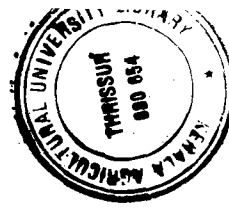# ETIOPATHOGENESIS AND THERAPEUTIC MANAGEMENT OF THIAMINE RESPONSIVE CAPRINE POLIOENCEPHALOMALACIA

By
USHA NARAYANA PILLAI

THESIS

Submitted in partial fulfilment of the
requirement for the degree

Doctor of Philosophy

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Clinical Medicine
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
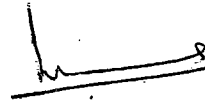MANNUTHY, THRISSUR - 680651
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I hereby declare that this thesis entitled "ETIOPATHOGENESIS AND THERAPEUTIC MANAGEMENT OF THIAMINE RESPONSIVE CAPRINE POLIOENCEPHALOMALACIA" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other University or Society.

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CERTIFICATE

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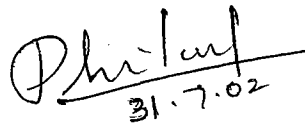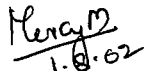
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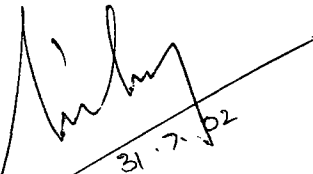
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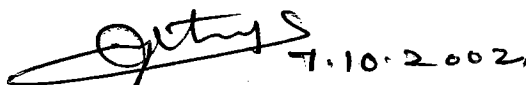Associate Professor and Head
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Dedicated to
My Parents

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LIST OF ABBREVIATIONS

CCN	-	Cerebrocortical necrosis
CNS	-	Central nervous system
dl	-	Decilitre
g	-	Gram
h	-	Hour
IU	-	International unit
mg	-	Milligram
μg	-	Microgram
PEM	-	Polioencephalomalacia
ppm	-	Parts per million
SRL	-	Strained rumen liquor
TK	-	Transketolase
TPP	-	Thiamine pyrophosphate

Introduction

INTRODUCTION

Goats (*Capra hircus*) are thought to be one of the earliest domesticated species. Through centuries it has remained as a popular prolific animal serving the needs of man. In these times of artificial foods and flavourings, the goat is becoming more and more popular for supplying an unadulterated source for milk and meat.

In India, goat population increased from 47.08 million in 1951 to 123 million in 2000 (FAO, 2000) and ranks first in the world. In Kerala, there were about 1.86 million goats as per livestock census 1996. During last decade goat population has increased considerably, indicating their importance in rural economy.

Goats by virtue of their size and reproductive efficiency have biological as well as managemental advantage over other farm animals. In our country goat rearing is predominantly practised by small and marginal farmers. Commercial goat farming is showing an increasing trend in Kerala.

The intensification of goat production has created complex health problems for which there are no simple and reliable diagnostic and therapeutic procedures. The solutions to these complex problems are not always readily apparent because of insufficient research on the etiology of the conditions and the control strategy to be adopted in the herds where the problems are occurring. Modern livestock producer is cost conscious, and anything the veterinarian does or recommends must be cost-effective. Therefore in food producing animal practice, the objective is to improve the efficiency of animal production using the most economical methods of management and the diagnosis, treatment and control of diseases.

Goats are hardy creatures when well, but bad patients when ill. A sick goat has to be nursed carefully and treated promptly for the correct condition (Hetherington, 1979). Under the changing scenario and stress of organized and intensive farming goats have become susceptible to many infectious and non-

infectious diseases. The conventional diseases of goats are well defined. However, there are certain emerging disease problems which need special attention. Polioencephalomalacia (PEM) or Cerebrocortical necrosis (CCN) which leads to serious economic losses to the farmers is one among these important problems. Polioencephalomalacia literally means softening of the grey matter of brain. It is a non-infectious neurodegenerative disease affecting many species of animals especially ruminants caused by multiple factors viz., deficiency of thiamine, copper and cobalt and excess sulphur in the diet. It is characterized clinically by depression, blindness, inco - ordination, opisthotonus and convulsions and pathologically by laminar cerebrocortical necrosis and malacia. In severe cases mortality may reach upto 100 per cent. This disease has world wide distribution among pasture and farm stock and is of great concern to the farming community.

In India, studies on clinical cases of polioencephalomalacia in goats have been meagre. Alteration in thiamine metabolism is considered to play a major role in the etiopathogenesis of almost all outbreaks of PEM (Radostits *et al.*, 2000).

Nair (1999) recorded an increasing trend in the occurrence of this disease in Kerala and suggested multifactorial etiology. In the light of the above observations, an investigation was undertaken on clinical cases of PEM in goats with the following objectives,

1. to find out the epidemiological aspects of polioencephalomalacia,
2. to study the etiopathogenesis and
3. to compare the efficacy of different lines of treatment.

Review of Literature

REVIEW OF LITERATURE

Polioencephalomalacia (PEM) or cerebrocortical necrosis (CCN) was reported for the first time as a distinct disease of cattle and sheep in USA by Jensen *et al.* (1956). The condition was characterized by sudden onset, anorexia, pressing of head on to a fixed object, progressive ataxia, opisthotonus, recumbency and convulsions and death in severe cases.

2.1 EPIDEMIOLOGY

Polioencephalomalacia was reported in sheep and cattle (Jensen *et al.*, 1956) horses (Cymbaluk *et al.*, 1978) goats (Smith, 1979; Tanwar, 1987; Fakhruddin *et al.*, 1987a) white tailed deer (Wobester and Runge, 1979) antelope (Blood *et al.*, 1979) dogs (Breud and Vendevelda, 1979) and cats (Jubb *et al.*, 1956).

Jensen *et al.* (1956) estimated annual incidence of PEM in USA ranged from 4.50 to 100.50 cases per 10,000 cattle and 70 cases per 1200 sheep. Hartley and Kater (1959) reported that the incidence of PEM in sheep ranged from one to 10 per cent. Morbidity rates in sheep and cattle were less than 10 per cent and mortality rate of PEM affected animals approached 100 per cent (Spence *et al.*, 1961). Fenwick (1967) reported PEM in 22 sheep, out of these 21 died and only one responded to treatment with thiamine. Verdura and Zamora (1970) noticed that 15 per cent of cattle fed with a diet based upon molasses and urea in large Cuban feedlot developed PEM during a six month period.

Mella *et al.* (1976) observed many cases of PEM in cattle fed with a new molasses urea based diet to fatten bulls. An incidence rate of 0.6 to one per cent in routine slaughter and 0.4 to 3.04 per cent in emergency slaughter was found to be due to the disease. Smith (1979) encountered six cases of PEM in goats. He observed more incidence during winter or early spring.

Polioencephalomalacia was first reported in India by Tanwar (1987) from the arid zone of Rajasthan. He observed the disease in 50 female goats of various

age groups. He reported the occurrence in late winter (January/February) mid summer (April) rainy season (August to October) and early winter (November). There was no correlation between the occurrence of PEM and environmental temperature, humidity and rainfall.

Hamlen et al. (1993) observed that most feedlot cases developed during late autumn and winter or early spring, a time for greater concentrate feeding. They noticed PEM in 11 out of 110 cattle exposed to a source of water containing sodium sulphate. Lonkar et al. (1993) observed PEM in 158 cases and the percentage incidence was 2.60. Though the disease was prevalent throughout the year, the occurrence was higher in late winter followed by mid summer, rainy season and early winter. Morbidity rate of 16 to 48 per cent and mortality rate of zero to eight per cent in PEM affected animals were reported by Jeffrey et al. (1994). Bulgin et al. (1996) found PEM unresponsive to thiamine treatment in ewes when they were moved to a field that had been sprayed with elemental sulphur.

Nair (1999) conducted a detailed investigation on PEM of goats in Kerala and reported that the disease occurred throughout the year with maximum incidence in the month of April followed by May, March and February and lesser incidence in January, August, September and October. Maliekal (2000) also reported that the disease occurred throughout the year in Kerala with maximum occurrence in the monsoon and post-monsoon period.

2.1.1 Age

Animals in the age group of 12 to18 months were mostly affected (Jensen et al., 1956). Spence et al. (1961) described 21 outbreaks in sheep and 12 in cattle. The age of affected sheep varied from lambs of six weeks to ewes of seven years or more but majority of the cases were in lambs of two to seven months. In cattle the age incidence ranged from 10 weeks to 11 months but the disease was most common in calves of three to seven months. Terlecki and Markson (1961)

reported that the age incidence of PEM ranged from two to seven months in sheep and 10 weeks to 11 months in cattle.

Gabbedy and Richards (1977) opined that PEM usually affected sheep and cattle in good physical condition of all ages throughout the year. Mortality rate in sheep flocks ranged from one to ten per cent. Smith (1979) encountered six cases of PEM in goats of two to two and a half months of age. The age group of PEM affected goats ranged from three to eight months (Fakhruddin et al., 1987b) and four months to four years (Tanwar, 1987). Incidence of PEM was mostly seen in adult goats (Lonkar et al., 1993). According to Maliekal (2000) animals in the age group of six months to five years were mostly affected where as George (2002) found that age of PEM affected goats ranged from one and a half months to two years.

2.1.2 Sex

Incidence of PEM in sheep was 38 per cent in males and 62 per cent in females (Jensen et al., 1956). Sobhanan (1981) opined that the incidence was more in females as male goats were disposed off at an early age and only females were maintained to the adult age. Out of 35 thiamine responsive PEM cases presented at the University Veterinary Hospital, Mannuthy 14.28 per cent (5) were males and the rest (30) were females (Maliekal, 2000).

2.2 ETIOLOGY
2.2.1 Diet

Many workers have associated PEM with dietary abnormalities leading to thiamine deficiency. Loew et al. (1970) and Brent (1976) reported that cattle maintained on high concentrate and low forage diet had decreased ruminal thiamine synthesis. Due to feeding of high concentrate ration, ruminal pH became acidic and bacterial flora changed to produce thiaminase precursors responsible for the destruction of ruminal thiamine.

Episodes of PEM have been associated with specific nutritional factors including high levels of dietary carbohydrates, molasses and urea (Mella et al., 1976). Heavy concentrate feeding leading to lactic acidosis could predispose to thiamine deficiency by changing the rumen flora and fauna, creating optimum pH for action of bacterial thiaminase and production of histamine, a potent cosubstrate for thiaminase action (Smith, 1979). The studies of Sobhanan (1981) and Maliekal (2000) showed that rice (a carbohydrate rich diet) formed the main component of concentrate ration of goats in Kerala, which possibly reduced rumen pH to produce a favourable environment for increased thiaminase production.

Sapienza (1981) opined that sudden change from roughage to concentrate resulted in rapid multiplication of gram positive bacteria and clostridium species, which inturn produced enzyme capable of reacting with thiamine in rumen and causing CNS disorders. Dietary composition most commonly associated with the development of PEM was high levels of concentrates and low quantities of roughage (Mcguirk, 1987). Polioencephalomalacia was commonly associated with milk replacer diets in kids or concentrate feeding in older goats (Radostits et al., 2000).

2.2.2 Thiamine

High level of thiaminase type I activity leading to intraruminal degradation of thiamine and possibly production of thiamine antimetabolites were attributed to be the factors responsible for thiamine deficiency or PEM (Edwin and Jackman, 1970; Edwin and Lewis, 1971). In cattle, sheep and goats, thiamine was supplied by the synthetic activity of ruminal microbes and a state of thiamine deficiency was believed to be due to thiamine destroying enzymes within the gastrointestinal tract. Thiaminase activity was detected in bacteria like Clostridium sporogenes and Bacillus thiaminolyticus (Morgan and Lawson, 1974; Roberts and Boyd, 1974; Shreeve and Edwin, 1974).

According to Brent (1976) rumen acidosis established rumen conditions conducive for the development of CCN due to decreased pH that was optimum for

thiaminase activity or proliferation of thiaminase producing bacteria. Boyd and Walton (1977) reported that the high thiaminase activity in the rumen of CCN affected animals could degrade all the thiamine usually ingested or synthesized. Edwin et al. (1982) reared calves on thiamine free synthetic milk and suggested that CCN might result from profound thiamine deprivation alone.

Edwin and Jackman (1982) observed that thiamine was an obligatory nutritional requirement for many forms of animal and plant life. In tissues it occurred as its diphosphate or pyrophosphate (TPP) and was a necessary coenzyme for several reactions in carbohydrate metabolism.

Several investigators have found markedly low concentrations of thiamine in the body tissues, low activity of thiamine dependent enzyme, transketolase in blood and high activity of thiamine destroying thiaminase in the gastrointestinal tract of animals with PEM (Jackman, 1985; Rammell and Hill, 1986; Fakhruddin et al., 1987a; Tanwar, 1987).

Gooneratne et al. (1989b) reported that inadequate microbial synthesis, impaired absorption and utilization of vitamin B_1, presence of vitamin B_1 antimetabolites and increased demand for vitamin B_1 or increased rate of B_1 excretion could lead to thiamine inadequacy. Thiamine deficiency was recorded in sheep exported live by sea. The high sheep density, competitive feeding conditions and high carbohydrate diet caused proliferation of thiaminase producing bacteria in the gastrointestinal tract (Thomas et al., 1990). Lonkar and Prasad (1994a) reported recovery of PEM affected animals following thiamine therapy.

Though the lesions of PEM were not suggestive of a single etiology, it was believed for long time that thiamine deficiency played a major role in the pathogenesis of PEM and early stages of PEM were responsive to paranteral administration of thiamine (Nair, 1999).

In sheep flocks, a drastic change in management such as shearing could precipitate PEM and a change in the diet from hay to corn silage resulted in

decrease in ruminal thiamine concentration (Radostits *et al.*, 2000). According to Maliekal (2000) and George (2002) carbohydrate rich diet could be one of the predisposing factors for the development of the disease in Kerala.

2.2.3 Sulphate

Feeding of rations containing high concentration of gypsum (CaSO$_4$, 2H$_2$O) or other sulphate salts such as sodium sulphate was noticed to be a common cause of several episodes of PEM (Raisbeck, 1982). High sulphate content of drinking water was associated with occurrence of PEM (Hibbs and Thilsted, 1983) since rumen microbes were capable of efficiently reducing sulphate to sulphide which was toxic to brain. Sadler *et al.* (1983) reported development of PEM in 20 out of 60 cattle when fed with 30 per cent roughage diet with an additional 0.72 per cent sulphate in the form of MgKSO$_4$.

The effect of high dietary sulphur supplementation on blood thiamine concentration was studied by Gooneratne *et al.* (1989b). They found that diet high in sulphur decreased the amount of vit. B$_1$ entering the duodenum probably due to reduced synthesis of vit. B$_1$. Sulphur toxicity was implicated as the cause of PEM in Hereford heifers exposed to saline well water (Beke and Hironaka, 1991). Gould *et al.* (1991) suggested that PEM could result from sulphide toxicosis following excess production of sulphide in the rumen.

Rousseaux *et al.* (1991) induced PEM in sheep by feeding high sulphur diet. A dose of 0.63 per cent sulphur in the diet produced PEM between the third and seventh week, which supported the hypothesis that PEM could result from excessive sulphide concentration in the rumen. McAllister *et al.* (1992) induced PEM in lambs by the administration of a sulphide solution into the oesophagus at 20 min intervals for 40 to 120 min and they indicated that this disease could be caused by sulphide toxicosis independent of the metabolic status of the animal. Olkowski *et al.* (1992) induced PEM in sheep with high sulphate diet. Sheep of about two months of age when fed with a diet rich in sulphur (0.63 per cent) developed clinical signs after three to eight weeks. Polioencephalomalacia

developed in mature Hereford crossbred cows on pasture supplemented with a water source containing elevated levels of sodium sulphate (7200 ppm) (Hamlen et al., 1993).

Jeffrey et al. (1994) observed CCN in calves and lambs fed concentrate ration with ammonium sulphate as urinary acidifier and were found non-responsive to thiamine therapy. Cummings et al. (1995) produced PEM in steers by feeding a diet added with sodium sulphate. The onset of signs of PEM was associated with increased sulphide concentration in the rumen fluid. Bulgin et al. (1996) observed PEM in ewes after they were moved to a field sprayed with elemental sulphur. An acute outbreak of PEM in lambs aged 15 to 32 days was observed by Low et al. (1996) after they were introduced to an ad lib concentrate ration containing 0.43 per cent sulphur. McAllister et al. (1997) observed an outbreak of PEM in feedlots with the introduction of high sulphate water during hot weather. Loneragan et al. (1998) reported that pathologic concentrations of ruminal hydrogen sulphide appeared to be a central component in the pathogenesis of sulphur associated PEM.

The dietary content of copper, zinc, iron and molybdenum might also have important modifying influences on sulphur toxicosis. Copper, zinc and iron form insoluble salts with sulphide and their low level would increase the bioavailability of sulphide in the rumen (Radostits et al., 2000).

2.2.4 Cobalt

Hartley et al. (1962) recorded an outbreak of PEM in cobalt deficient sheep in New Zealand. MacPherson et al. (1976); Mann et al. (1983) stated that animals maintained on cobalt deficient diet had low levels of thiamine in tissues.

2.2.5 Water deprivation – Sodium ion toxicosis.

Padovan (1980) described PEM in cattle subjected to water restriction and this disease was due to either deprivation of water or subsistence on water with a high sodium concentration.

2.2.6 Amprolium

Spicer and Horton (1981) induced PEM in sheep using amprolium at the rate of 1g per kg body weight. Fakhruddin *et al.* (1987a) produced PEM in goats by giving amprolium at the rate of 4.50 g as drench by stomach tube daily in three divided doses till the development of symptoms. Amprolium was reported to antagonize absorption of thiamine in intestine by interfering with its phosphorylation (Lonkar and Prasad, 1992b).

Chahar *et al.* (1993) induced PEM in sheep and symptoms developed 40 to 77 days after administration of amprolium. They observed a decrease in the level of thiamine in the serum and rumen contents. Lonkar and Prasad (1994a) observed wide individual variation regarding the total quantity of amprolium required to induce PEM in goats. The quantity required varied from 8.29 to 23.8 g per kg body weight while the time required varied from 57 to 119 days. Polioencephalomalacia was induced in buffalo calves aged 6 to 12 months by drenching amprolium at the rate of 300 mg per kg body weight per day for 25 to 55 days (Tanwar and Malik 1995). They found biochemical changes characteristic of PEM after oral administration of amprolium for 4 to 6 weeks.

Nair (1999) failed to induce PEM in goats by feeding amprolium even at a dose rate of 350 mg per kg body weight for 45 days. Reproduction of clinical signs simulating PEM could be the result of "amprolium poisoning" since amprolium was detected in the brain tissue of experimentally affected animals (Radostits *et al.*, 2000).

2.2.7 Plants and pasture

Evans *et al.* (1975) experimentally induced PEM by feeding diets containing 15 to 25 per cent dried bracken fern rhizomes, a natural source of type I thiaminase. Galitzer and Ochme (1978) observed symptoms and lesions similar to that of PEM in *Kochia scoparia* poisoning in cattle and concluded that plant thiaminase might be involved. Polioencephalomalacia was experimentally produced in horses by feeding Nardoo fern (Pritchard and Eggleston (1978).

Dickie and Berryman (1979) observed heavy mortality in cattle grazed on *Kochia scoparia* pasture and the clinical signs were similar to that of PEM. Hamlen *et al.* (1993) reported CCN in animals when they were shifted from over- grazed to lush pasture. Hill and Ebbet (1997) diagnosed PEM clinically in heifers grazing on chou-moellier (Brassica sps). High sulphur content of the plant and recent change in grazing management were the most likely causes of the outbreak.

2.3 PATHOGENESIS
2.3.1 Thiamine

There are considerable evidences to show that an aberration of thiamine metabolism has a major role in the pathogenesis of PEM.

The biochemical role of thiamine was first revealed by the classical studies of Peters (1936), who demonstrated its implication in carbohydrate utilization. Thiamine mainly as thiamine pyrophosphate played an important role as a co-enzyme in carbohydrate metabolism both in the TCA cycle and the pentose phosphate pathway. Inhibition of carbohydrate metabolism in thiamine deficiency resulted in elevated levels of blood lactate, pyruvate, oxaloglutarate and lowered erythrocyte transketolase activity. This deficient state had general effects on metabolic activity that terminated in energy dependent type of neuronal necrosis (Edwin and Lewis, 1971; Edwin and Jackman, 1973; Rammell and Hill, 1986).

Thiamine pyrophospate was a coenzyme for the decarboxylation of not only pyruvate but also glyoxylate, 2-oxobutyrate, 2-oxoisovalerate, 2-oxoisocaprate, 2-oxo-β methylvalerate hydroxypyruvate, 2-oxoglutarate and 2-oxoadipate. So deficiency would lead to a failure in biosynthesis of one or more enzymes, the relevant substrates would tend to accumulate and could produce clinical signs of thiamine deficiency (Edwin and Jackman, 1981).

Brent and Bartley (1984) did not consider CCN to be due to failure of thiamine synthesis in the rumen or due to any defect in its absorption from the intestines. In the rumen, thiaminase in the presence of a cosubstrate not only

destroyed thiamine but also created thiamine analogues that inhibited one or more thiamine requiring reactions necessary for energy metabolism in CNS.

Thiamine was necessary for the production of thiamine diphosphate, a coenzyme which played a role in the activation of transketolase. Transketolase found in the glial cells and erythrocytes was an important enzyme involved in glucose metabolism. Since the brain was glucose dependent, glial cell transketolase played an important role in brain metabolism (Hamlen et al., 1993).

