheep even at Livestock Development Farm Lampatan was reported as high as 20 per cent. This figure may go up even about 40 per cent in farmers migrative Baruwal sheep flock in the mid hill region. The Kage are the predominant type of sheep in the middle belt particularly in the valley areas. The causes of such mortality in sheep has not so far been studied particularly in the valley of mid hill region of Nepal.

Materials and Methods

The postmortem reports of ten years (1977 - 1987/88) maintained at Livestock Development Farm, Lampatan were analysed to identify the causes of mortality. Total number of dead sheep involved in this study was 607. Five different types of sheep: Kage, Polworth 50% X Kage 50%, Polworth 75% X Kage 25%, Borderleicester 50% X Kage 50%, and Borderleicester 75% X Kage 25% were considered for this study. The mortality records were analysed grouping yearwise, monthwise and breed wise. The causes of death described in the postmortem reports were as many as 43. These causes of death were again regrouped into internal parasites, infectious and non-infectious diseases.

Results and Discussion

The analysis of postmortem reports indicated that the causes of death among sheep was 27 per cent from malnutrition, 24 per cent from internal parasites, 23 per cent

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reported unknown due to lack of diagnostic facilities and the remaining from contagious, non-contagious and many other causes.

Maximum number of sheep died during the month of August / September which decreased lowest in May / June. The high mortality rate was associated with rainy season, with lambing season (January-April) and with unavailability of feed in pasture.

The highest mortality 48 per cent was observed in Polworth crossbreds and the least seven to eight per cent was found in Borderleicester crossbreds. Season wise and breed wise mortality was highly significant (5 per cent).

The highest mortality was observed in the year 1986/87 and lowest in 1977/78. During that year majority of the death, about 65 per cent, was due to non-infectious conditions like debility, anaemia, lack of milk and accidental injuries. About 25 per cent of the death was caused by internal parasites like tape worm, hook worm and *Haemonchus*. The remaining percentage of death was due to suspected infectious diseases like Haemorrhagic Septicemia, Black Quarter and Tetanus. Pneumonia was another important disease causing 7 per cent mortality in sheep. The tendency of higher mortality due to pneumonia was recorded in the months of July / August probably due to monsoon and winter stress as observed in migratory sheep (Karki, 1925).

The findings indicated the existence of genotype X environmental interaction under inadequate feeding and improper management system of Livestock Development Farm, Lampatan, Pokhara.

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STUDY OF THE DRUG SENSITIVITY PATTERN OF MASTITIS MILK ISOLATES OF CATTLE AND BUFFALOES IN KATHMANDU VALLEY

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Summary

Study of the antibiotic sensitivity pattern of 441 different bacterial strains isolated from mastitis milk of cattle and buffaloes revealed that almost all of the isolates were found resistant to the drugs tested with a varying degree of resistancy ranging from 11.11 per cent to cent per cent. *Staphylococcus aureus* was found to be the most common bacteria causing mastitis followed by *Streptococci* and *E. Coli*.

Introduction

Mastitis, the commonest problem among cattle husbandry in Nepal is usually caused by various strains of pathogenic bacteria which are very often resistant to commonly used antibiotics and chemotherapeutic agents and thus create the therapeutic problem in clinical practice, especially in the field. The development of the antibiotic resistance with udder isolates is a regular phenomenon indicating a necessity of a periodical study of their sensitivity to different antibiotics, so that the treatment can be modified accordingly (Babu *et. al.*, 1979). In our country very little study has been done in the antibiotic sensitivity against microorganisms resulting mastitis. The present study has attempted to detect the common bacteria causing mastitis among cattle and buffaloes and testing their sensitivity pattern against different antibiotics. The knowledge gained might be helpful for the large number of Veterinary practitioners.

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Materials and Methods

During the years 1981 to 1986, a total of 765 milk samples from 599 cows and 166 buffaloes clinically suffering from mastitis were received at Central Diagnostic and Research Laboratory (CDRL), Tripureswar and were studied to determine the bacterial causes. The milk samples were first tested with Mastoid Reagent (Sodium Hydroxide 15 g; Teepol 5 g; Bromothymol 0.1 g and distilled water add to 100 ml.) and were subjected to bacteriological culture. The intensity of mastitis was determined based on the degree of gel formation of the milk mixing 0.5 ml. of reagent with 10 ml. of milk sample in a sterile petridish.

The isolation and identification of the causative agents were carried out according to the standard bacteriological technique (Robert *et. al.* 1957). A total of 441 bacterial strains belonging to eleven different genera were isolated, of which only 106 strains were subjected to the antibiotic sensitivity test with commonly used ten different antibiotics using the paper disc method followed by Cruickshank *et. al.*, (1975).

Results and Discussion

Out of 765 mastitis milk samples from clinically suffering cows and buffaloes 548 (71.64 per cent) were found positive to mastoid reagent. First, second, third and fourth degree of mastitis positive reaction was recorded among 27.58 per cent, 17.38 per cent, 14.50 per cent and 12.15 per cent of cases respectively. Very strong positive cases of mastitis were found in cross bred cattle (13.68 per cent) than that of buffaloes (6.62 per cent) (Table - 1).

Table - 1: Examination of Milk for Mastitis with Mastoid Reagent Test.

Animals	Total Sample	Gradation of Reaction of Milk Sample:				
		—	+	++	+++	++++
Cow	599	173 (28.88)	154 (25.70)	98 (16.36)	92 (15.35)	82 (13.68)
Buffaloes	166	44 (26.50)	57 (34.33)	35 (21.08)	19 (11.44)	1 (6.62)
Total	765	217 (28.36)	211 (27.58)	133 (17.38)	111 (14.50)	93 (12.15)

Note:- (—) = Negative or no viscid mass formed
 (++) = Distinct positive; gel formation
 (+++) = Strong positive; distinct gel formation
 (++++) = Very strong positive;
 Number in parenthesis indicate percentage.