Deficiency of thiamine resulted in increased blood concentration of pyruvate, reduction in lactate pyruvate ratio and depression of erythrocyte transketolase. Brain had a greater dependence on the pentose phosphate pathway for glucose metabolism in which transketolase was a rate limiting enzyme. Odema of intracellular compartment principally involving astrocytes and satellite cells was suggested to be due to reduction in ATP production following a defect in carbohydrate metabolism in the astrocyte. Such changes decreased the activities of ATP dependent sodium and water transport in the neurons and resulted in intraneuronal swelling (Lonkar and Prasad 1994a; Radostits et al., 1994).

2.3.2 Sulphate

Raisbeck (1982) and Brent and Bartley (1984) reported that sulphite produced from sulphate by rumen microflora caused PEM by a mechanism similar to that attributed to ruminal thiaminase. Durand and Komisarczuk (1988) explained that in rumen, sulphur changed to hydrogen sulphide by the action of rumen micro-organisms. Sulphide was absorbed across the rumen wall at a rate much faster than ammonia and absorption rate was a function of the sulphide concentration and rumen pH. Sulphide also had a direct paralytic effect on the carotid body and this could also inhibit respiration.

Excess dietary sulphur could decrease blood thiamine concentration and this deficiency was sufficient to account for signs and lesions of PEM (Gooneratne et al., 1989a). Excess dietary sulphur exerted an effect on availability of B_1 in ruminants and could be due to possible occurrence of B_1-S divalent

cations in the rumen (Gooneratne *et al.*, 1989b). Short and Edwards (1989) observed that sulphide affected oxidative metabolism by inhibiting the action of catalase, peroxidase, dopaoxidase, carbonic anhydrase, dehydrogenase and peptidases.

The toxic effect of excess dietary sulphur is unique in ruminants. The brain is the target organ. Sulphite derived radicals could cause lipid peroxidation and damage to brain tissue. It was shown that a deficiency of thiamine increased the intensity of lipid peroxidation. Free thiamine was thought to protect the cells by scavenging potentially toxic intermediates generated by the myeloperoxidase hydrogen peroxide halide system (Olkowski *et al.*, 1992). Sulphide inhibited cellular respiration leading to hypoxia and it might be sufficient to create neuronal necrosis and polioencephalomalacia (Radostits *et al.*, 1994).

Bulgin *et al.* (1996) stated that sulphide combined with haemoglobin to create sulfhaemoglobin, reducing the oxygen carrying capacity of blood.

Ruminal sulphide after systemic absorption, might cause decreased ATP production and necrosis of grey matter as neurons were highly susceptible to ATP depletion (McAllister *et al.*, 1997). Loneragan *et al.* (1998) detected blood vessel degeneration in brain and generalized vasculopathy in sulphur toxicosis.

2.4 CLINICAL SIGNS

Jensen *et al.* (1956) observed two forms of PEM in cattle based on severity of symptoms. In the more severe type, the animals were often found prostrate and comatose. They showed severe muscular tremors, twitching of ears and eyelids and convulsions. Those affected with the sub-acute form occasionally pushed the head against solid objects, and were bilaterally blind with excessive salivation and bulbar paralysis.

Temporary blindness, partial protrusion of tongue as though partially paralysed, ruminal atony, ataxia and nystagmus were noticed by Dickie *et al.* (1979). Anorexia, marked depression, weakness of limbs, progressive ataxia of

hind limbs, incoordination of movements, staggering gait, salivation, loss of vision, aimless walking, head pressing, opisthotonus, hyperaesthesia and paddling movements were observed in amprolium induced PEM in goats (Sobhanan, 1981; Fakhruddin *et al.*, 1987a; Tanwar *et al.*, 1987).

Jackman (1985) observed a short period of dullness, wandering aimlessly in wide circles, ataxia, collapse with intermittent convulsions, hyperaesthesia, nystagmus, opisthotonus, trismus and clonic extensor spasms in ruminants. According to Gauri and Vashistha (1988) in naturally occurring PEM in goats there was anorexia followed by depression, progressive dullness, ataxia and staggering gait. In recumbency, the animals had stiffness all over the body, extensor rigidity of both hind limbs, opisthotonus, paddling, convulsions, temporary blindness, nystagmus, absence of menace reflex and normal pupillary response.

Sagar *et al.* (1990) observed impaired vision, decreased response to external stimuli and abnormal gait in calves with PEM. Anorexia, depression, head pressing, blindness, twitching of facial muscles and absence of menace response were reported and in terminal stages, paddling, grinding of teeth, dyspnoea, coma and death were recorded by Lonkar and Prasad, (1992a). Syamasundar and Malik (1993) observed ataxia, kyphosis, lacrimation, opisthotonus, torticollis, sternal/lateral recumbency, coma and death in PEM affected buffaloes.

Tanwar and Malik (1995) observed lacrimation, depression, dullness, anorexia, rigid stance, raising of head, standing motionless for several hours, kyphosis, blindness, nystagmus, hyperaesthesia, convulsions, extensor rigidity of thoracic limbs, paddling and opisthotonus as clinical symptoms in amprolium induced PEM in buffalo calves.

The symptoms observed by Nair (1999) included anorexia, opisthotonus, occasional nystagmus, shivering of the whole body and finally paralysis of the

limbs. Animal pressed their head against hard objects and there was loss of eye preservation reflex.

Maliekal (2000) observed signs such as excitement, depression, aimless wandering, anorexia, ataxia, rolling of eye balls, hyperaesthesia on slight provocation, tonic/clonic convulsions, opisthotonus and star gazing posture in the early stages of PEM in goats. Early stages of the disease in goats were characterized by dullness, depression, staggering gait, head pressing, head tilt and lateral deviation of head (George, 2002).

Pulse and respiratory rates were normal in affected animals except in some cases which showed laboured breathing (Tanwar *et al.*, 1983; Tanwar, 1987; Fakhruddin *et al.*, 1987a). However Sobhanan (1981) reported weak and irregular pulse. Temperature was usually normal but elevated in extreme convulsive phase only (Sobhanan, 1981; Fakhruddin *et al.*, 1987a). Temperature of 38 to 40°C was reported by Tanwar *et al.* (1983).

2.5 RUMEN FLUID
2.5.1 pH

pH of rumen fluid in healthy goats was found to be 7.08 ± 0.06 by Lal *et al.* (1990). 6.88 ± 0.065 by Basak *et al.*, (1993) 6.93 ± 0.27 by Shihabudheen (1998) and 7.08 ± 0.30 by Maliekal (2000).

Tanwar (1987) and Lonkar and Prasad (1994b) observed that pH of rumen liquor did not show any significant difference between control and PEM induced group.

2.5.2 Physical characters
2.5.2.1 *Colour*

Normal colour of rumen liquor in goats varied from olive to brownish green as observed by Dirksen (1979) olive green by Shihabudheen (1998) and Maliekal (2000).

McAllister *et al.* (1997) observed greyish discolouration of ruminal fluid in sulphur induced PEM.

2.5.2.2 *Consistency*

Thick consistency of rumen liquor was reported by Pillai (1988); Shihabudheen (1998) semi liquid (Pradhan *et al.*, 1988) and viscous by Mohan *et al.* (1992) in normal goats.

The consistency of rumen liquor was found to be thick in PEM affected cattle (McAllister *et al.*, 1997) and in goats (Maliekal, 2000).

2.5.2.3 *Odour*

Rumen fluid from healthy animals had an aromatic odour and this depended on the nature of rumen contents (Dirksen, 1979; Mohan *et al.*, 1992; Shihabudheen, 1998; Maliekal, 2000). Pradhan *et al.* (1988) reported an aromatic and goatish odour for the rumen fluid.

Maliekal (2000) reported that the odour of rumen liquor of PEM affected goats was aromatic.

2.5.3 Biochemical characters

2.5.3.1 *Total volatile fatty acids (TVFA)*

Total volatile fatty acids level in normal goats on maintenance ration was 70.08 ± 1.89 mEq/l (Rai *et al.*, 1972), 60.17 ± 4.91 mEq/l (Shihabudheen, 1998), 70.66 ± 2.28 mEq/l (Maliekal, 2000). Total volatile fatty acids concentration in the rumen liquor of PEM affected goats was 67 ± 2.80 mEq/l (Maliekal, 2000).

2.5.3.2 *Sulphate*

The reduction of inorganic sulphate to sulphide takes place in the rumen and the concentration of sulphide in the rumen is very low and usually less than 0.80 µmole/ml. The concentration of total sulphur in the rumen liquor ranged from 10 to 12 mg/100ml and in parotid saliva 0 to 14 µg/ml (Bray, 1969).

The onset of clinical signs in sulfur induced PEM corresponded with the onset of increased ruminal sulphide concentration (Gould *et al.*, 1991). Sulphide concentration in ruminal fluid of steers with clinical signs of PEM and in clinically normal penmates ranged from 144 to 649 μmol/l and 20 to 514 μmol/l respectively (McAllister *et al.*, 1997).

The hydrogen sulphide (H_2S) concentration in ruminal gas cap ranged from 3.20 to 18.77 mg/l with a mean of 12.19 ± 1.64 mg/l in PEM affected calves. Since H_2S concentration in ruminal gas cap were reflective of total ruminal sulphide content it could be used as an on farm diagnostic aid (Loneragan *et al.*, 1998).

2.5.3.3 *Copper and Cobalt*

The literature reviewed did not throw any light on the ruminal copper and cobalt level in healthy ruminants.

2.5.3.4 *Thiamine*

Bechdel *et al.* (1928) reported that thiamine synthesis took place in rumen and that rumen flora were closely associated with this synthetic process. Herriek (1975) observed that thiamine antimetabolites were present in many fungi and bacteria which had grown on spoiled feeds could block synthesis or absorption of thiamine in the rumen.

Gupta *et al.* (1976) noted significantly low rumen thiamine levels in rumen dysfunctions of cattle and buffaloes with respective values of 1.99 ± 0.33 and 1.44 ± 0.23 μg per cent when compared to 7.04 ± 1.04 and 5.81 ± 1.26 μg per cent respectively in healthy animals and attributed the fall in rumen thiamine either to deranged synthesis or destruction of thiamine in the rumen.

Rumen thiamine levels reported in healthy calves were 6.54 ± 1.60 μg/dl (Fakhruddin *et al.*, 1987b) and in goats 6.80 to 8.60 μg/100ml (Tanwar, 1987), 5.94 ± 0.637 μg per cent (Gauri and Vashistha, 1988), 0.40 to 0.70 mg/l

(Thomas *et al.*, 1990), 9.90 ± 0.31 µg per cent (Lonkar and Prasad, 1994), 4.80 ± 0.69 µg per cent (Singh *et al.*, 2000).

Fakhruddin *et al.* (1987b) reported that the level of rumen thiamine was reduced to 1.14 ± 0.16 µg per cent at the onset of clinical signs in cattle and this was responsible for the prominent neurologic signs.

Tanwar (1987) and Gauri and Vashistha (1988) reported that mean thiamine content of rumen liquor was reduced to 1.13 ± 0.339 µg/100ml and 0.400 µg/100ml respectively in PEM affected goats. The mean value of thiamine level in rumen content was 6.61 ± 0.77 µg per cent at the start of experiment and 1.01 ± 0.35 µg per cent at the onset of clinical signs in PEM induced goats (Fakhruddin *et al.*, 1987a).

Mean value of thiamine levels in rumen contents observed before the start of experiment was 6.81 ± 1.04 µg per cent which came down to 1.33 ± 0.21 µg per cent at the onset of clinical signs and again increased to 5.52 ± 0.38 µg per cent after recovery in sheep (Chahar *et al.*, 1993).

Lonkar and Prasad (1994b) conducted an experimental study in goats and reported that at the onset of clinical signs the thiamine level in rumen liquor significantly dropped (1.20 µg per cent) as against the pre-experimental value of 9.40 µg per cent and the value recorded in control counter parts were 9.20 µg per cent. On recovery, the level again increased and were comparable to the normal values. Rumen liquor thiamine level was found to be 0.52 ± 0.25 µg/dl in PEM affected calves (Singh *et al.*, 2000).

2.6 SERUM BIOCHEMISTRY
2.6.1 Glucose

Normal blood glucose level in healthy goats was reported to be 54.66 ± 2.70 mg per cent (Varma, 1967), 52.39 ± 0.92 mg/dl (Pillai, 1988), 59.66 ± 0.98 mg/dl (Lal *et al.*, 1990), 48.24 ± 1.70mg/dl (Das and Misra, 1991), 30 to 65 mg/dl

(Radostits *et al.*, 1994) 47.76 ± 10.23 mg/dl (Shihabudheen, 1998) and 52.83 ± 2.76 mg/dl (Maliekal, 2000).

Blood glucose level was raised to 127.5 mg/100 ml (normal value of 50 to 80 mg/100ml) in an outbreak of PEM in calves (Pass, 1968). Lilja (1973) and Bakker *et al.* (1980) observed a steep increase in blood glucose level just before onset of clinical signs in sheep and calves. Blood glucose level was elevated only when clinical signs were present in sheep and the degree of elevation was associated with severity of clinical signs (Spicer and Horton, 1981). Thomas (1986) recorded a significant rise in blood glucose level to 9.80 mmol/l from reference value of 2 to 6 mmol/l in PEM affected goats.

Glucose level ranged from 136.10 mg/dl to 208.20 mg/dl in amprolium induced PEM in bovines (Kasahara *et al.*, 1989). A steep rise in the blood glucose level from 45.90 to 79.00 mg/dl at the onset of clinical signs and subsequent decline to pre-experimental level after recovery was recorded by Lonkar and Prasad (1993). Syamasundar and Malik (1993) observed a significant elevation in the glucose level at the onset of clinical signs (125.95 ± 11.32 mg/dl) in amprolium induced PEM in buffalo calves. Mean glucose level of 98.50 ± 8.83 mg/dl was observed in PEM affected goats (Maliekal, 2000).

2.6.2 Magnesium

Serum magnesium level in healthy goats was found to be 2.33 mg/dl (Boss and Wanner, 1977), 2.02 mg/dl (Sobhanan, 1981) 2.13 ± 0.06 mg/dl (Pyne *et al.*, 1982), 2.33 ± 0.18 mg/dl (Shihabudheen, 1998) and 1.08 ± 0.15 mg/dl (Maliekal, 2000).

Normal serum magnesium level was recorded in an outbreak of PEM in calves (Pass, 1968), sheep (Spence *et al.*, 1961) lambs (Thornber *et al*, 1981) goats (Sobhanan, 1981, Maliekal, 2000), buffaloes (Tanwar *et al.*, 1994).

Gooneratne *et al.* (1989a) reported that the metabolism of magnesium and thiamine were interdependent. Hamlen *et al.* (1993) observed mild to moderate

elevation in serum magnesium level (1.48 mmol/l; reference range 0.80 to 1.32 mmol/l) in PEM affected cattle.

2.6.3 Copper

Plasma copper concentration in healthy goat was reported to be 75 to 100 µg/100ml (MacPherson et al., 1976), 93.19 µg/dl (Sobhanan, 1981), 0.42 mg/l (Gooneratne et al., 1989a). The mean serum copper concentration in heifers ranged from 7.90 to 18.80 µmol/l (Hill and Ebbet, 1997).

Sobhanan (1981) recorded serum copper value ranging from 67.68 to 130 µg/dl with a mean value of 91.21 µg/dl in natural cases of PEM in goats. Concentration of plasma copper in steers which developed PEM was 0.35 mg/l (Gooneratne, 1989a) while copper level of less than 0.30 mg/l was associated with copper deficiency in cattle. Hamlen et al. (1993) observed plasma copper level of 10.90 ± 1.68 µmol/l in PEM of cattle that consumed water with elevated level of sodium sulphate. Hill and Ebbet (1997) recorded mean serum copper concentration of 2.20 µmol/l with a range of 0.60 to 7.60 µmol/l in polioencephalomalacia affected cattle fed with choumoellier (Brassica sps.)

2.6.4 Cobalt

Serum cobalt levels reported in healthy sheep were 75 to 100 µg per cent (MacPherson et al., 1976).

Hartley et al. (1962) recorded an outbreak of PEM in cobalt deficient sheep in New Zealand.

MacPherson et al. (1976) stated that sheep maintained on a ration designed to produce cobalt deficiency in steers not only became cobalt deficient but also were affected by cerebrocortical necrosis. Cobalt supplementation was effective in preventing the development of CCN. However, MacPherson et al. (1976) failed to confirm further cases of PEM in a group of sheep depleted of cobalt. Mann et al.

(1983) reported that some of the animals maintained on a cobalt-deficient diet had low levels of liver thiamine and they attributed it to defective storage.

2.6.5 Sulphate

Blood levels of inorganic sulphate was positively related to rumen total sulphur and sulphide levels but the upper levels of blood inorganic sulphate were regulated by renal excretion (Comar and Bronner, 1960). Blood inorganic sulphate levels varied markedly over the day between 2 and 6 mg per cent (Bray, 1969).

Accumulation of sulphide in the rumen generally could have an adverse effect on the animal's nutrition. Some quantity of the sulphide passed directly into the blood and exerted toxic effect only in extreme cases (Kandylis, 1984).

2.6.6 Lactate

The lactate concentration in the blood of healthy goats was found to be 13.02 ± 1.02 mg per cent (Pillai, 1988), 9.27 ± 0.39 mg per cent (Lal *et al.*, 1990), 10.72 ± 0.84 mg per cent (Das and Misra, 1991), 9.80 mg per cent (Lonkar and Prasad, 1993) 16.78 ± 0.78 mg per cent (Shihabudheen, 1998) and 16.73 ± 0.48 mg/dl (Maliekal, 2000).

Blood lactate level ranged from 5.25 to 11.06 mmol/l in PEM affected sheep and blood lactate level above 6 mmol/l was indicative of thiamine deficiency (Chick *et al.*, 1981). Lactate values showed marked variations throughout the experimental period in amprolium induced PEM in sheep (Spicer and Horton, 1981).

Thomas (1986) observed an increase in blood lactate level (4.20 mmol/l) in PEM affected sheep and the control values ranged from 0.9 to 2.4 mmol/l. Blood lactic acid level of 54 mg per cent was recorded in calves showing neurological signs of PEM (Kasahara *et al.*, 1989). Blood lactate levels of 47.75 ± 5.60 mg/dl were recorded by Syamasundar and Malik (1993) in amprolium induced PEM in buffalo calves.

An increase in lactic acid level from 9.80 to 28.10 mg per cent was observed at the onset of clinical signs of induced PEM in goats and level became normal on recovery (Lonkar and Prasad, 1993). The mean blood lactate concentration increased about four fold from 1.2 to 4.4 mmol/l at the onset of clinical signs in amprolium fed calves (Tanwar *et al.*, 1994). Maliekal (2000) observed an increased level of lactate 72.06 ± 5.42 mg per cent in naturally occurring PEM cases in goats.

2.6.7 Pyruvate

Blood pyruvic acid content in healthy goats was 1.10 mg per cent (Lonkar and Prasad, 1993) 2.12 ± 0.505 mg/dl (Shihabudheen, 1998) and 2.32 ± 0.07 mg/dl (Maliekal, 2000).

Blood pyruvate levels in experimentally induced PEM ranged from 1.4 to 5.4 mg per cent and the values got reduced by an average of 60 per cent after treatment with thiamine (Pill, 1967). Bakker *et al.* (1980) observed a gradual increase in blood pyruvate level with a steep increase just before the onset of clinical signs in experimentally induced ovine polioencephalomalacia. Blood pyruvate level ranged from 0.08 to 0.26 mmol/l in PEM affected sheep (Chick *et al.*, 1981).

Spicer and Horton (1981) reported that pyruvate levels were elevated in some cases after the appearance of clinical signs but showed wide variation from sheep to sheep. Pyruvic acid level upto 3.76 mg per cent was observed in PEM affected calves (Kasahara *et al.*, 1989).

The levels of pyruvic acid showed only slight increase in the initial stage but at the onset of clinical signs the level abruptly increased (1.2 to 3.5 mg per cent) and returned to pre-experimental value (1.2 mg per cent) following treatment with thiamine (Lonkar and Prasad, 1993). In amprolium induced PEM in buffalo calves, a significant elevation in blood pyruvate level from normal value of 0.86 ± 0.04 to 2.56 ± 0.12 mg per cent was recorded by Syamasundar and Malik (1993).

The mean blood pyruvic acid concentration increased about six fold from 0.05 to 0.30 mmol/l at the onset of clinical signs of amprolium induced PEM in calves (Tanwar et al., 1994). A mean value of 5.07 ± 0.34 mg/dl was recorded in naturally occurring cases of PEM in goats (Maliekal, 2000).

2.6.8 Lactate pyruvate ratio

Lactate pyruvate ratio of 6.17 ± 0.42 was recorded by Shihabudheen (1998) in healthy goats.

Reduction in lactate pyruvate ratio was observed by Mella et al. (1976) in PEM affected bovines. Lonkar and Prasad (1993) observed a lactate pyruvate ratio of 8.5 at the onset of clinical symptoms in induced CCN in goats. However a lactate pyruvate ratio of 20.53 ± 1.36 was noted by Syamsunder and Malik (1993) in PEM affected calves.

Tanwar et al. (1994) observed reduction in lactate pyruvate ratio from 24.40 ± 1.80 to 14.79 ± 0.71 at the onset of clinical signs in amprolium fed calves.

2.6.9 Thiamine

Thiamine concentration in blood of healthy goats was reported to be 4.1 to 5 µg/dl (Tanwar, 1987), 4.01 ± 0.450 µg per cent (Gauri and Vashistha, 1988) and 66 to 178 nmol/l (Rammell and Hill, 1988) 70 to 180 nmol/l for cattle and 71 to 187 nmol/l for sheep (Hill et al., 1987).

Thiamine determination in animals with PEM showed no significant difference between normal and affected animals despite the evidence of thiamine diphosphate deficiency (Loew and Dunlop, 1972a).

Total blood thiamine concentration decreased significantly from the normal value of 26.40 ± 2.30 µg/100ml to 15.20 ± 3.10 µg/100ml during the first week of induction of PEM in sheep (Loew and Dunlop, 1972b). Fakhruddin et al., (1987b) reported serum thiamine level of 1.14 ± 0.16 µg/dl in PEM affected calves.

Blood thiamine level below 17 µg/l indicated thiamine deficiency. The mean thiamine level in the blood serum of PEM affected goats was 3.85 ± 0.06 µg per cent initially and 0.50 ± 0.20 µg per cent at the onset of clinical signs (Hill *et al.*, 1987). Tanwar (1987) estimated blood thiamine level of 0.40 to 1.40 µg/dl in PEM affected goats. Blood thiamine content of PEM affected goats was less than 66 nmol/l with a mean of 29 nmol/l (Rammell and Hill, 1988).

A concentration of less than 40 µg/l of B_1 in blood could be considered as marginal (Gooneratne *et al.*, 1989b). The same authors observed that the concentrations of B_1 in two affected animals were 14.70 and 23.50 µg/l

Gould *et al.* (1991) experimentally produced PEM through excess feeding of sulphur without significant changes in tissue, blood, cerebrospinal fluid, brain and liver thiamine levels. Olkowski (1992) stated that sulphur induced PEM was not likely to be caused by overt thiamine deficiency but thiamine played a major role in the prevention of clinical signs and alleviation of PEM lesions.

Hamlen *et al.* (1993) classified thiamine status in animal into normal with value of 75 to 185 nmol/l, marginal 50 to 75 nmol/l and deficient less than 50.0 nmol/l. The serum thiamine level at the onset of clinical symptoms was significantly low as per Lonkar and Prasad (1993).

Blood thiamine concentrations during an outbreak of PEM were similar in affected and clinically normal steers, and all the values were within reference range of 35 to 75 µg/l (McAllister, 1997).

Loneragan (1998) stated that total blood thiamine in steers affected with dietary sulphur induced PEM was 98.7 ± 4.1 nmol/l (reference range 75 to 185 nmol/l).

2.6.10 Erythrocyte transketolase (TK) and Per cent TPP effect

Transketolase is an enzyme of the hexose monophosphate shunt which uses thiamine pyrophosphate (TPP) as coenzyme and magnesium as cofactor.

Brin *et al.* (1960) employed the following classification to categorize the degree of thiamine deficiency in human.

Normal   0 to 15 per cent TPP effect

Marginal 15 to 24 per cent TPP effect

Severe   > 25 per cent TPP effect

Erythrocyte transketolase (TK) one of the best indicators of thiamine status, was depressed in PEM affected sheep (MacPherson *et al.*, 1976; Grigat and Mathison, 1983).

Edwin *et al.* (1979) stated that in both cattle and sheep, per cent TPP effect tended to be markedly elevated from their respective normal values of 15 and 23 to 172 and 122 per cent in CCN. Sobhanan (1981) observed highly significant increase in the geometric mean of TPP effect (58.69 per cent) in PEM affected goats when compared to healthy goats (20.29 per cent).

In induced PEM of sheep, transketolase level began to decrease well before the onset of clinical signs. This was accompanied by a corresponding rise in TPP effect which suggested the existence of a sub clinical form of PEM (Spicer and Horton, 1981).

Edwin *et al.* (1982) recorded 310 to 392 per cent TPP effect in pre-ruminant calves reared on thiamine free milk. Jackman (1985) noticed TPP effect of 120 to 240 per cent with an average of (172) and 96 to 152 with an average of 122 in CCN affected cattle and sheep respectively. Goats with PEM were found to have low TK activity (35 ± 5 to 18 ± 2 IU/l) with elevated TPP effects (47 ± 20 to 54 ± 4 per cent) Thomas *et al.* (1987).

Per cent TPP effect started increasing rapidly and reached 149.31 ± 18.80 and 146.01 ± 21.00 in treated and control calves respectively. Transketolase activity and per cent TPP effect reached pre experimental values on day 6 of treatment (Syamasundar *et al.*, 1993). Tanwar *et al.* (1994) recorded TK activity

of 14 ± 2.14 IU/l and TPP effect of 352 ± 24 per cent at the onset of clinical signs in amprolium induced PEM in buffalo calves.

2.7 TREATMENT

Pill (1967) suggested that thiamine therapy was useful in cases of PEM. Thiamine appeared to be beneficial at the dose rate of 1g/1000 lb body weight in cattle in conjunction with hypertonic solution and dexamethasone (Pass, 1968). Daly (1968) reported that thiamine was beneficial in cases of PEM at the dose range of 300 to 400 mg for ewe and 1.60 g for the cows.

Smith (1979) recommended intravenous, intramuscular or subcutaneous administration of thiamine at the dose rate of 6.6 to 11mg/kg body weight repeated every six hours for 24 hours as the treatment of PEM in cattle and sheep. Tanwar   et al. (1983) and Jackman (1985) suggested that goats showing typical signs of PEM should be treated with thiamine paranterally as early as possible. Thiaminase activities in the ruminal fluid of ovine PEM were repressed within two hours after oral administration of thiamine propyl disulphide and thiamine hydrochloride (Thomas, 1986).

Thiamine @ 6.62 to 11 mg/kg body weight was the drug of choice for treatment of animals affected with PEM preferably half the dose intravenously and remaining half intramuscularly to be repeated after an interval of 24 hours for three days (Fakhruddin et al. (1987b). Gauri and Vashistha (1988) recommended two ml dexamethasone and mannitol @ 1.5 g/kg body weight along with thiamine in advanced cases of PEM.

Gooneratne et al. (1989b) reported that B_1 and other related compounds could repress extracellular enzyme thiaminase and hence a critical concentration of extracellular B_1 in the rumen fluid might be an important factor for the control of thiaminase production.

Bulgin et al. (1996) found that oral administration of Bismuth carbonate had beneficial effect in PEM due to sulfur toxicosis. They also recommended oral

administration of a broad-spectrum antibiotic to kill sulphide producing rumen flora. Treatment with dexamethasone (0.20 mg/kg body weight. IV, once), thiamine (10 mg/kg body weight IV, once) and sodium ceftiofur (1.1 mg/kg body weight.) was recommended for sulphur induced PEM in calves (Loneragan *et al.*, 1998).

In PEM, the drug of choice was thiamine hydrochloride @ 5 mg/kg body weight. intravenously every three hours on the first day and to be repeated once daily for three days. When treatment was given in the initial stages of the disease a beneficial response within one to six hours was common and recovery occurred in 24 h. Oral administration of thiamine hydrochloride @ of 1 g for goats and 5 g for calves as a drench was indicated when thiaminase was thought to be in the alimentary tract (Radostits *et al.*, 2000).

There was no specific treatment for PEM caused by sulphate toxicity (Radostits *et al.*, 2000).

Thiamine hydrochloride was the drug of choice preferably half dose by intravenous route and half dose by intramuscular route for three days in the early stages of the disease (Singh *et al.*, 2000). Treatment with thiamine hydrochloride @ 50 mg/kg body weight intravenously twice daily along with 15 g bismuth carbonate orally for four days was found very effective in naturally occurring cases of PEM in goats (George, 2002).

2.8 AUTOFLUORESCENCE

Lee and Little (1980) reported that brain autofluorescence at the area of cortical necrosis could be utilized as a method to diagnose cases of PEM. Degradation of the lipid material within the macrophages was responsible for autofluorescence.

Markson and Wells (1982) stated that the areas of CCN were identified by autofluorescence of fresh or formalin fixed tissue under ultraviolet light. Gould *et al.* (1991) recorded blue green autofluorescence in calves affected with PEM.

Ricardo *et al.* (1991) observed creamy white autofluorescence in the brains of calves in induced PEM under UV illumination. McAllister *et al.* (1992) reported that autofluorescent lesions were present in the cerebrocortical grey matter, twenty four hours after sulphide administration.

Formalin fixed brain slices of amprolium fed calves showed a greenish yellow autofluorescence when viewed under UV light at 365 nm as per Tanwar *et al.* (1993). Bulgin *et al.* (1996) observed yellow tan autofluorescence in elemental sulfur toxicosis of sheep. Yellowish green autofluorescence of cortical areas of brain in calves were observed by Sagar *et al.* (1996).

2.9 PATHOLOGY

Dow *et al.* (1963) observed widespread vacuolation and laminar necrosis of cerebral cortex in sodium sulphate induced PEM in pigs. Morgan (1973) reported that the important histological changes in PEM were oedema of astrocytes and degeneration of other cortical elements which was probably secondary. No changes were observed consistently in tissues other than the brain. Brain collected from a range cattle died of PEM was moist, soft and swollen with flattened gyri. On histopathology laminar cortical necrosis was observed (Dickie *et al.*, 1979).

Spicer and Horton (1981) observed swelling and cerebral coning together with separation of the cortex from the white matter in amprolium induced PEM. Microscopically the cerebrocortical grey matter showed varying degrees of changes from acute neuronal necrosis to almost complete destruction of the cerebral cortex. There was capillary endothelial hypertrophy and mild perivascular lymphoid cuffing. Moderate to severe encephalitis characterized predominantly by microgliosis, perivascular lymphocytic infiltration and reactive changes in the astrocytes were present in the white matter. According to Jackman (1985) gross appearance of brain in PEM revealed irregular areas of yellowish discolouration of cerebral gyri.

Enlarged soft and spongy brain with yellowish discolouration particularly of cerebral hemisphere was observed in PEM of goats. Histopathology of brain tissue revealed focal areas of microcavitation, loose matrix and edematous brain tissue with congested vessels. A few lymphocytes and monocytes were seen in the perivascular space (Fakhruddin *et al.*, 1987a). Yellowish discolouration of the dorsal cerebral cortex, especially the occipital lobes was observed in PEM of goats (Tanwar, 1987; Tanwar *et al.*, 1993). The histological lesions consisted of focal necrosis of the cerebral cortex with shrunken neurons, perineuronal vacuolation and dilatation of perivascular space.

Cerebrocortical and subcortical necrosis with microvascular fibrinoid necrosis in the thalamic region were the characteristic lesions in sulphate induced PEM in goats (Olkowski *et al.*, 1992).

Chahar *et al.* (1993) noticed that in PEM affected sheep brain was grossly softened and yellowish. Histologically necrotic changes were observed in cerebral cortex losing their nuclear and cytoplasmic details. Hamlen *et al.* (1993) reported that the brain grossly appeared soft, multifocally flattened and yellowish. Histopathologically, brain showed bilateral and multifocal acute necrosis of cortical grey matter.

According to Lonkar and Prasad (1994a), in amprolium induced CCN in goats, gross lesions of brain included edema and discrete yellowish foci on the cerebral hemispheres. Cerebral lesions were characterized by congestion, edema, microcavitation laminar necrosis, increased perineuronal and perivascular spaces, neuronal degeneration with shrunken angular and triangular neurons, malacic foci, extravasation of erythrocytes, gliosis, satellitosis, perivascular cuffing and prominence of capillary endothelium confined to cerebral cortex.

Loneragan *et al.* (1998) observed that segmental areas of cerebral cortex had pale staining vacuolated neuropil and shrunken angular necrotic neurons with homogenous eosinophilic cytoplasm and pyknotic nuclei. Focal areas of haemorrhage in the thalamus and degeneration of veins and venules of the mid

brain were also noted. A low number of macrophages, lymphocytes and plymorphonuclear cells were evident in the perivascular space.

Nair (1999) studied histological lesions of the brain in experimental and natural cases of PEM in goats. It was characterized by diffuse laminar cortical degeneration and necrosis, occasional neuronal swelling, glial cell reaction and white matter vacuolation. Vascular changes predominated in sodium sulphate induced PEM. A predominant perivascular accumulation of lymphocytes, gitter cells and monocytes were seen in natural cases.

Histopathology of brain of naturally occurring cases of PEM in goats revealed necrosis of neurons of pyramidal and fusiform layers of cerebral cortex with neuronophagia, satellitosis, perivascular edema, perivascular cuffing and neovascularisation of grey matter (George, 2002).

2.10 ULTRASTRUCTURAL PATHOLOGY

Morgan (1974) studied the detailed ultrastructural pathology of brain in ovine PEM. Consistent features of oedematous cortex were swollen, watery astrocytes and satellite cells, many of which showed an apparent increase in nuclear volume. Organellae were dispersed in the electronlucent cytoplasm. Mitochondria were either small and dense or hypertrophied. Smooth and rough endoplasmic reticulum were preserved. Myelinated axons were intermittently separated from their myelin sheath by an electronlucent space. At the areas of edema, many axons showed increased density of axoplasm. Neurons were compressed and showed variable electron density.

Necrotic neurons showed distension of golgi sacules. Golgi sacules showed loss of compact arrangements and ultimately appeared as an array of bizarre membranous fragment and vesicles.

Ultrastructural investigation by Nair (1999) revealed the basic reaction of the brain tissue to be similar in both the experimental and natural cases of PEM in goats except for their intensity. Ultrastructural lesions were characterized by

neuronal swelling, membrane lysis, segregation of the filamentous and granular component of nucleolus, cytoplasmic organellar damage such as fragmentation of RER, partial degranulation of ribosomes, mitochondrial swelling, cristolysis and complete disappearance of organellae. Neuropil spongiosis and splitting of myelin at the intra period line and multiple vacuolations of the white matter were characteristic.

Materials and Methods

MATERIALS AND METHODS

3.1 EPIDEMIOLOGY

Detailed data on the occurrence of polioencephalomalacia were collected from the records maintained in twenty randomly selected Veterinary hospitals in Kerala state covering all the fourteen districts and personally interviewing the field veterinarians through questionnaire. Data were collected for the years 1997 and 1998. Month wise data on the total number of cases, total cases in goats and PEM cases were collected. Information was gathered on the possible predisposing factors and suspected etiology. Details on the age, sex and the symptoms manifested were also collected.

Data on the occurrence of PEM in goats for a period of three years (1999 to 2001) were collected from the records maintained in the Kerala Agricultural University Veterinary Hospital at Mannuthy.

3.2 THERAPEUTIC TRIAL

The study was conducted in the Department of Clinical Medicine, College of Veterinary and Animal Sciences, Mannuthy during the period from 1999 to 2001. Normal (control) values of various parameters were worked out by collecting samples from six apparently healthy animals maintained under identical conditions. Goats brought to the University Veterinary Hospital, Mannuthy with clinical signs suggestive of PEM were subjected to detailed anamnesis and clinical examination. Twenty three cases diagnosed as PEM were utilized for the present study. Two cases were in an advanced stage and died on the second day of treatment. Post-mortem examination was conducted on these animals and samples from the CNS – brain, spinal cord and sciatic plexus were collected for pathological examination. Animals were treated at random with three schedules of treatment so that each schedule consisted of seven animals each (Group I, II and III). All the animals were kept under observation till recovery or death.

Group I - Animals in this group were treated with thiamine hydrochloride (Sisco Research Laboratory) at the dose rate of 50 mg/kg body weight (dissolved in 10 ml of distilled water). Half the dose was given intravenously and other half intra muscularly once daily for four days.

Group II – Animals in this group were treated as in group I along with 500 mg of oxytetracycline orally once (on the day of admission).

Group III – Animals in this group were treated as in group I along with 1 g of thiamine hydrochloride orally once (on the day of admission).

The efficacy of these treatments were compared.

3.3 SAMPLING AND ANALYSIS

Detailed history was collected from the owners of each case. The patient data, present and past history, feeding practices, managemental practices, duration of the disease, possible predisposing factors etc. were recorded as per the proforma given in appendix.

Samples of rumen fluid and blood collected from the clinical cases on zero (before treatment), 48 and 96 hours of experiment were analysed for various parameters under study.

3.3.1 Rumen fluid

Rumen fluid (20 ml) was collected from the diseased goats with the help of rumen fluid extraction apparatus into 25 ml capacity air tight screw capped vials for analysis. At the time of collection of samples, pH of rumen liquor was determined by placing a drop directly on a strip of standard indicator paper (BDH). Rumen contents were strained through a four folded muslin cloth and strained rumen liquor (SRL) was centrifuged for 10 min. at 6000 rpm. The supernatant was stored at $-20^{\circ}C$ till the analyses were completed. Physical characters and protozoal activity of rumen fluid were determined as per the method of Misra and Tripathy (1963). Total volatile fatty acids, sulphate, copper

and cobalt were estimated as per the methods of Barnett and Reid (1957), Snell and Snell (1963) and atomic absorption spectrophotometric methods (Perkin – Elmer model 3380) respectively.

3.3.1.1 *Thiamine*

Thiamine content in the rumen liquor was estimated by thiochrome method (Myint and Houser, 1965) as described below.

Two ml SRL was autoclaved for 30 min. at 121°C after treating with 0.40 ml of 1 N hydrochloric acid and 3.60 ml of N/10 hydrochloric acid. The mixture was further hydrolysed with 4 ml of one per cent diastase in 2 N sodium acetate by overnight incubation in a water bath at 37°C after adding 1 drop of toluene. While gently swirling a 125 ml flask containing 2.50 ml of 30 per cent sodium hydroxide and 0.30 ml of 0.10 per cent freshly prepared potassium ferricyanate solution, 2 ml of the hydrolysate was added to it. The mixture was shaken for 50 sec. and 0.10 ml of 3 per cent, hydrogen peroxide (H_2O_2) was added to it. Further 10 ml of isobutanol was added and shaken for 90 sec. and centrifuged at 800 to 1000 rpm for 30 sec. The isobutanol layer was pipetted off and readings taken in a Fluorometer, using 7-60 as primary and 2-A as secondary filters. The procedure was repeated for preparing sample blank by omitting the addition of 0.10 per cent potassium ferricyanate. Two ml of freshly prepared thiamine hydrochloride working standard (0.1/μg/ml in N/10 HCl) was subjected to the same procedure as for the sample. The concentration of thiamine was calculated using the formula:

$$(S - SB) \times \frac{5}{2} \times \frac{\mu g \text{ Std. thiamine HCl in 2 ml}}{\text{Std. S} - \text{Std. SB}} \text{, where}$$

S - sample reading

SB - sample blank

Std. S - standard sample

Std. SB - standard sample blank

5/2 – Dilution factor

3.3.2 Blood

Eight ml (approximately) of blood was collected from the jugular vein of affected goats in dry glass vials with EDTA @ 1-2 mg/ml as anticoagulent for the preparation of haemolyzate for the estimation of transketolase (TK) and per cent thiamine pyrophosphate (TPP) effect.

Fifteen ml of blood was collected in another test tube for separating serum. Sera thus separated were stored at -20°C till further analyses. The following standard procedures were used for the analysis of various parameters. Glucose was estimated as per Trinder (1969). Serum copper and cobalt were estimated using atomic absorption spectrophotometer (Perkin - Elmer model 3380). Serum sulphate and magnesium were estimated as per the method of Snell and Snell (1963) and Oser (1965) respectively. Lactate was determined as per the method of Noll (1974) and pyruvate by the method of Czhok and Lamprecht (1974).

3.3.2.1 *Estimation of erythrocyte TK level and per cent TPP effect*

Erythrocyte transketolase activity and per cent thiamine pyrophosphate (TPP) effect were measured by the method of Schouten *et al.* (1964) modified by Thomas *et al.* (1990).

The specific reaction for its estimation consisted of incubating the source of the enzyme (erythrocyte haemolysate) with the substrate, ribose-5-phosphate with and without added thiamine pyrophosphate (TPP), which was the coenzyme for transketolase. The resultant sedoheptulose-7-phosphate was then estimated spectrophotometrically after reaction with a chromogenic reagent. Any increase due to added TPP (expressed as per cent TPP effect) was interpreted as showing a lack of thiamine in the blood sample. The higher the per cent TPP effect the greater the degree of thiamine deficiency.

3.3.2.1.1 *Reagents*

The following reagents were prepared as described:

- Ringers buffer – pH 7.4 (volume 512 ml)

NaCl (9.0/l) : 16 ml

KCl (11.5 g/l) : 412 ml

Mg SO$_4$ 7 H$_2$O (38.2 g/l) : 4 ml

K$_2$HPO$_4$ (17.5 g/l) : 80 ml

The pH was adjusted to 7.4 with 1 M HCl.

- Ribose-5-phosphate, disodium salt-0.018M pH 7.4

  Ribose-5-phosphate, disodium salt, 493.38 mg (Sigma F.W. 274.07)

  Ringers' buffer 100 ml

  (Stored frozen in five ml aliquots, stable for 2 weeks at -60°C).

- Thiamine pyrophosphate – 0.002M pH 7.4

  Thiamine pyrophosphate, 92.10 mg (Sigma, F.W. 460.5)

  Ringer's buffer 100ml

  (Stored frozen in 5 ml aliquots, stable for 2 weeks at - 60°C).

- Trichloroacetic acid – 15% (Stored at room temperature)

- Sulphuric acid – water mixture – 7.2M H$_2$SO$_4$ (Stored at room temperature)

  Concentrated sulphuric acid, 564 ml

  Distilled water 224 ml (Add Sulphuric acid drop by drop to water).