Table - 2: Antibiotic Resistance Pattern of Mastitis Milk Isolates.

S. No.	Organisms	Isolated from		Tested	Antibiotic Resistance Percentage										
		Cows			P	Am	Str	G	Pol	T	Ch	Ox	Sul	Neo	
		Buffaloes	Total												
1	<i>Staph. aureus</i>	217	46	263	51	11.76	19.6	6.07	41.17	88.23	21.56	41.17	23.52	58.33	-
2	<i>Staph. epidermidis</i>	37	4	41	-	-	-	-	-	-	-	-	-	-	-
3	<i>Streptococcus</i> sps.	36	22	58	12	8.33	25	100	16.66	91.66	16.66	41.66	33.33	83.33	-
4	<i>Escherichia coli</i>	23	25	48	16	100	33	-	0	0	33	33	-	-	0
5	<i>Citrobacter</i> sps.	6	-	6	4	100	50	50	50	50	100	100	-	-	100
6	<i>Enterobacter</i> sps.	8	-	8	8	100	0	25	25	100	12.5	12.5	25	50	10
7	<i>Corynebacterium</i> sps.	6	-	6	6	50	0	100	0	0	0	-	66.66	-	0
8	<i>Aeromonas</i> sps.	6	-	6	4	100	0	0	0	-	25	0	50	-	25
9	<i>Bacillus</i> sps.	2	-	2	2	0	0	100	0	-	0	0	0	-	0
10	<i>Diplococcus</i> sps.	2	-	2	2	0	0	-	0	0	0	0	0	0	0
11	<i>Klebsiella</i> sp.	1	-	1	1	100	0	0	0	100	0	0	0	-	-
Total		344	97	441	106	.	.	.	.	.	.	.	.	.	.

Note : P = Penicillin Am = Ampicillin Str = Streptomycin Pol = Polymyxin T = Tetracycline
 Ox = Oxytetracycline Sul = Sulphathalazole Neo = Neomycin Ch = Chloramphenicol G = Gentamicin
 O = Sensitive - = Not tested

Among the 441 bacterial isolates isolated from cows (344) and buffaloes (97) the largest number were *Staphylococcus aureus* (263) followed by *Streptococcus sp.* (58), *E. coli* (48), *Staph. epidermidis* (41), *Enterobacter sp.* (8), *Corynebacterium sp.* (6), *Citrobacter sp.* (6), *Aeromonas sp.* (6), *Bacillus sp.* (2), *Diplococci* (2) and *Klebsiella sp.* (1) (Table - 2).

It is evident from Table - 2 that out of 51 isolates of *Staphylococcus aureus* tested for sensitivity, 11.76 percent of them were found to be resistant to Penicillin. The isolates were highly resistant to Streptomycin (96.07 per cent) followed by Polymyxin - B (88.23 per cent), Sulphathalazole (58.33 per cent), Gentamicin (41.17 per cent), Chloramphenicol (41.17 per cent), Tetracycline (21.56 per cent) and Oxytetracycline (23.52 per cent). Babu *et. al* (1979) recorded strains of *Staphylococci* resistant to Penicillin (15.37 percent), Tetracycline (13.64 per cent) and Chloramphenicol (26.4 per cent). According to Panduranga Rao *et. al.* (1966) the isolates were completely resistant to Terramycin, Auriomycin and Chloromycitin where as Murthy and Makholia (1963) found 40 per cent resistant to Chloramphenicol.

All the 12 isolates of *Streptococci* isolated from milk were highly resistant to Streptomycin (100 per cent) followed by Polymyxin - B (91.66 per cent) and Sulphathalazole (83.33 per cent). A moderate to mild degree of resistancy was also observed with Ampicillin (25 per cent), Tetracycline (16.66 per cent), Gentamicin (16.66 per cent), Oxytetracycline (33.33 per cent), and Chloramphenicol (41.66 per cent) together with 91.66 per cent sensitivity to Penicillin. It has also been reported by Babu *et. al.* (1979) that *Streptococci* isolated from cow milk were completely sensitive to Penicillin and nearly to Streptomycin (91.65 per cent), where as one out of eight strains from buffaloes was found to be resistant to Penicillin and completely sensitive to Dihydrostreptomycin.

Among six strains of *Corynebacterium sp.* isolated from cows milk 50 per cent, 66.6 per cent and 100 per cent resistancy was observed to Penicillin, Oxytetracycline and Streptomycin respectively but were found completely sensitive to all the other antibiotics tested.

The two isolates of *Diplococci* from cows were sensitive to all the antibiotics tested.

The six isolates of *Aeromonas* sp. isolated from cows were completely resistant to Penicillin and fairly to Oxytetracycline (50 per cent), Tetracycline (25 per cent) and Neomycin (25 per cent) but were found sensitive to other antibiotics tested.

Among the 48 isolates of *E. coli* 16 were tested for sensitivity. They were found completely resistant to Penicillin, moderately (33 per cent) to Ampicillin, Tetracycline and Chloramphenicol and were completely sensitive to Gentamicin, Polymyxin, and Neomycin.

It could be concluded that many of the common mastitis milk isolates had developed a mild to high degree of resistancy to most of the commonly used antibiotics. This situation further demands the regular monitoring of antibiotic sensitivity test and use the relevant antibiotics in Nepal.

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