- L-cysteine hydrochloride – 3% (Prepare fresh, hold at 4°C)

- Standard Sedoheptulose

  Stock standard sedoheptulose solution – 0.01M Sedoheptulose anhydride monohydrate, 210.20 mg (Sigma, F.W. 210.2) was dissolved in 100 ml distilled water and stored frozen in 10 ml aliquots in polypropelene tubes.

Working standard sedoheptulose solution – 1 to 4 μmol/ml. It was prepared by diluting 1, 2, 3 and 4 ml each of stock standard sedoheptulose solution to 10 ml with distilled water.

3.3.2.1.2 *Procedure*

The following procedure was adopted to measure erythrocyte TK activity and TPP effect:

- Eight ml of blood was collected in a centrifuge tube containing EDTA as anticoagulant. It was then centrifuged for 5 min. at 3000 rpm. Plasma and buffy coat were removed. The packed erythrocytes were frozen at -20°C. After complete freezing, sample was thawed to facilitate haemolysis.

- Fourteen tubes were labelled each in duplicate as B (Blank), TK (Transketolase activity), TPP (thiamine pyrophosphate effect), S_1, S_2, S_3 and S_4 (standards 1 to 4 μmol/ml). To each tube, 0.20 ml Ringer's buffer was added except in TPP tubes, in which 0.20 ml thiamine pyrophosphate solution was added.

- To tubes marked B, TK and TPP, 0.20 ml of hemolysate was added while in each of the four standard tubes, 0.20 ml of 1.0, 2.0, 3.0 and 4.0 μmoles of working standard sedoheptulose solution was added.

- All the tubes were capped with parafilm.

- Hemolysates containing tubes were again frozen and thawed to ensure complete haemolysis of erythrocytes. The contents in all the tubes were mixed well.

- All the tubes were incubated at 37°C for 30 min. The contents in all the tubes were mixed well carefully.

- To blank tubes, 0.40 ml of 15 per cent trichloroacetic acid was added. Then 0.40 ml of Ribose-5-phosphate solution was added to all the tubes and mixed well.

- All the tubes were reincubated at 37°C for 30 min. Then to all tubes, 0.40 ml of 15 per cent trichloroacetic acid was added except in blanks. The contents in the tubes were well mixed.

- All the tubes were centrifuged at 3000 rpm for 20 min. From each tube 0.20 ml clear supernatant was transferred into appropriately labelled tubes. To each tube 4.80 ml of sulphuric acid-water solution was added. It was mixed well and all the tubes were immersed in a 100°C boiling water bath for 4 min.

- All the tubes were cooled to room temperature. To each tube 0.20 ml of 3 per cent L-cysteine hydrochloride solution was added and mixed well. The tubes were allowed to stand overnight for colour development.

- Samples TK, TPP and standards were read against blank on spectrophotometer at 510 and 540 nm.

3.3.2.1.3 *Calculation*

The transketolase activity in the blood is expressed in International Unit. One International Unit of erythrocyte transketolase (TK) activity is expressed as number of micromoles of sedoheptulose-7-phosphate formed per min. per litre of packed RBC under the described conditions. It was determined with the following formula:

$$TK = \frac{\Delta OD\ sample}{\Delta OD\ standard} \times \frac{Concentration\ of\ standard}{30} \times 1000$$

where,

30   = Time in minutes

1000   = Factor for per litre of packed RBC

$$= \Delta OD \text{ sample} \times \frac{1000}{30} \times \frac{\text{Concentration of standard}}{\Delta OD \text{ standard}}$$

$$= \Delta OD \text{ sample} \times 33.33 \times \frac{1}{\text{gradient of standard curve}}$$

The gradient of standard curve was determined by dividing each ΔOD of standard value of 1.0, 2.0 3.0 and 4.0 μmoles concentration with 1, 2, 3 and 4 respectively and then taking the mean of all the values.

3.3.2.1.4 *Per cent TPP effect*

It was calculated as the relative enhancement of enzyme activity after saturation of the apoenzyme with TPP *in vitro* with the following equation:

$$\% \text{ TPP effect} = \left(\frac{\text{TK activity in tube TPP} \times 100}{\text{TK activity in tube TK}} \right) - 100$$

3.3.2.2 *Total thiamine hydrochloride in blood serum*

This was measured by thiochrome method of Myint and Houser (1965) this was done as for whole blood with the following changes. Added 0.20 ml of N HCl, 3.80 ml of N/10 HCl and 2.0 ml of one per cent diastase to 2.0 ml of blood serum making the final volume as 8.0 ml. Thiochrome measurement was done with 2.50 ml of serum filtrate and for calculation a dilution correction factor of 8/5 was used.

3.3.2.2.1 *Oxidation of thiochrome and measurement*

In a 125 ml flask 2.50 ml of 30 per cent (w/v) NaOH was mixed with 0.30 ml of 0.10 per cent (w/v) K_3Fe $(CN)_6$. While the flask was swirled gently 2 ml of blood filtrate (hydrolysate) was added slowly. The mixture was shaken for 50 sec. before adding 0.10 ml of 3.0 per cent H_2O_2. The mixture was again shaken with 10 ml of Isobutanol (Columbia Organic Chemical Co.) for 90 sec. followed by centrifugation at 800 to 1000 rpm for 30 sec. The Isobutanol layer was pipetted off and read in the fluorophotometer set at 100 with 0.25 μg/ml quinine sulfate

standard solution in N/10 H_2SO_4. The procedure was repeated for the Sample Blank, omitting the 0.10 per cent $K_3Fe (CN)_6$.

3.3.2.2.2 U.S.P. *Thiamine hydrochloride reference standard solution and measurement*

Thiamine hydrochloride standard stock solution of 100 μg/ml in 20 per cent (v/v) ethyl alcohol was prepared and adjusted to a pH of 3.5. Fresh thiamine hydrochloride standard solution of 0.10 μg/ml in N/10 HCl was then prepared daily from this stock solution. The same procedure was followed with 2.0 ml of this 0.1 μg/ml standard solution as with the blood filtrate and blood filtrate blank.

3.3.2.2.3 *Calculation*

The thiamine hydrochloride (μg/ml) of whole blood was calculated as follows: $(S.SB) \times 5/2 \times \dfrac{\text{μg standard thiamine hydrochloride in 2 ml}}{(\text{Standard S} - \text{Standard SB})}$, where

S - Sample reading

SB - Sample blank reading

Standard S - Standard reading

Standard SB - Standard sample blank reading.

3.4 STATISTICAL ANALYSIS

Statistical analysis was conducted according to the method described by Snedecor and Cochran (1980).

3.5 AUTO FLUORESCENCE

Neutral buffered formalin fixed slices of the brain from different regions of the cerebrum were examined for autofluorescence in a UV cabinet at 360 nm wave length (Markson and Wells, 1982).

3.6 PATHOLOGY

Two animals did not respond to the therapy and succumbed to the disease. Portions of CNS from these animals were collected for detailed examination. The brain, spinal cord and plexus were preserved immediately in 10 per cent buffered neutral formalin. After 24 h. of fixation, sections were sliced into small pieces to ensure examination of a wide range of neuroanatomical structures. Several regions of the CNS comprising of the anterior, middle and caudal parts of cerebral hemispheres, hippocampus, caudate nucleus, mid brain, thalamus, cerebellum, pons, medulla oblongata, cervical region of the spinal cord and plexus were fixed in neutral buffered formalin until further processing.

Preserved tissues were processed and paraffin sections prepared. The sections were stained with Haematoxylin and Eosin (Sheehan and Hrapchack, 1980) for histopathological studies.

3.7 ULTRA STRUCTURAL PATHOLOGY

A few tissues from different parts of the brain were also collected and fixed immediately in 2.50 per cent phosphate buffered glutaraldehyde, rinsed in phosphate buffer and post fixed in one per cent osmium tetroxide. Tissue blocks were processed through graded acetone series and embedded in spurr. Sections were cut with glass knives in a Leick ultracut. Thin sections were picked up on uncoated copper grids, stained with uranyl acetate and lead citrate and examined in a Hitachi 600 A electronmicroscope at an accelerating voltage of 50 KV.

Results

RESULT

Twenty one cases which exhibited signs suggestive of PEM were subjected to detailed clinical investigation and the tentative diagnosis was confirmed. These animals were subjected to therapeutic trials. Two animals brought to the hospital with severe clinical symptoms succumbed to the disease. Post-mortem examination was conducted on these animals and samples from the CNS were collected for pathological examination.

4.1 EPIDEMIOLOGY

The number of cases reported from the 20 veterinary hospitals of the fourteen districts in Kerala are set out (Table 1 and Fig. 1). The study revealed that out of the total number of cases 15.39 per cent were of goats. Average percentage occurrence of PEM during 1997 and 1998 were 1.09 and 1.19 per cent respectively. The mean percentage occurrence of PEM for the above two years came to 1.14 per cent. The disease was not recorded in certain parts of Kerala but went upto 3.61 per cent in other parts. The highest prevalence of PEM was recorded in Malappuram district (3.61 per cent) followed by Thrissur (3.17 per cent), Idukki (2.51 per cent) and Kozhikode (1.98 per cent). The disease was recorded throughout the year (Table 2 and Fig. 2). Maximum occurrence was noted during the month of October followed by December, November and September and comparatively low incidence was observed in the months of May, June, July and August.

The responses of the veterinarians of the selected hospitals with regard to certain queries were as follows.

Nutrition deficiency, particularly thiamine was considered by many as the causative factor for the occurrence of the disease. This observation was based on the effectiveness of the treatment with thiamine @ 10 mg/kg body weight during the initial phase of the disease. Some veterinarians opined that as only a few animals in a group were affected at a time, the relationship of vitamin / mineral deficiency with the occurrence of PEM could not be a simple one. Majority

Table 1: District-wise distribution of PEM cases during the year 1997 and 1998

District	Total No. of cases	Total No. of cases in goats	Total No. of PEM cases	Percentage incidence among total cases in goats
Thiruvananthapuram	14623	3615	13	0.35
Kollam	7896	2850	30	1.05
Pathanamthitta	23180	4852	33	0.68
Idukki	29492	3977	100	2.51
Kottayam	21668	4100	33	0.80
Alappuzha	9483	2001	8	0.40
Ernakulam	12855	3529	28	0.80
Thrissur	7271	756	24	3.17
Palakkad	13278	2673	21	0.79
Malappuram	2714	443	16	3.61
Kozhikode	11646	2469	49	1.98
Wyanad	54316	1833	19	1.03
Kannur	5461	243	4	1.65
Kasargod	13603	1592	19	1.19
Total	227486	34933	397	

Total cases in goat – 15.39 per cent
PEM cases   – 1.14 per cent

Fig. 1 District - wise distribution of PEM cases during the year 1997 and 1998
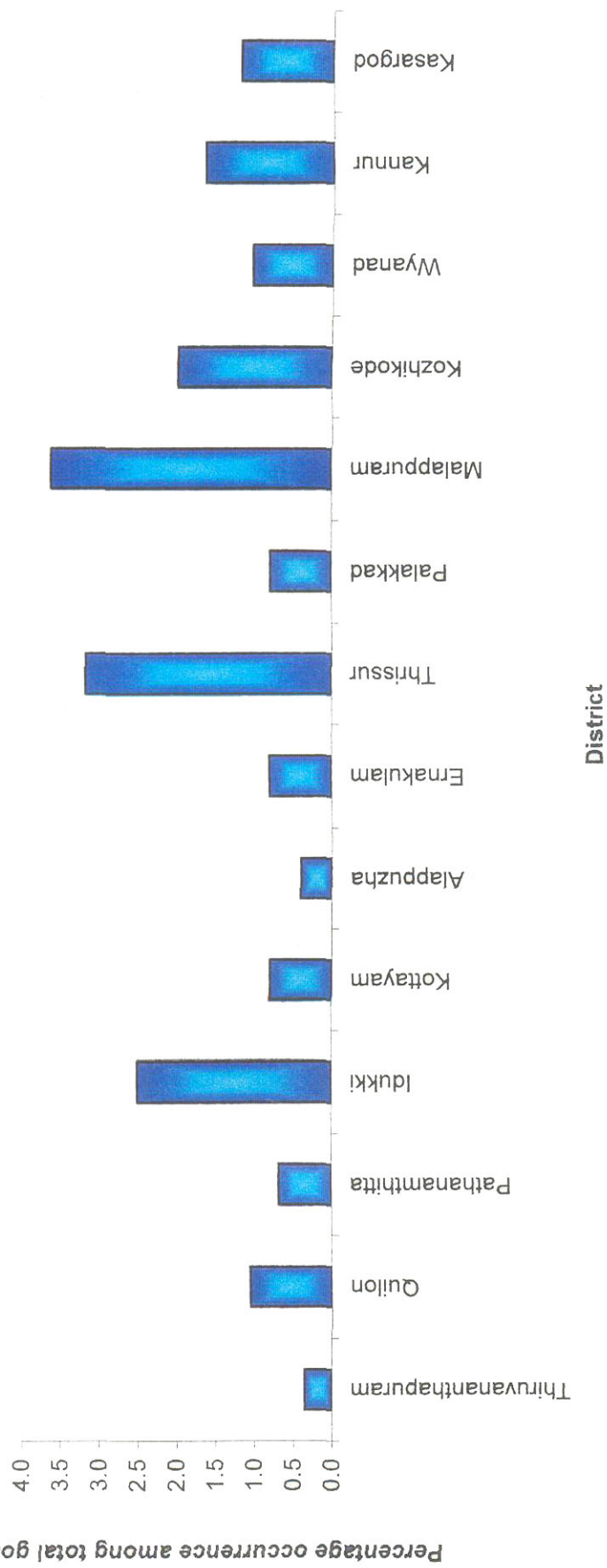
Table 2: Month-wise occurrence of PEM among goats during 1997 to 1998 (values in percentage)

Place	Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Attukal (Trivandrum)	1997	0.99	-	2.27	1.08	-	-	-	-	1.17	-	1.35	-
	1998	0.86	-	-	1.13	0.98	2.29	-	1.37	-	1.05	-	-
Aruvikkara (Trivandrum)	1997	-	-	-	-	-	-	-	-	-	-	-	-
	1998	-	-	-	-	-	-	-	-	-	-	-	-
Parakkadavu (Kollam)	1997	1.04	-	2.22	0.94	1.63	0.93	0.76	1.56	2.19	-	-	1.57
	1998	-	0.69	1.32	1.81	1.01	1.22	1.35	-	-	1.60	1.53	0.73
Ranni (Pathanamthitta)	1997	-	-	-	-	-	-	-	-	-	-	-	-
	1998	-	-	-	-	-	-	-	-	-	-	-	-
Thiruvalla (Pathanamthitta)	1997	0.79	0.71	0.61	1.31	0.71	1.23	1.29	-	0.82	2.05	0.90	-
	1998	1.40	-	2.43	0.92	1.39	1.23	0.88	1.14	-	0.77	1.47	1.66
Cherthala (Allappey)	1997	-	-	-	-	-	-	-	-	-	1.28	-	-
	1998	-	-	-	0.81	-	-	-	-	-	-	-	7.69
Pala (Kottayam)	1997	0.6	-	1.14	0.4	-	0.41	1.26	-	0.74	0.94	-	0.78
	1998	1.05	1.48	1.21	1.37	1.85	-	2.54	1.5	0.69	-	1.9	2.04
Thodupuzha (Idukki)	1997	2.02	12.22	4.08	1.88	3.1	2.72	1.31	2.2	1.62	3.57	4.86	3.2
	1998	2.18	0.74	0.62	1.94	2.67	3.03	2.87	2.63	2.71	2.56	3.06	4.03
Valakode (Idukki)	1997	-	-	-	-	-	-	-	-	-	-	-	-
	1998	-	-	-	-	-	-	-	-	-	-	-	-
Pancode (Ernakulam)	1997	0.3	-	0.69	1.02	1.6	-	0.98	0.90	0.92	-	1.47	0.76
	1998	0.69	-	1.32	0.76	1.41	0.87	1.83	-	1.84	0.78	0.93	0.88

Contd......

Place	Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Odakkali (Ernakulam)	1997	-	-	-	-	-	-	2.63	-	-	3.44	-	-
	1998	-	-	-	-	-	-	-	-	-	-	-	-
Velikulangara (Thrissur)	1997	4.16	7.14	5.4	-	-	3.33	3.44	2.63	3.03	-	10	-
	1998	2.94	6.66	4.76	3.12	-	2.5	-	-	2.38	2.63	-	-
Alathur (Palakkad)	1997	-	1.04	2.24	1.96	-	-	1.05	-	0.75	1.73	2.35	2.29
	1998	0.82	-	0.64	0.75	-	-	0.95	-	1.73	0.78	-	1.04
Karuvarakundu (Malappuram)	1997	4.54	-	8.33	-	-	6.25	-	-	18.7	-	-	2.77
	1998	-	-	-	-	4.54	11.11	9.09	-	-	10	13.6	3.33
Chelanoor (Kozhikkodu)	1997	2.65	2.83	2.06	7.35	1.17	1.26	2.38	1.96	1.09	1.04	1.81	1.19
	1998	1.86	-	1.82	3.12	-	-	2.53	1.44	1.66	1.9	1.08	2.02
Kalpetta (Wayanad)	1997	-	-	1.56	1.47	-	2.94	-	-	0.89	1.02	-	1.38
	1998	0.94	1.28	-	2.17	4.83	1.61	-	0.98	-	2.66	1.53	-
Kolacherymukku (Kannur)	1997	-	-	-	-	-	-	-	-	-	33.33	-	-
	1998	12.5	-	-	-	-	-	-	6.25	-	-	-	7.69
Kodakkad (Kasargod)	1997	3.84	-	3.22	4.76	2.22	-	3.57	-	4.54	4.0	-	6.45
	1998	-	6.25	7.14	-	2.56	-	2.38	3.03	-	3.7	-	2.85
Ravaneswaram (Kasargod)	1997	-	-	-	4.0	-	-	-	-	-	-	-	-
	1998	-	-	-	-	-	-	-	-	-	-	-	-
Trikaripur (Kasargod)	1997	-	-	-	-	-	-	-	-	-	-	-	-
	1998	-	-	-	-	-	-	-	-	-	-	-	-
Total		1.15	1.03	1.17	1.10	0.79	1.07	1.08	0.69	1.19	2.02	1.20	1.36

Fig. 2: Month-wise occurrence of PEM among goats during 1997 to 1998
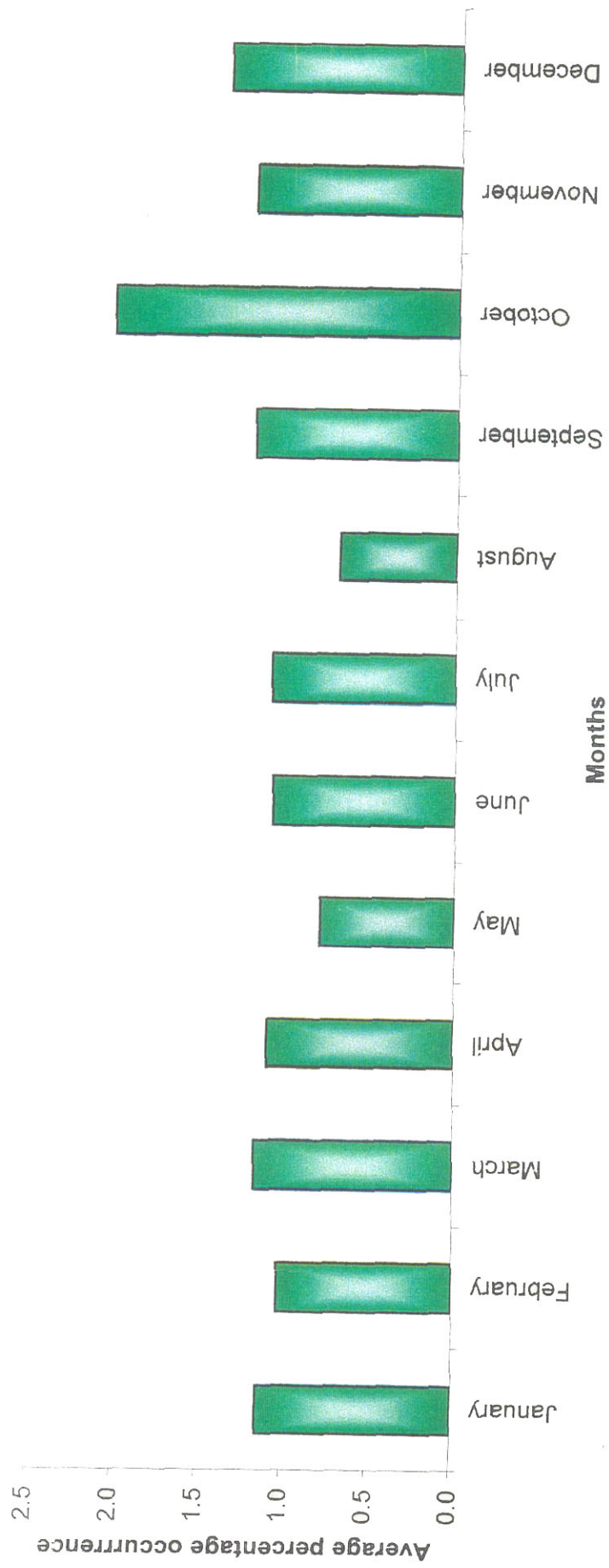
attributed feeding of cooked rice and high amount of concentrates without adequate roughage as the most probable predisposing factor for the disease.

A small percentage of veterinarians suggested the possibility of plant toxicoses. One field veterinarian reported the usage of sulphur dusting powder as a fungicide in rubber plantations as the probable dietary cause of this condition. No relationship was attributed between the occurrence of the disease and the water provided.

Eighty nine per cent of the veterinarians who have recorded PEM reported that there was no sex influence on the occurrence of this disease. The age group of the affected animals, as per the opinion and observation of veterinarians suggested that goats of one to three years of age were more susceptible. The managemental system followed by majority of farmers in the state was semi-intensive.

Treatment pattern adopted by field veterinarians included injection of vitamin B_1 at a dose rate of 10 mg/kg/day along with supportive therapy with parentral administration of drugs like calcium, dextrose saline and liver extracts. The percentage of treatment success ranged from 50 to 98 per cent. The mortality rate increased when the cases were presented to the hospital in advanced stage of the disease.

The data collected on the occurrence of diseases of goats from the University Veterinary Hospital during the period from 1999 to 2001 revealed that 17.20 per cent of the cases were that of goats and out of this 1.24 per cent were PEM (Table 3 and Fig. 3). Females were found to be more prone to this condition. Out of 66 thiamine responsive PEM presented to the veterinary hospital, 53 goats (80 per cent) were females and the rest were males (Fig. 4). The age of the affected animals ranged from one month to five years with maximum occurrence in the age group of six months to two years.

The history revealed that none of these animals were previously affected with a similar disease. History did not suggest any predisposing causes for the development of the disease. Most of them were usually fed with rice-gruel and

Table 3: Month – wise occurrence of PEM in the college hospital during 1999-2001 (values in percentage)

Month	Total no. of cases	No. of cases in goats	No. of PEM	PEM cases in %
January	2404	479	7	1.46
February	2338	399	3	0.75
March	2723	532	3	0.56
April	2168	399	4	1.00
May	2289	418	5	1.19
June	2321	384	5	1.30
July	3120	510	7	1.37
August	2641	348	10	2.87
September	2518	482	6	1.24
October	2889	495	4	0.80
November	2554	464	8	1.72
December	2895	394	4	1.02
Total	30860	5304	66	

Total goat cases =17.20 per cent

PEM cases among goat cases =1.24 per cent

Fig. 3: Month - wise occurrence of PEM in college hospital during 1999 to 2001
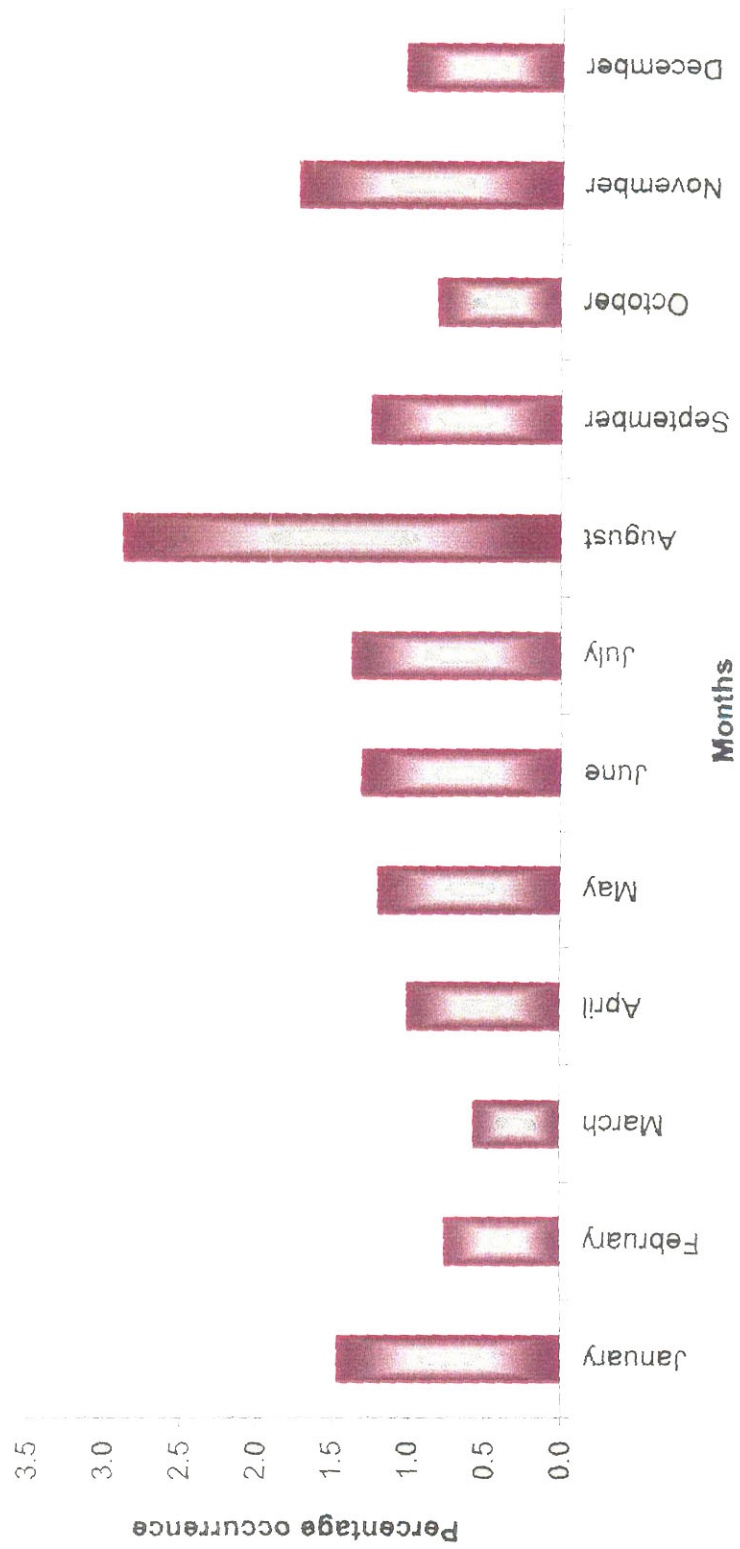
Fig. 4: Sex wise frequency of PEM among goats in college hospital

Female
Male

20%

80%
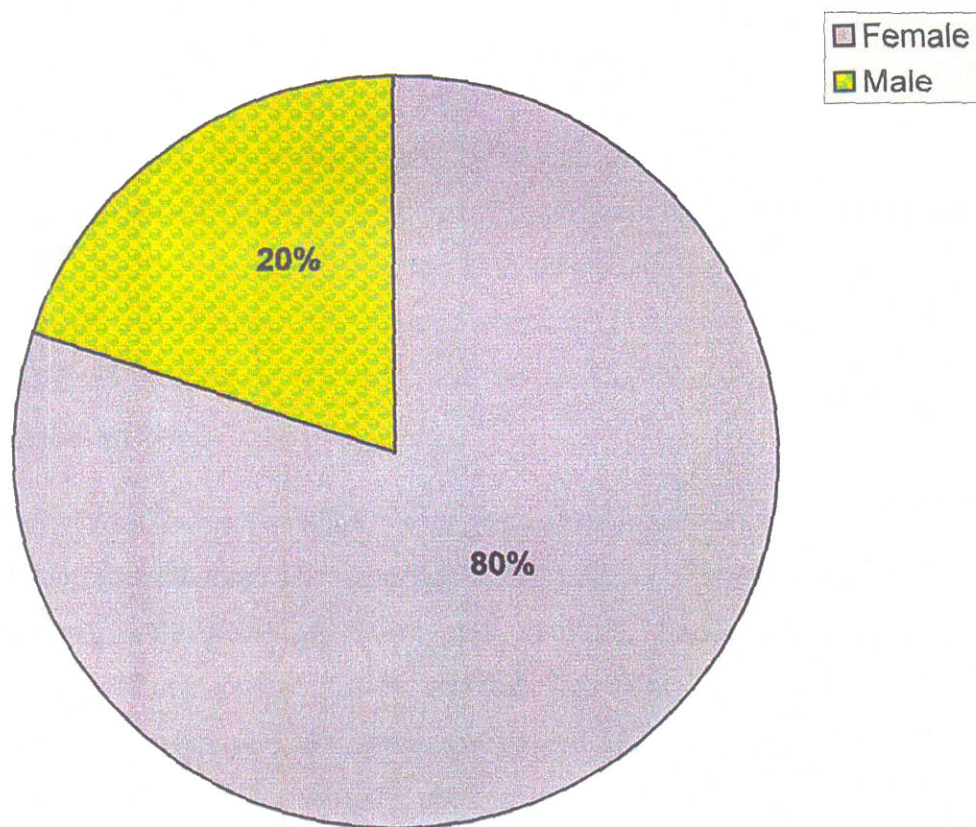
small quantities of ground nut-cake or coconut-cake and jack tree leaves. Usually they were sent out in the afternoon for browsing.

The occurrence was more in the month of August (2.87 per cent), followed by November (1.72 per cent), January (1.46 per cent), July (1.37 per cent) and June (1.30 per cent). Occurrence was less in the months of March (0.56 per cent) and February (0.75 per cent).

4.2 THERAPEUTIC TRIALS
4.2.1 Clinical signs

Clinical signs observed before treatment and during the course of treatment are presented. In the early stages of the disease the following symptoms were reported by the owners. Most of the animals were dull and depressed. Some of the animals were excited. Continuous/intermittent bleating followed by low carriage of head, leaning on the walls, aimless wandering, ataxia, and rolling of eye balls were reported.

Out of the 21 cases of PEM, seven goats were presented to the hospital in a state of lateral recumbency. The important clinical signs observed were nystagmus hyperaesthesia, with paddling movements of limb, tonic/clonic convulsion, opisthotonus, and star-gazing posture. Animals lied down only on one side. On turning to the opposite side, the animals struggled violently and returned to the earlier position. Most of the animals used to eat green leaves and concentrates even when they were in lateral recumbency. Salivation and grinding of teeth with slight protrusion of tongue were noted in two affected animals. Menace response was sluggish to absent but pupillary reflex was normal. Corneal opacity and blindness developed in two animals due to prolonged recumbency and self inflicted injury to the eye. Miosis was observed in four cases. Clinical signs in mild cases included dullness, low carriage of head, lateral kink of neck, leaning on the walls, pressing of head against solid objects, broad based stance, aimless wandering, ataxia, staggering gait, swaying of hind quarters and frequent falling down.

Plate 1: Goat affected with PEM before treatment

Plate 2: Goat affected with PEM – third day of treatment

Plate 3: Goat affected with PEM – fifth day of treatment

Plate 4: Goat affected with PEM – radial paralysis

Plate 5: Goat affected with PEM - after recovery

Plate 1

Plate 2

Plate 3

Plate 4

Plate 5

Goat affected with PEM – Clinical signs

Plate 6 : Pressing of head on hard object

Plate 7 : Opisthotonus

Plate 8 : Head tilt

Plate 9 : Lateral deviation of neck

Plate 10 : Broad based stance

Plate 6

Plate 7

Plate 8

Plate 9

Plate 10

Dribbling of urine was observed in one male goat. During the course of treatment one of the severely affected animals in Group I developed radial paralysis and it recovered after physiotherapy (Plates 1 to 10).

The rate of respiration in the healthy group was 30.00 ± 1.93 / minute. In animals of group I, II and III it was 36.57 ± 4.37, 37.42 ± 3.19 and 48.00 ± 7.42 per minute respectively on the day of admission. A statistically significant increase in respiration rate ($p \leq 0.05$) was noticed only in group III when compared to normal value. The variations noticed at 48 h and 96 h were only marginal.

Pulse rate in healthy control was 78.50 ± 2.55 / minute. At zero hour the corresponding values were 90.71 ± 5.72, 90.00 ± 7.99, and 94.86 ± 6.05 per minute in animals of group I, II and III respectively. The zero hour value was significantly higher ($p \leq 0.05$) in group III and became comparable to normal value by 48 h.

The mean body temperature in healthy control was 102.26 ± 0.17°F. The variations noticed in the diseased goats during the period of study were marginal and within normal range (Table 4a and 4b).

Conjunctival mucous membrane of all affected animals were pale roseate except in four cases in which mucous membrane along with the whole eye was reddened due to rubbing and occasional hitting of head on the ground.

Rumen motility was not affected in majority of the cases except for a slight decrease in the intensity (sluggish) in three cases.

4.3 RUMEN FLUID
4.3.1 pH

The mean pH of the rumen liquor in normal animals ranged from 6 to 8 with a mean value of 7.0 ± 0.25. The mean pH of the rumen liquor at zero hour in group I, II and III was 7.0 ± 0.21, 7.0 ± 0.21 and 7.14 ± 0.26 respectively. There was no statistically significant difference between the normal value and zero hour value. The variations observed in pH in different groups of animals during the

Table 4a : Mean clinical data of normal goats (mean ± SE)

Respiration (per minute)	Pulse (per minute)	Temperature (°F)
30.0 ± 1.93	78.50 ± 2.55	102.26 ± 0.17

Table 4b : Mean clinical data of animals of Group I, II and III at 0, 48 and 96 h of therapeutic trial (mean ± SE)

Group	Respiration (per minute)			Pulse (per minute)			Temperature (°F)		
	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h
I	36.57 ± 4.37	34.0 ± 1.53	31.71 ± 1.48	90.71 ± 5.72	82.29 ± 3.19	81.71 ± 3.79	101.81 ± 0.52	101.94 ± 0.30	102.09 ± 0.26
II	37.42 ± 3.19	44.14 ± 5.44	40.86 ± 2.92	90.00 ± 7.99	82.57 ± 3.23	74.29 ± 1.27	102.51 ± 0.46	102.11 ± 4.4	101.94 ± 0.24
III	48.00 ± 7.42*	35.29 ± 1.78	34.0 ± 1.69	94.86 ± 6.05*	84.57 ± 1.62	78.0 ± 3.60	102.51 ± 0.28	102.34 ± 0.23	101.89 ± 0.16

* - Significant at $p \leq 0.05$ when compared with normal values

observation period were only marginal and within the normal range (Table 5a and 5b).

4.3.2 Physical characters

Rumen liquor collected from the goats in the normal and diseased groups at zero, 48 and 96 hour of study had greenish yellow/olive green colour, aromatic odour and thick consistency with heavy concentration of protozoa. No significant difference was noticed in the physical characters of rumen liquor of all the groups when compared with normal.

4.3.2.1 *Protozoan motility*

Protozoan motility in normal animals and animals of Group I, II and III at different hours of observation ranged from moderate (++) to vigorous (+++) (Table 5a and 5b).

4.4 BIOCHEMICAL CHARACTERS
4.4.1 Total volatile fatty acids (TVFA)

Total volatile fatty acids concentration in the rumen liquor of healthy animals was 55.00 ± 1.87 mEq/l.

The mean TVFA concentration of group I, II and III before the treatment were 55.60 ± 2.63, 53.86 ± 2.95 and 52.86 ± 3.92 mEq/l respectively and were not significantly different from the normal value. Variations noticed after the initiation of therapy were only marginal and within the normal range (Table 5a and 5b).

4.4.2 Sulphate

The mean sulphate concentration in rumen liquor of healthy animals was 6.10 ± 0.58 mg/dl. In animals of group I, II and III it was 6.41 ± 0.70 mg/dl, 5.54 ± 0.47 mg/dl and 6.97 ± 0.72 mg/dl respectively at zero hour. The differences observed between the normal and zero hour values of diseased goats were not

Table 5a : Rumen liquor - pH, protozoal activity and TVFA in normal goats (mean ± SE)

pH	Protozoal activity	TVFA (mEq/l)
7.0 ± 0.25	(++) – (+++)	55.0 ± 1.87

Table 5b : Rumen liquor - pH, protozoal activity and TVFA in animals of Group I, II and III at 0, 48 and 96 h of therapeutic trial (mean ± SE)

Group	pH			Protozoal activity			TVFA (mEq/l)		
	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h
I	7.0 ± 0.21	7.0 ± 0.22	7.14 ± 0.26	(++) – (+++)	(++) – (+++)	(++) – (+++)	55.60 ± 2.63	53.96 ± 3.84	56.29 ± 3.44
II	7.0 ± 0.21	6.86 ± 0.26	7.14 ± 0.14	(++) – (+++)	(++) – (+++)	(++) – (+++)	53.86 ± 2.95	58.29 ± 5.07	57.0 ± 4.44
III	7.14 ± 0.26	7.0 ± 0.22	7.14 ± 0.26	(++) – (+++)	(++) – (+++)	(++) – (+++)	52.86 ± 3.92	55.29 ± 3.86	51.29 ± 4.39

significant. Variations noticed at 48, and 96 h in group I, II and III were only marginal and within the normal range (Table 6a and 6b).

4.4.3 Copper

The mean copper content of rumen fluid of healthy animals was 1.12 ± 0.08 ppm. In diseased groups, the mean concentration of copper ranged from 0.86 ± 0.07 to 1.13 ± 0.07 ppm. No statistically significant difference was observed between normal and diseased groups (Table 6a and 6b).

4.4.4 Cobalt

The mean cobalt content of rumen liquor of healthy animals was 0.23 ± 0.02 ppm.

The cobalt concentration was 0.27 ± 0.02 ppm in group I, 0.22 ± 0.02 ppm in group II and 0.22 ± 0.03 ppm in group III animals at zero hour. No significant difference noticed at zero, 48 and 96 h in cobalt level in animals of group I, II and III when compared to normal value (Table 6a and 6b).

4.4.5 Thiamine

Rumen fluid thiamine level in healthy animals was 5.55 ± 0.24 µg/dl. In animals of group I, II and III the values were 2.11 ± 0.23, 2.27 ± 0.49 and 2.09 ± 0.42 µg/dl respectively at zero hour. A statistically significant decrease (p≤ 0.01) was noticed in the thiamine concentration at zero hour in animals of group I, II and III when compared to normal value.

At 48h, the thiamine concentration was 3.45 ± 0.37 µg/dl in group I, 3.91 ± 0.42 µg/dl in group II and 4.23 ± 0.52 µg/dl in group III. Rumen liquor thiamine levels in group I, II and III were elevated at 48 h when compared with their zero hour value. But the values were significantly low (p≤ 0.01) in group I and II when compared with normal value. In group III, the mean thiamine level was comparable with that of normal value at 48 h and it was possible only at 96 h in group I and II.

Table 6a : Rumen liquor - sulphate, copper, cobalt and thiamine level in normal goats (mean ± SE)

Sulphate (mg/dl)	Copper (ppm)	Cobalt (ppm)	Thiamine (µg/dl)
6.1 ± 0.58	1.12 ± 0.08	0.23 ± 0.02	5.55 ± 0.24

Table 6b : Rumen liquor - sulphate, copper, cobalt and thiamine level in animals of Group I, II and III at 0, 48 and 96 h of therapeutic trial (mean ± SE)

Group	Sulphate (mg per cent)			Copper (ppm)			Cobalt (ppm)			Thiamine (µg/dl)		
	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h
I	6.41 ± 0.70	6.79 ± 0.77	6.37 ± 0.42	1.04 ± 0.09	0.96 ± 0.11	1.03 ± 0.11	0.27 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	$2.11^{Aa} \pm 0.23**$	$3.45^{Ab} \pm 0.37**$	$4.59^{B} \pm 0.37$
II	5.54 ± 0.47	5.99 ± 0.85	5.5 ± 0.36	1.06 ± 0.09	1.13 ± 0.07	0.89 ± 0.09	0.22 ± 0.02	0.23 ± 0.02	0.21 ± 0.01	$2.27^{Aa} \pm 0.49**$	$3.91^{Ab} \pm 0.42**$	$5.93^{B} \pm 0.68$
III	6.97 ± 0.72	6.97 ± 0.67	6.8 ± 0.31	0.86 ± 0.08	0.95 ± 0.09	0.86 ± 0.07	0.22 ± 0.03	0.24 ± 0.02	0.25 ± 0.01	$2.09^{A} \pm 0.42**$	$4.23^{B} \pm 0.52$	$6.09^{C} \pm 0.66$

** - Significant ($p \leq 0.01$) when compared with normal values
* - Significant ($p \leq 0.05$) when compared with normal values
Means in the row under the same parameter with different superscripts differ
A, B, C ($p \leq 0.01$)
a, b, c ($p \leq 0.05$)

Fig. 5 Average rumen liquor thiamine content of normal and diseased animals

Fig. 6 Average serum glucose level in normal and diseased animals
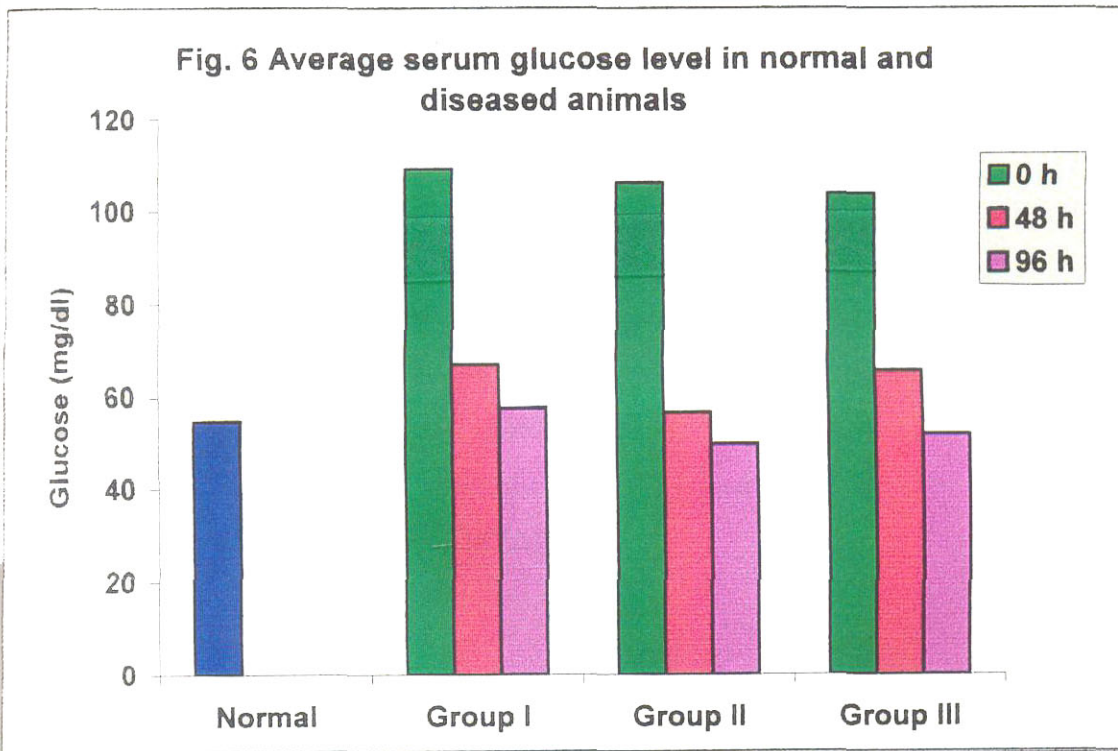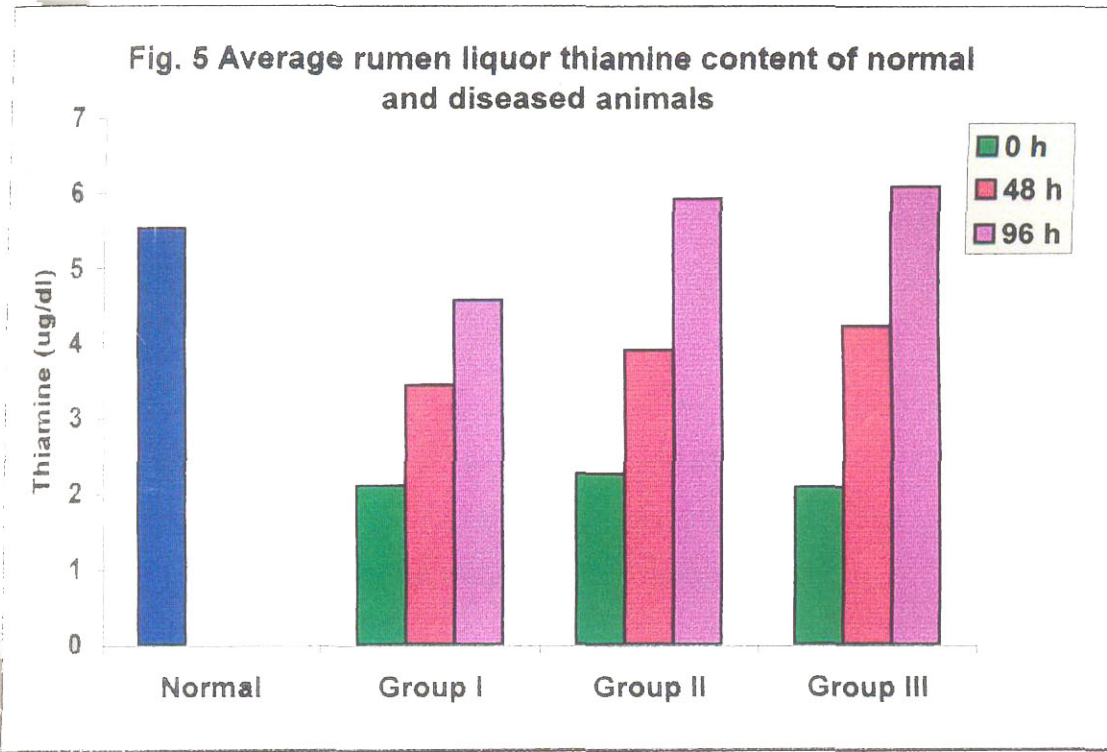
A statistically significant increase was observed (p≤ 0.01) in all the groups by 96 h when compared with corresponding pre-treatment values (Table 6a, 6b and Fig. 5). Pair wise comparison (ANOVA) did not show significant variations among the three groups.

4.5 SERUM BIOCHEMISTRY

4.5.1 Glucose

The mean serum glucose level was 54.50 ± 3.53 mg/dl in healthy goats. In animals of group I, II and III the values were 109.07 ± 11.64 mg/dl, 106.07 ± 11.32 mg/dl and 103.64 ± 7.36 mg/dl respectively at zero hour. The values were significantly high (p≤ 0.01) when compared with normal value.

Statistically significant (p≤0.01) decrease in glucose levels 66.79 ± 4.71 mg/dl in group I, 56.50 ± 3.81 mg/dl in group II and 65.40 ± 5.72 mg/dl in group III were observed at 48 h after initiation of treatment and was comparable to normal value.

At 96 h glucose level decreased significantly (p≤ 0.05) in group I and III from their 48 h value to 57.46 ± 1.97 mg/dl and 51.64 ± 4.11 mg/dl respectively with non significant change in group II (Table 7a, 7b and Fig.6).

4.5.2 Magnesium

Serum magnesium level in healthy animals was found to be 1.95 ± 0.08 mg/dl. Serum magnesium level in the three groups of animals during the pre-treatment and post – treatment periods were not significantly different from control values. The variations observed during the course of treatment were also not statistically significant (Table 7a and 7b).

4.5.3 Copper

Mean plasma copper level in healthy goats was 0.97 ± 0.09 ppm. The pre-treatment values of diseased goats were not significantly different from the normal value. Variations noticed in the plasma copper level at zero, 48 and 96 h in

Table 7a: Serum – glucose, magnesium, copper and cobalt level in normal goats (mean ± SE)

Glucose (mg/dl)	Magnesium (mg/dl)	Copper (ppm)	Cobalt (ppm)
54.50 ± 3.53	1.95 ± 0.08	0.97 ± 0.09	0.08 ± 0.01

Table 7a : Serum glucose, magnesium, copper and cobalt level in animals of Group I, II and III at 0, 48 and 96 h of therapeutic trials (mean ± SE)

Group	Glucose (mg/dl)			Magnesium (mg /dl)			Copper (ppm)			Cobalt (ppm)		
	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h
I	109.07^{Aa} ± 11.64**	66.79^{Bb} ± 4.71	57.46^{Bc} ± 1.97	1.96 ± 0.14	1.88 ± 0.15	1.89 ± 0.18	0.92 ± 0.13	1.01 ± 0.11	0.85 ± 0.09	0.08 ± 0.01	0.081 ± 0.01	0.084 ± 0.01
II	106.07^{A} ± 11.32**	56.50^{B} ± 3.81	49.46^{B} ± 4.11	2.14 ± 0.21	1.98 ± 0.19	2.17 ± 0.21	0.85 ± 0.08	0.83 ± 0.12	1.05 ± 0.12	0.08 ± 0.01	0.084 ± 0.01	0.073 ± 0.01
III	103.64^{Aa} ± 7.36**	65.4^{Bb} ± 5.72	51.64^{Bc} ± 4.11	1.98 ± 0.19	1.9 ± 0.17	1.81 ± 0.16	0.76 ± 0.08	0.86 ± 0.07	0.86 ± 0.05	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.02

** - Significant p≤0.01 when compared with normal
Means within the same row of the same parameter with different superscript differ
A, B - p≤0.01
a, b, c - p≤0.05

animals of group I, II and III were only marginal and within normal range (Table 7a and 7b).

4.5.4 Cobalt

Mean serum cobalt level was found to be 0.08 ± 0.01 ppm in the healthy group. Mean serum cobalt level at zero hour was found to be 0.08 ± 0.01 ppm, 0.08 ± 0.01 ppm and 0.09 ± 0.01 ppm in group I, II and III respectively. No statistically significant differences were noticed between the diseased groups and healthy group of goats. In all groups the post - treatment values were not significantly different from the pre-treatment values (Table 7a and 7b).

4.5.5 Sulphate

The mean value of serum sulphate was found to be 3.37 ± 0.39 mg/dl in healthy group. The increase observed in serum sulphate level at zero hour in animals of group I, II and III was not statistically significant. Variations in the serum sulphate level observed during the course of treatment in the present study were only marginal and not significant (Table 8a and 8b).

4.5.6 Lactate

Mean serum lactate level in healthy animals was 14.95 ± 1.44 mg/dl. A significant increase ($p \leq 0.01$) was noticed in the serum lactate level at zero hour in animals of groups I, II and III and at 48 h in group I ($p \leq 0.01$) and group III ($p \leq 0.05$) when compared to the normal value. In group I the serum lactate level got reduced from 51.14 ± 6.34 mg per cent at zero hour to 17.6 ± 2.14 mg per cent at 96 h. In group II it got reduced from 43.39 ± 7.72 to 15.42 ± 1.98 mg per cent at 96 h and in group III it got reduced to 13.89 ± 1.67 from zero hour value of 60.14 ± 10.54 mg per cent. The values at 48 and 96 h were significantly lower than that of the zero hour value in all the group. The values at 96 h of treatment were comparable to that of the normal value (Table 8a, 8b and Fig. 7).

4.5.7 Pyruvate

The serum pyruvate level in healthy animals was found to be 1.82 ± 0.14 mg per cent. A significant increase (p≤ 0.01) in serum pyruvate level was noticed at zero hour in animals of group I, II and III, when compared with the healthy goats.

In animals of group I, the pyruvate level decreased from 4.53 ± 0.13 at zero hour to 3.37 ± 0.15 mg per cent at 48 h which was statistically significant (p≤0.01). In group II the level decreased from 3.86 ± 0.16 at zero hour to 2.16 ± 0.25 mg per cent at 48 h and in group III decreased from 4.03 ± 0.40 to 2.63 ± 0.09 mg per cent. The values observed at 48 h in group II and III animals were not statistically significant when compared to the normal value. At 96 h, there were no significant differences between the three groups and normal value (Table 8a, 8b and Fig. 8).

4.5.8 Lactate pyruvate ratio

The lactate pyruvate ratio in healthy group was 8.45 ± 1.01. A Significant increase in L : P ratio (p≤0.01) was noticed at zero hour in group III only and became comparable to normal value at 48 h. No significant difference was noticed in the L : P ratio at zero, 48 h and 96 h in group I and II, when compared to normal value (Table 8a and 8b).

4.5.9 Thiamine

Mean serum thiamine level in healthy group was 4.05 ± 0.39 µg/dl. A significantly low value (p≤0.01) was noticed in serum thiamine level at zero hour in group I, II and III when compared to healthy goats. The zero hour values were 2.01 ± 0.38, 2.19 ± 0.23 and 2.29 ± 0.39 µg/dl in groups I, II and III respectively. Thiamine levels were increased in animals of group I, II and III animals by 48h and the values were comparable to the normal value. At 96 h, the serum thiamine level were increase significantly (p≤0.05) in all the groups when compared to the

Table 8a: Serum – sulphate, lactate, pyruvate and L :P ratio in normal goats

Sulphate (mg/dl)	Lactate (mg/dl)	Pyruvate (mg/dl)	L : P
3.37 ± 0.39	14.95 ± 1.44	1.82 ± 0.14	8.45 ± 1.01

Table 8b : Serum – sulphate, lactate, pyruvate and L :P ratio in animals of Group I, II and III at 0, 48 and 96 h of therapeutic trials (mean ± SE)

Group	Sulphate (mg/dl)			Lactate (mg/dl)			Pyruvate (mg/dl)			L : P		
	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h
I	3.9 ± 0.46	3.31 ± 0.45	3.17 ± 0.30	51.14^{A}± 6.34**	31.97^{B} ± 3.68**	17.60^{C} ± 2.14	4.53^{A}± 0.13**	3.37^{B} ± 0.15**	2.28^{C} ± 0.32	11.17 ± 1.15	9.34 ± 0.75	8.6 ± 1.46
II	3.77 ± 0.64	3.06 ± 0.18	3.1 ± 0.37	43.39^{A}± 7.72**	21.36^{A} ± 2.27	15.42^{B} ± 1.98	3.86^{A} ± 0.16**	2.16^{Bb} ± 0.25	1.73^{Bc} ± 0.14	11.64 ± 1.92	10.24 ± 1.52	8.24 ± 0.91
III	4.24 ± 0.31	4.81 ± 0.81	3.83 ± 0.35	60.14^{A}± 10.54**	25.89^{B} ± 3.75*	13.89^{C} ± 1.67	4.03^{Aa} ± 0.40**	2.63^{b} ± 0.29	2.11^{Bc} ± 0.25	14.67^{Aa} ± 2.0	9.76^{Aa} ± 0.74	6.39^{B} ± 0.51

** - Significant (p≤0.01) when compared with control
Means within the same row of the same parameter with different superscripts differ
A, B, C - p≤0.01
a, b, c - p≤0.05

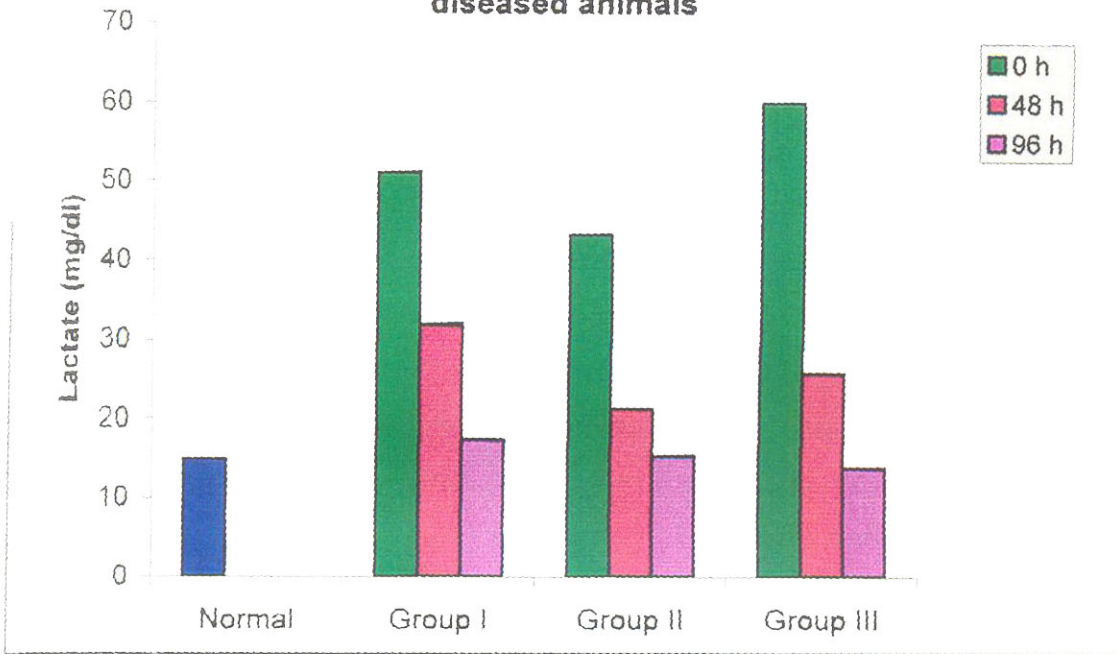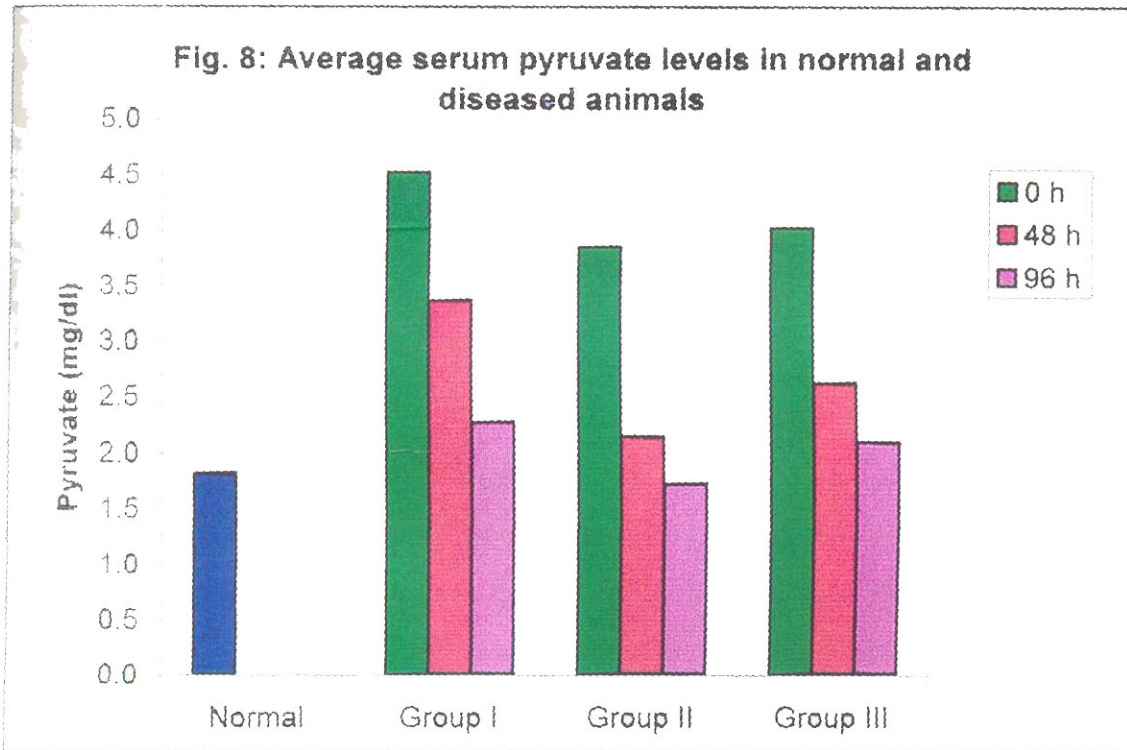Fig. 7: Average serum lactate levels in normal and diseased animals

Fig. 8: Average serum pyruvate levels in normal and diseased animals

normal value. The values were 5.49 ± 0.46, 5.30 ± 0.43 and 5.49 ± 0.37 μg/dl in groups I, II and III respectively (Table 9a, 9b and Fig. 9).

4.5.10 Erythrocyte transketolase and per cent TPP effect

The normal erythrocyte transketolase level and per cent TPP effect in healthy goats were 54.14 ± 6.45 IU/l and 17.40 ± 4.10 per cent respectively. In group I animals erythrocyte TK value increased from 33.89 ± 4.21 at zero hour to 57.29 ± 3.58 IU/l at 96 h, group II animals it got increased from 43.57 ± 5.44 to 55.43 ± 5.95 IU/l and in group III it got increased from 32.26 ± 3.94 to 48.39 ± 4.42 IU/l at 96 h. A highly significant higher value in per cent TPP effect was observed at zero hour in all the three groups when compared to the normal value. The values were 81.26 ± 13.17, 60.74 ± 4.20, 79.93 ± 10.66 per cent for groups I, II and III respectively. There was increase in TK level and decrease in TPP effects in group I, II and III at 48 h when compared to the zero hour values and the values were comparable to the normal value. The per cent TPP effect was decreased non significantly from 14.75 ± 7.68 at 48 h to 3.90 ± 1.84 per cent at 96 h in group I and from 18.61 ± 5.08 at 48 h to 8.56 ± 0.89 per cent at 96 h in group II and significantly ($p \leq 0.05$) from 29.14 ± 9.71 at 48 h to 3.79 ± 1.88 per cent at 96 h in group III (Table 9a, 9b and Fig. 10,11).

Pair-wise comparison (ANOVA) did not show significant difference among the three groups.

4.6 RESPONSE TO THERAPY
Group I

All the animals in group I responded to the treatment, with parentral administration of thiamine @ 50 mg/kg body weight daily for four days and started showing improvement in the very next day after initiation of treatment. Three animals (42.85 per cent), became completely normal clinically by fourth day and three (42.85 per cent) on fifth day. The animal that had developed radial paralysis recovered completely.

Table 9a : Thiamine, TK and per cent TPP effect in normal goats

Thiamine (µg/dl)	TK (IU/l)	Per cent TPP effect
4.05 ± 0.39	54.14 ± 6.45	17.40 ± 4.10

Table 9b: Thiamine, TK and per cent TPP effect in animals of Group I, II and III at 0, 48 and 96 h of therapeutic trials (mean ± SE)

Group	Thiamine (µg/dl)			TK (IU/l)			Per cent TPP effect		
	0 h	48 h	96 h	0 h	48 h	96 h	0 h	48 h	96 h
I	2.01^A ± 0.38**	3.84^B ± 0.39	5.49^C ± 0.46*	33.89^{Aa} ± 4.21*	41.96^a ± 3.87	57.29^B ± 3.58	81.26^A ± 13.17**	14.75^{Bb} ± 7.68	3.90^{Bb} ± 1.84
II	2.19^{Aa} ± 0.23**	3.84^{Aa} ± 0.56	5.30^B ± 0.43*	43.57^a ± 5.44	52.45^b ± 5.19	55.43^b ± 5.95	60.74^A ± 4.20**	18.61^{Bb} ± 5.08	8.56^{Bb} ± 0.89
III	2.29^A ± 0.39**	3.97^{Bb} ± 0.62	5.49^{Bc} ± 0.37*	32.26^A ± 3.94**	43.57^{Bb} ± 5.87	48.39^{Bb} ± 4.42	79.93^A ± 10.66**	29.14^{Bb} ± 9.71	3.79^{Bc} ± 1.88

** - Significant $p \leq 0.01$ when compared the normal

* - Significant $p \leq 0.05$ when compared to normal

Means within the same row of the same parameter with different superscript differ

A, B - $p \leq 0.01$

a, b, c - $p \leq 0.05$

Fig. 9: Average serum thiamine in normal and diseased animals
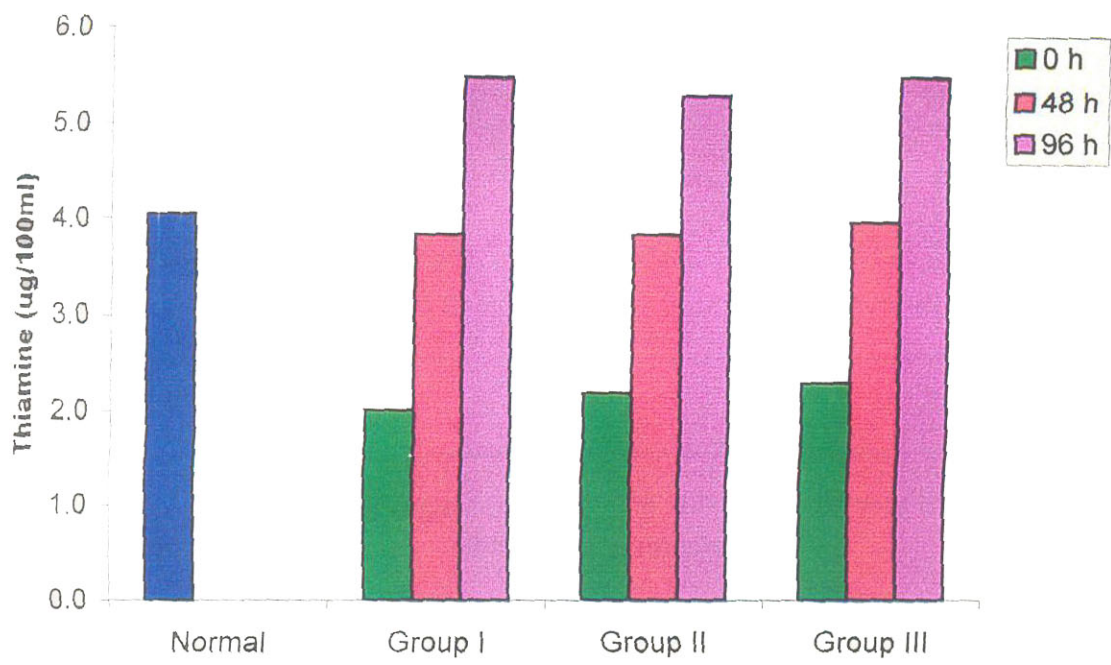
Fig. 10: Average erythrocyte transketolase level in normal and diseased animals
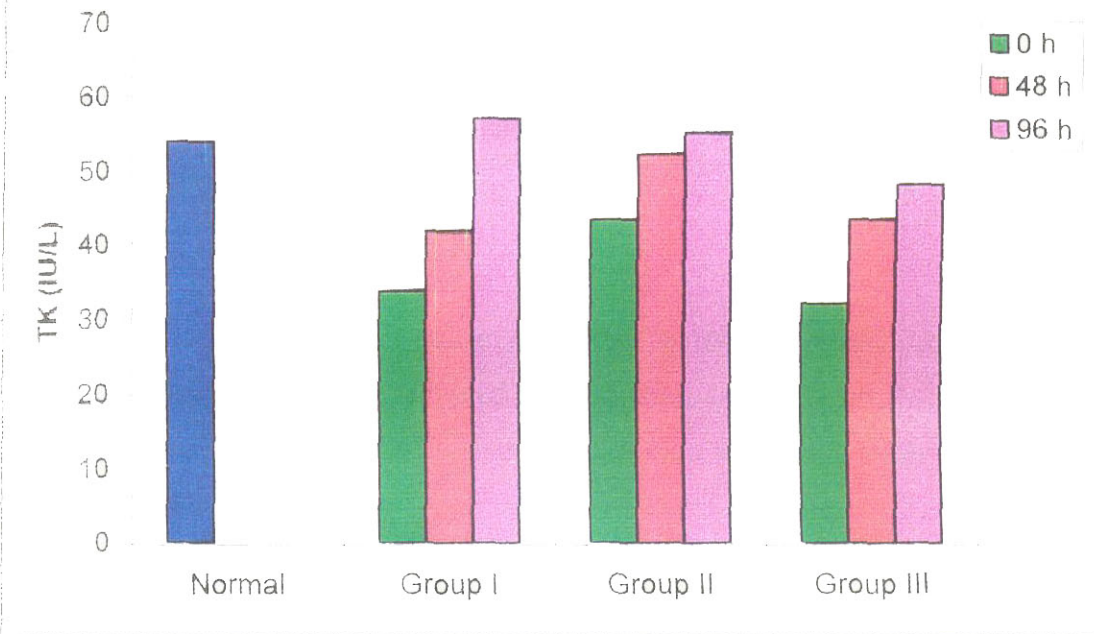
Fig. 11: Average per cent TPP effect in normal and diseased animals
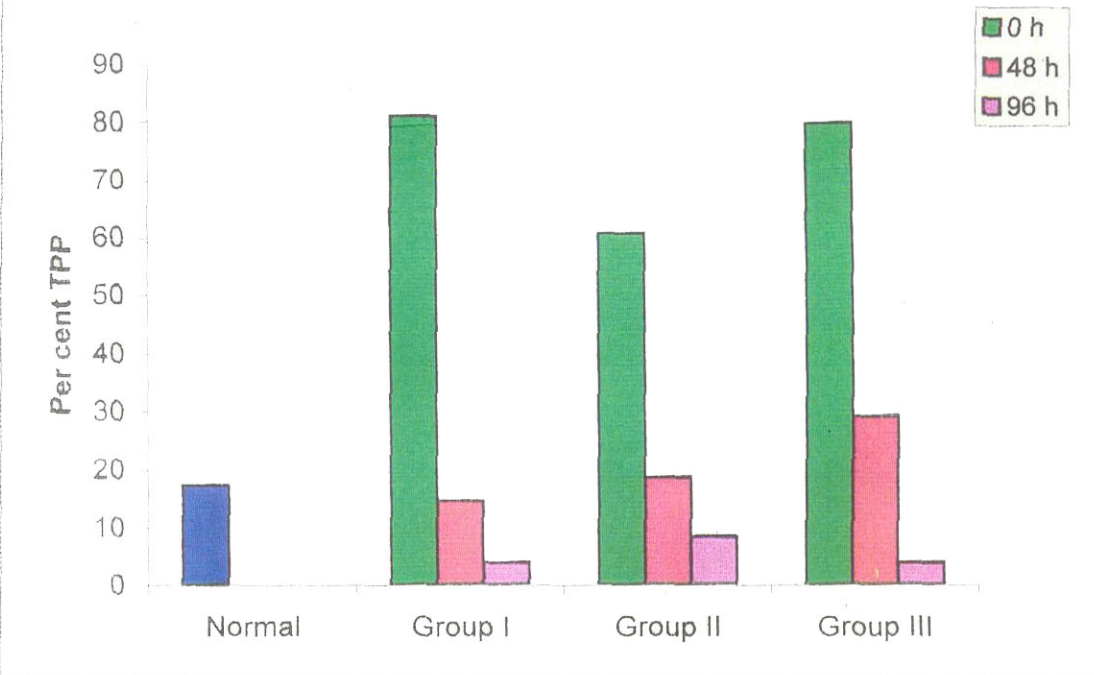
Group II

All the animals responded to the treatment with oxytetracycline @ 500 mg
orally on the day of admission along with parentral administration of thiamine as
in group I. One animal (14.20 per cent) became completely normal after three
days of treatment. Five (71.40 per cent) on the fourth day and one (14.20 per cent)
on the fifth day of treatment. No complications developed in this group.

Group III

All the animals showed quick responses to the therapeutic regimen
employed. The line of treatment adopted was 1g thiamine orally on the day of
admission along with parentral administration of thiamine as in group I. The
animals were able to walk / stand by second / third day. Four animals (57.14 per
cent) required only three days treatment, to become clinically normal, two animals
(28.5 per cent) required four days treatment and one animal (14.20 per cent)
required five days treatment (Table 10).

Table 10: Response to treatment in different days of observation

Group No.	Percentage recovery on 3rd day	Percentage recovery on 4th day	Percentage recovery on 5th day	Percentage recovery on 10th day
I	-	42.85	42.85	14.30
II	14.30	71.40	14.30	-
III	57.20	28.50	14.30	-

4.7 AUTOFLUORESCENCE

Brain collected from the animals that died during the study period did not
reveal autofluorescence under UV illumination.

4.8 PATHOLOGY

Pathologic observations were made on two goats which died on the second day after being presented for treatment. The brain on gross examination showed extensive meningeal congestion. Whole brain was soft, swollen and there was diffuse yellowish discolouration of the cerebral grey matter. The gyri were moderately swollen. Dark brown pin head sized spots of necrosis were seen in the grey and white matter (Plate 11) The pons, medulla oblongata and spinal cord did not reveal any gross lesions.

Neurons of the superficial, middle and deeper cortical laminae of cerebral grey matter showed varying degrees of degeneration and necrosis. Superficial and mid-cortical laminar neurons appeared as round condensed basophilic structures surrounded by perineuronal space giving the parenchyma a diffuse spongy appearance (Plate 12). Predominant gliosis and neovascularization characterized by the presence of numerous capillary sprouts were observed in the area. The fusiform and pyramidal layers of the cerebral cortex contained neurons which were shrunken and dark staining (Plate 13). Perineuronal edema, capillary congestion and endothelial damage were also observed in these segments. The glial reaction was less prominent in these regions. A moderate degree of hypocellularity was also observed. Satellitosis, perivascular and perineuronal edema, swelling of astrocytic nuclei, swelling and vacuolation of neurons, peripheral and central chromatolysis and fading of neurons into the substances of the neuropil were observed in certain other region of the cerebral cortex (Plate 14). The deeper cortical laminae close to the white matter revealed extensive neuronal damage, hypocellularity, congestion of vessels with endothelial cell damage and perivascular cuffing with a mixed population of cells predominantly mononuclears (Plate 15 and 16). Neutrophils, lymphocytes and microglial cells were seen accumulated as aggregates or sheets in the junction between the grey matter and white matter (Plate 17 and 18). These inflammatory cells were also seen spreading into the white matter. The neurons and glial cells were not discernible or were liquefied and in many places appeared as eosinophilic debri.

Plate 11 : Brain - Swollen with flattening of gyri and congestion of blood vessels

Plate 11

Plate 12 : Cerebrum - Superficial and mid cortical laminar neurons, perineuronal space, spongy appearance to the parenchyma – H&E × 250

Plate 13 : Cerebrum - Gliosis and neovascularization – H&E × 250

Plate 14 : Cerebrum - Satellitosis, perivascular and perineuronal oedema – H&E × 250

Plate 15 &16 : Cerebrum - Perivascular cuffing with mixed population of cells – H&E × 250, 400

Plate 12
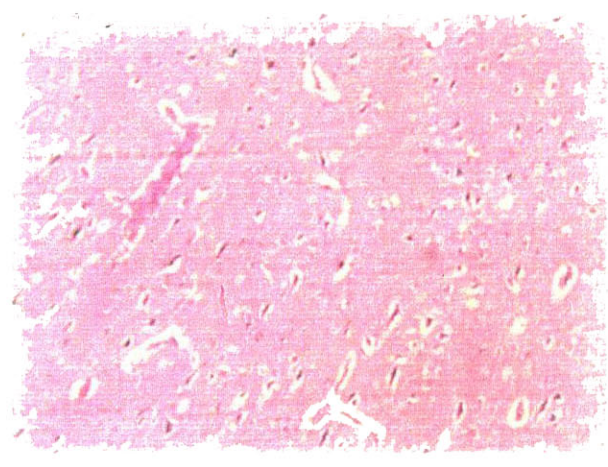
Plate 13
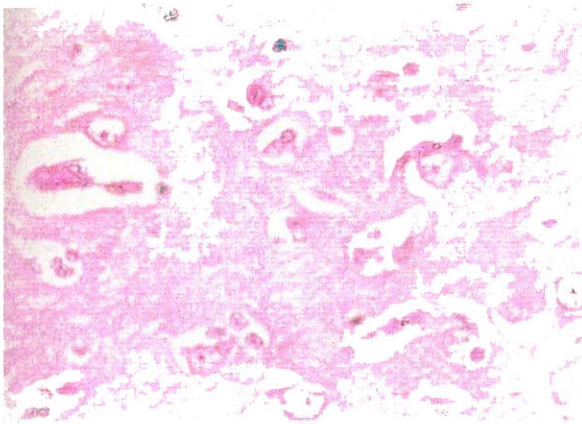
Plate 14

Plate 15
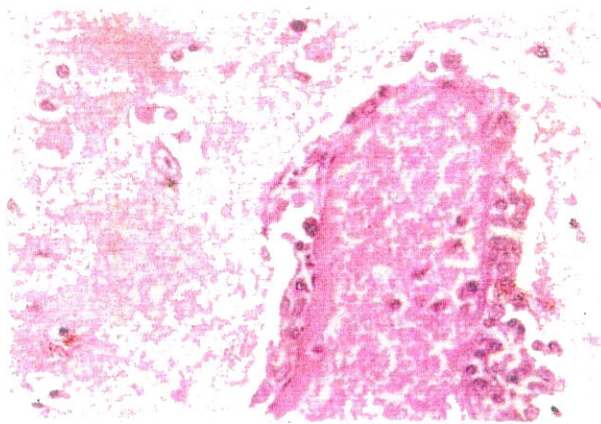
Plate 16

The white matter in most of places appeared normal with linear arrays of oligodendroglial cells.

Purkinje cell degeneration was observed in certain foliae of the cerebellum (Plate 19). The granular cell layer appeared less cellular. The interfolial capillaries were obliterated with endothelial cells.

The pons and medulla oblongata showed no characteristic histological lesions.

Capillary congestion, thrombosis, pericapillary and intracapillary accumulations of neutrophils and mononuclear cells were the lesions present in the ventro-lateral column of the spinal cord (Plate 20 and 21). This was observed only in one animal.

All the plexuses remained normal with fascicles of varying sizes delimited by perineurium and bound together by epineurium containing less dense connective tissue and vasavasorum.

4.9 ULTRASTRUCTURAL PATHOLOGY

Cerebrocortical neurons showed various stages of degeneration and necrosis. The changes were pronounced in the neuropil and cell body of the neurons. Most of the neurons appeared condensed with a predominance of heterochromatin which appeared as electron dense clumps in the nucleoplasm. Certain other neurons appeared as electron dense angular bodies amidst lysed neuropil contents. Irregularity and nuclear membrane breakage, and even loss and dispersal of chromatin were observed in some of the neurons in the gyri. Nucleolar details were not observable in most of the neurons.

In the neuronal cytoplasm, the organelles were found dispersed in a loose matrix. Matrix vacuolation was prominent and there is loss of organellar details. Clumps of dilated endoplasmic reticulum and pleomorphic and condensed mitochondria were seen occasionally. Ribosomes were seen dispersed in the loose matrix.

Plate 17 & 18 : Cerebrum - Focal accumulations of neutrophils and lymphocytes at cortico medullary region– H&E × 250, 400

Plate 19 : Cerebellum – Purkinje cell degeneration and obliteration of interfolial capillaries with endothelial cells– H&E × 250

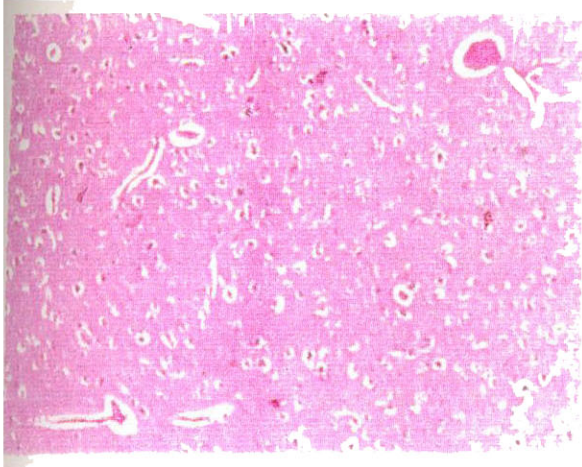Plate 20 & 21: Spinal cord – Capillary congestion, pericapillary and intracapillary accumulations of neutrophils and mononuclear cells – H&E × 250, 400
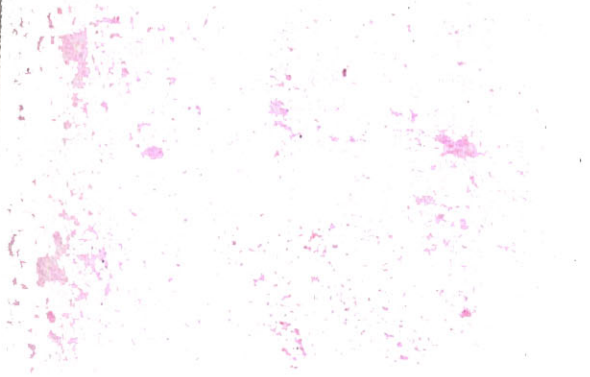
Plate 17
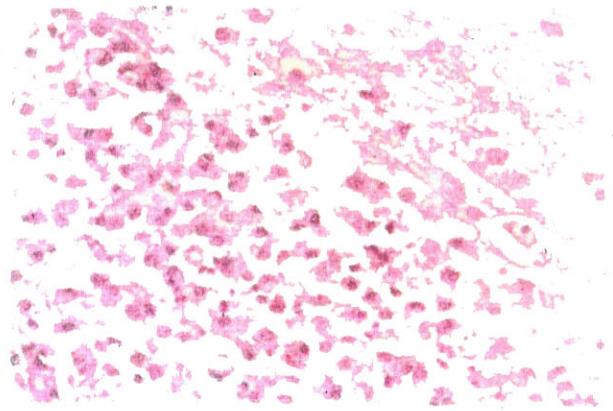
Plate 18
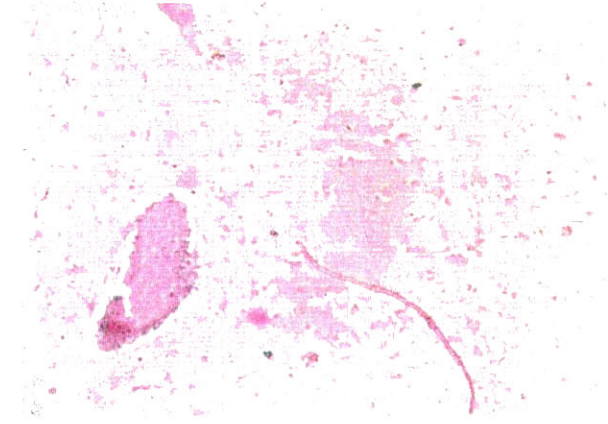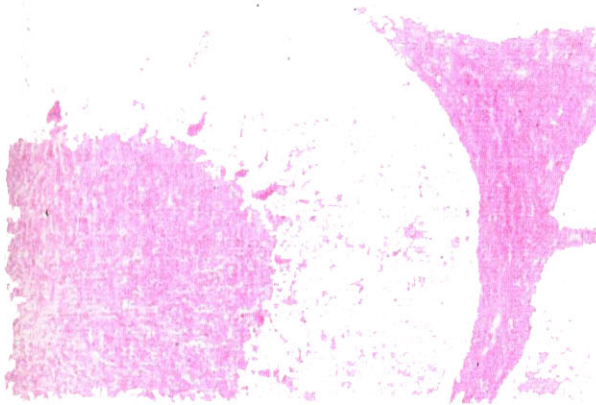

Plate 19
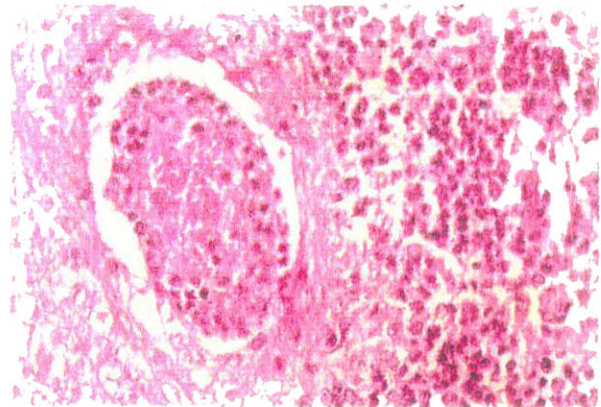
Plate 20
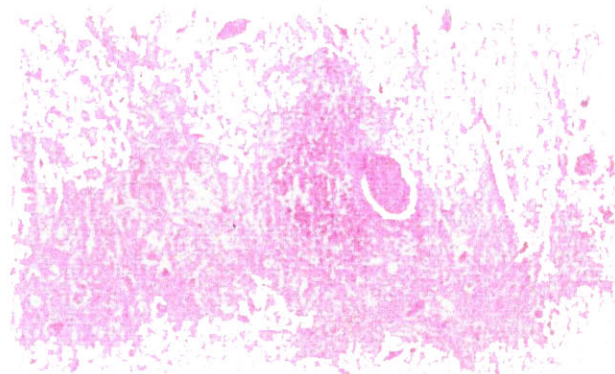
Plate 21

There was a predominance of microglial cells around and away from the damaged neurons. Most of them appeared swollen with disruption of nuclear membrane. The euchromatin appeared as granular clumps in the lucent nucleoplasm. The heterochromatin was very much condensed and homogenous and appeared as one or two large electron dense bodies (Plates 22). There was total disruption of the cytoplasm and the organelles appeared dispersed in the oedematous and lytic neuropil. Clumps of mitochondria could be seen at certain locations. They appeared electron dense. Lysis of the cell wall has caused damage to the cell components in the neuropil. Spiltting and ballooning of myelin and vacuoles of varying sizes were seen along with necrosed and homogenized axons. Organelles were not discernible in most of the axoplasm. In some, the axoplasm appeared granular and in others it was homogenized. Damaged mitochondria were seen in most of the axons. Axons with well wrapped and dense myelin could also be observed amidst the damaged ones (Plate 23).

Plate 22: E/M - Cerebral cortex microglial cells: nuclear membrane damage, euchromatin aggregation and disruption of cytoplasmic organelle. Neuropil oedema and lysis of components and presence of groups of mitochondria - 6000

Plate 23: Cerebrum white matter myelin splitting, intramyelinic vacuolation and necrosis of axons - 12000
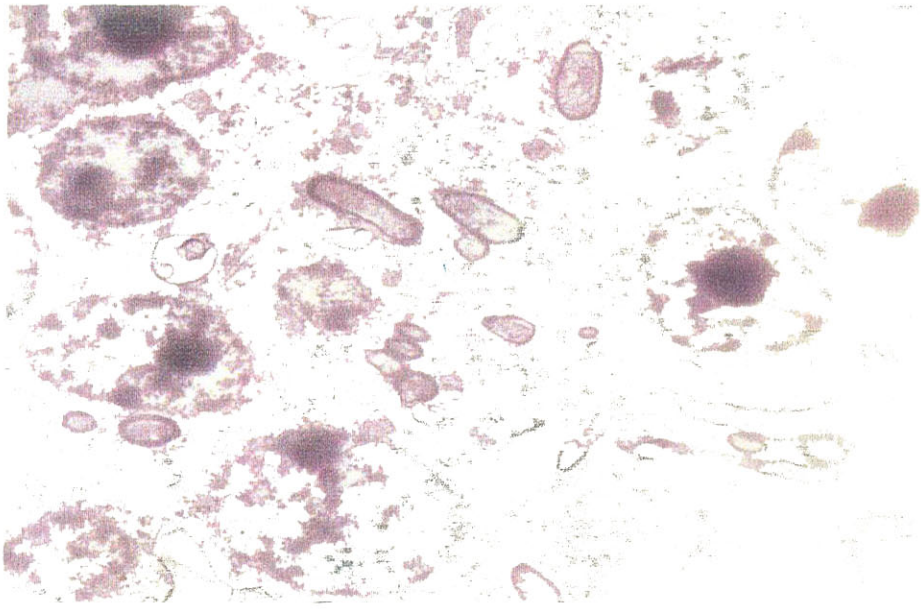
Plate 22
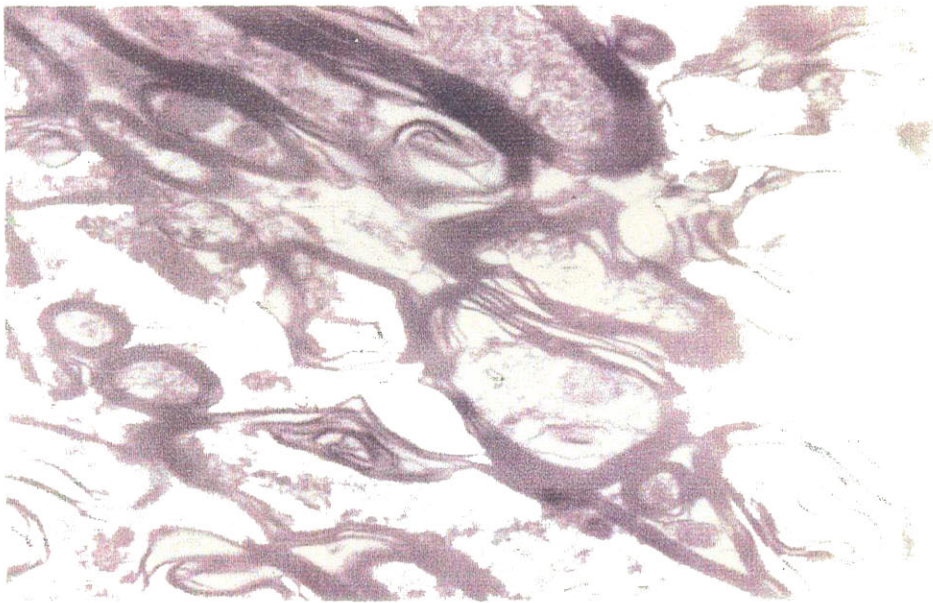
Plate 23

Discussion

DISCUSSION

5.1 EPIDEMIOLOGY

The data on the occurrence of PEM were collected from different parts of Kerala. In many Veterinary hospitals diagnosis was not properly made and data were not recorded in a systematic manner. This was a serious limitation for an epidemiological study.

Data on the occurrence of the disease suggested that goat cases constituted 15.39 per cent of the total number of cases presented at the hospital. Of these 1.14 per cent exhibited symptoms suggestive of polioencephalomalacia. Incidence of PEM in College hospital was found to be 1.24 per cent among the total cases in goats. An incidence of 1.43 per cent was reported by Sobhanan (1981) in Kerala and 2.44 per cent by Lonkar et al. (1993) in goats in Rajasthan.

The study revealed highest rate of occurrence in Malappuram followed by Thrissur, Idukki and Kozhikode. Goat population was also comparatively high in these districts.

Although the disease occurred throughout the year maximum incidence was noted during the month of October followed by November, December and January (Post monsoon period). Jensen et al. (1956); Tanwar (1987); Maliekal (2000) observed that PEM occurred when animals were shifted to lush pasture. Thiaminase mimicking substances present in some of the lush pasture may cause destruction of thiamine in the rumen (Dickie and Berryman, 1979). Common roughages eaten by goats need screening for the presence of substances simulating thiaminase. Fakhruddin et al. (1987b) noticed more cases of PEM in winter season. According to Tanwar (1987) there was no correlation between occurrence of PEM and environmental temperature, humidity and rainfall. Little and Sorensen (1969) could not observe any seasonal influence on the occurrence of this disease. Hamlen et al. (1993) recorded more cases during late autumn and winter or early spring, when the goats were fed with more quantity of concentrates. Lonkar et al. (1993) recorded the disease throughout the year.

The present study revealed that the disease was more prevalent in females and this could be due to more number of females in the population. Male goats are either disposed off or slaughtered at an early age, where as females are retained for breeding and milk production. Lonkar *et al.* (1993) also reported similar findings.

The age group of the affected animals varied from one month to five years. Similar observations were made by Gabbedy and Richards (1977). Edwin and Lewis (1971) experimentally produced PEM in pre-ruminant animals by feeding thiamine low diet. Increased requirement of thiamine in rapidly growing animals and absence of adequate microbial synthesis in pre-ruminant stage make them susceptible to polioencephalomalacia. Similar observations were made by Smith (1979) and Jubb and Huxtable (1991). According to Thomas (1986) activity of thiaminase is more in pre-ruminant lambs. Under normal conditions microbial synthesis alone may not meet the total vitamin requirement of host and dietary sources, therefore, may be essential (Ranjan, 1993). So any alteration in the microflora or digestive function may cause thiamine deficiency in ruminants.

In Kerala, cooked rice (a carbohydrate rich diet) forms a major component of the concentrate fed to goats. A high carbohydrate diet would require relatively more thiamine for its effective utilization. The irregular or excess feeding of rice often accounted for majority of ruminal dysfunctions with lowering of pH in goats (Aleyas and Vijayan, 1981) and encourage proliferation of undesirable bacteria (Sobhanan, 1981). These normally quiescent organisms may destroy thiamine in rumen and there by increase the incidence of PEM. Sapienza (1981) also reported similar findings in concentrate fed animals.

5.2 THERAPEUTIC TRIALS
5.2.1 Clinical signs

The basic clinical data collected from the diseased animals did not show statistically significant differences from the healthy animals except for the rate of respiration and pulse at zero hour in group III. Initial increase in the rate of

respiration and pulse in group III could be due to excitement, occasional tremors and increased serum lactate level. Most of the animals of this group were below six months of age and had severe form of the disease. Thomas (1986) also observed more severe signs in younger animals.

Tanwar *et al.* (1983) reported increased rate of respiration and pulse with laboured breathing and normal temperature in PEM cases. Similar findings were reported by Smith (1979); Fakhurddin *et al.* (1987b); Maliekal (2000). Mucous membrane of all the affected animals were pale roseate except in two cases in which mucous membrane along with whole eye was reddened due to rubbing and occasional hitting of the head on the ground. Similar findings were also reported by Tanwar (1987).

Rumen motility was not affected in majority of the cases except for a non significant decrease in severe cases. If large quantities of hydrogen sulphide were present in the rumen, the rumen motility would have been depressed (Dougherty *et al.*, 1965).

In the initial stages of the disease, frequent bleating, low carriage of head, leaning on to the wall, aimless wandering, rolling of eye ball and upward turning of the head were reported by owners. Seven animals were presented to the hospital in a state of lateral recumbency. Even in lateral recumbency most of them consumed concentrates and leaves in small quantities. Similar observations were made by Markson *et al.* (1974); Pierson and Jenson (1975); Gauri and Vashistha (1988); Tanwar (1995), Gould (1998). According to Little and Sorensen (1969) the disease syndrome start without any premonitory signs.

Nystagmus (either vertical or horizontal) a constant finding in the present study was also reported in PEM by Jensen *et al.* (1956); Little and Sorensen (1969); Blood *et al.* (1979); Lonkar and Prasad (1992a); Maliekal (2000); George (2002). Corneal opacity that developed in two of the animals was traumatic in origin due to frequent shaking and rubbing of the head on cement floor of the shed. Similar findings were recorded by Lonkar and Prasad (1992a).

Another characteristic and constant finding noticed in this study was that the affected animals preferred to lie only on one side and on turning to the other side the animals struggled violently and returned to the original position. This was in accordance with the findings of Maliekal (2000); George (2002). This observation suggests that although the disease affects brain diffusely, the two halves of the cerebrum may not be equally affected. An animal with cerebral lesion that compulsively circles tend to circle towards the side of the lesion (Braund, 1995).

The neurological signs observed suggested the involvement of cerebral cortex and cerebellum. Clinical signs like lameness, tremors, opisthotonus and intermittent convulsions also indicated disturbances in cerebellar function or imbalance in neurotransmitter system. The cerebellum is a central point in the CNS for the organization of movement (Llinas, 1975). The blindness observed in two cases could be due to involvement of the visual cortex. Based on the degree of involvement symptoms appeared in the form of nystagmus, loss of eye preservation reflex and finally blindness (Nair, 1999). He reported histological lesions in all the layers of cerebral cortex. Radial paralysis observed in one case might be one of the polyneuritic symptoms of thiamine deficiency (Edwin and Lewis, 1971; Jana and Ghosh, 2000). Alteration in the behaviour and posture occurred probably due to brain lesions which lead to loss of integrity of cranial nerves (Singh et al., 2000). Inability to rise during the advanced and severe stages of the disease could also be due to the involvement of the brain stem as suggested by Radostits et al. (1994).

5.3 RUMEN FLUID

5.3.1 pH

No statistically significant difference was noted in the pH of rumen liquor of group I, II and III animals when compared with the healthy group. Normal rumen liquor pH observed in the present study agreed with the observations of Loew and Dunlop (1972); Tanwar (1987); Lonkar and Prasad (1994b). Slightly acidic rumen pH was reported in PEM cases that were fed high quantity of

concentrates as rumen pH was influenced by the nature of feeds and fodders (Mella *et al.*, 1976). According to Brent (1976) the rumen fluid pH optimum for the action of bacterial thiaminase I was 5.0. This may suggest lesser involvement of bacterial thiaminase I in the pathogenesis of PEM, encountered in this study.

5.3.2 Physical characters

The colour, consistency and odour of rumen fluid were same in all the groups and agreed with the observations made by Maliekal (2000). Maliekal (2000) opined that although changes in the rumen pH were not marked, their could be alterations in the rumen environment. This could induce microbial changes in the rumen. The odour was aromatic. Rumen fluid of sheep with sulfur induced PEM had pH of 6 to 6.5 with strong odour of rotten egg (Bulgin *et al.*, 1996).

5.3.3 Protozoal activity

The protozoal activity in the healthy and diseased groups ranged from moderate (++) to vigorous (+++) and agreed with the observations made by Maliekal (2000). This finding suggested that there were no marked changes in the rumen environment of PEM affected goats.

5.4 BIOCHEMICAL CHARACTERS

5.4.1 Total volatile fatty acids

No statistically significant difference was observed in TVFA values of diseased groups when compared with healthy group. The findings were comparable with the values reported by Lal *et al.* (1992); Basak *et al.* (1993); Maliekal (2000).

5.4.2 Copper, Cobalt and Sulphate

References regarding the normal level of copper, cobalt and sulphate in rumen liquor of goats were not available. No statistically significant difference was observed in rumen copper, cobalt and sulphate level of diseased groups when

compared to healthy goats. The level of these microminerals in the rumen fluid mainly depended on the type of food, mineral composition of food-stuff and the interaction between feed type and mineral composition (Shuttle, 1986). As the appetite of the animals were not affected there was no possibility for any major changes in the level of these minerals. Bray (1969) observed that inorganic sulphate got reduced to sulphide in the rumen. A sulphide concentration of 14.70 μmols/ml rumen fluid did not produce any toxic effect. In ruminants, the neurotoxic effects of sulphide apparently are mediated via eructation of hydrogen sulphide from the rumen and absorption through the lungs. The present study suggested that occurrence of PEM in Kerala was not related to excess sulphate/ deficiency of copper or cobalt in the diet.

5.4.3 Thiamine

The mean thiamine concentration in rumen liquor of healthy group was found to be 5.55 $\pm$ 0.24 μg/dl. This was comparable with the values reported by Gauri and Vashistha (1988); Singh et al. (2000).

The thiamine content of rumen liquor was 2.11 $\pm$ 0.230 μg/dl, 2.27 $\pm$ 0.49 μg/dl and 2.09 $\pm$ 0.42 μg/dl respectively in group I, II and III at zero hour of study. The values were significantly low when compared to the normal values but slightly higher than the values reported by Chahar et al. (1993); Lonkar and Prasad (1994b); Singh et al. (2000) in PEM affected goats. The difference might be due to variations in the severity of the disease, feeding and managemental practices. Ruminants are unique in that they are able to synthesize B vitamins including thiamine in their rumen. Requirements are increased during pregnancy and lactation and also when the carbohydrate content of the diet is high. The daily thiamine requirement of an adult animal has been estimated as 2 to 4 mg. Intraruminal synthesis of thiamine is estimated to be 1 to 3.5 mg/day. This implies that unless there is significant amount of thiamine in the pasture or other feeds, the ruminant may often be on the border line deficiency (Rammell and Hill, 1986). Thiamine deficiency occurs in ruminants when ruminal synthesis decreases or thiamine is hydrolysed by thiaminase in the rumen. Edwin and Jackman (1973)

detected an adequate amount of thiaminase in rumen content of PEM affected animals that could degrade all the thiamine usually ingested or synthesized. In the recovered animals rumen thiamine level reached normal level and this could be due to altered rumen microflora. Orally administered thiamine represses the activity of ruminal thiaminase which might be responsible for more significant increase in ruminal thiamine level at 48 h in group III. Thomas (1986) suggested that the oral administration of thiamine is effective to repress the activity of ruminal thiaminase. Fakhruddin *et al.* (1987a) reported similar findings in cattle. The negative thiamine balance produced results in depletion of tissue thiamine reserve and this would lead to the clinical condition, PEM.

5.5 SERUM BIOCHEMISTRY

5.5.1 Glucose

The mean blood glucose level in the healthy group was 54.50 ± 3.53 mg/dl. This was comparable with the values reported by Pillai, (1988); Maliekal (2000). Statistically significant increase was (p≤0.01) noted in group I, II and III animals at zero hour and rapidly returned to normal level by 48 h after treatment. Similar observations were made by Lonkar and Prasad (1993); Syamasundar and Malik (1993); Maliekal (2000). The degree of elevation could be correlated with the severity of clinical signs. Trauma has been shown to cause increase in glucagon leading to hyperglycaemia (Brockman and Manny, 1976) or it might reflect an inability to utilize glucose (Mella *et al.* (1975). Elevation in glucose level might also represent an adrenergic response.

5.5.2 Magnesium

Normal serum magnesium level in healthy group was 1.95 ± 0.08 mg/dl. This was comparable to the value reported by Sobhanan (1981); Shihabudheen (1998) in healthy goats.

No statistically significant difference was observed in serum magnesium level in diseased groups when compared to the healthy group. Normal magnesium level was recorded in outbreaks of PEM in sheep and cattle (Spence *et al.*, 1961)

in lambs (Thornber *et al*, 1981) in buffalo calves (Syamasundar and Malik, (1993) and in goats (Maliekal, 2000). The present finding suggest the magnesium is not having any direct role in the etiology of PEM.

5.5.3 Copper

Mean serum copper level in the healthy group was 0.97 ± 0.09 ppm. This was comparable to the value reported by Ghosh (1998) in goats and Dhami *et al.* (2001) in bulls. No statistically significant difference was observed in serum copper levels between the diseased groups and healthy group. Similar observations were made by McDonald (1982). The mean value of copper in PEM affected animal was 0.35 mg/l and copper level less than 0.30 mg/l only was considered copper deficiency in cattle (Gooneratne *et al.* 1989b). The results ruled out the possibility of copper playing a role in the etiopathogenesis of thiamine responsive PEM.

5.5.4 Cobalt

The mean serum cobalt level in healthy group was 0.08 ± 0.01 ppm. This was comparable to the value reported by Ghosh (1998) in goats, Samanta *et al.* (1995); Day *et al.* (2001) in cattle. No statistically significant difference was observed in diseased groups when compared with the normal values. Hartley *et al.* (1962) and MacPherson *et al.* (1976) recorded outbreaks of polioencephalomalacia in cobalt deficient sheep and steers respectively. MacPherson *et al.* (1976) stated that cobalt supplementation was effective in preventing the development of conditions favourable to the production of CCN. The present study suggested that cobalt does not play any role in the etiopathogenesis of PEM in Kerala.

3.5.5 Sulphate

Normal level of sulphate in the healthy goats was 3.37 ± 0.39 mg/dl. This was comparable with the value of 2 to 6 mg per cent reported by Bray (1969). No statistically significant differences were observed in serum sulphate level in

diseased animals. There were several reports that development of PEM in cattle and sheep were associated with high sulphur intake in the form of sulphates in feed and water (Gooneratne et al., 1989a; Gooneratne et al., 1989b; Sagar et al., 1990). In most of the animals affected with PEM related to high sulphate intake, thiamine treatment was not of value (Gooneratne et al., 1989b). In the present study, all the animals responded to thiamine treatment. Therefore chances of sulphur induced PEM need be considered only when affected animals do not respond to thiamine treatment. In addition there were no clinical signs (twitching of muscles, signs of pain, laboured breathing and smell of hydrogen sulphide to breath) that were suggestive of sulphur toxicosis.

5.5.6 Lactate, Pyruvate and L : P ratio

The normal levels of serum lactate, pyruvate and L : P ratio in healthy goats were 14.95 ± 1.44 mg/dl, 1.82 ± 0.14 mg/dl and 8.45 ± 1.01 respectively. This was comparable to the values obtained by Shihabudheen (1988); Maliekal (2000).

Statistically significant increase in lactate level ($p \le 0.01$) was noted in group I, II and III animals at zero hour, with respective values of 51.14 ± 6.34 mg/dl, 43.39 ± 7.72 mg/dl and 60.14 ± 10.54 mg/dl. The serum lactate level in diseased groups declined gradually over a period of zero to 48 h and became comparable to normal value by 96 h of study. The rate of reduction was more marked in group III. The significant increase in lactate levels of serum was in agreement with the findings of Lonkar and Prasad (1993); Syamasundar and Malik (1993); Maliekal (2000).

Increase in the lactate level at the onset of clinical symptoms was reported in CCN or thiamine deficiency suggesting a link between CCN and lactate level (Mueller and Asplund, 1981). Peters (1967) suggested that pyruvate and lactate accumulated in blood because tissues were unable to oxidise pyruvate properly in thiamine deficiency. Low et al. (1970); Markson et al. (1974) postulated that pyruvate metabolism was TPP dependent. Hence in thiamine deficiency there is

rise in blood pyruvate level. Robbins *et al.* (1984) stated that higher concentration of pyruvic acid which occurs in thiamine deficiency might prove itself toxic to the neurons. Higher levels of lactate and pyruvate could also lead to acidosis and associated clinical signs (Benevenga *et al.*, 1990). Lactate is readily formed from pyruvate by lactate dehydrogenase. An increase in pyruvate can lead to increased lactate level. Increased absorption of lactate from the rumen of affected animals might also have contributed to the increased serum lactate level. However, Mella *et al.* (1976) found decreased level of lactate in PEM.

Statistically significant increase ($p \leq 0.01$) in pyruvate level was observed in group I, II and III at zero hour. In group I, pyruvate level decreased from 4.53 ± 0.13 mg/dl at zero hour to 3.37 ± 0.15 mg/dl at 48 h. This was significantly high when compared to normal values. But pyruvate level at 48 h was comparable to normal value in group II and III. The significant increase in pyruvate level at zero hour in the present study indicated inhibition of carbohydrate metabolism and was in agreement with the findings of Lonkar and Prasad (1993); Syamasunder and Malik (1993); Maliekal (2000). According to Thompson and Cumings (1957); Pill (1967) a fall (60 per cent) in blood pyruvate level in response to treatment with thiamine was considered to be direct evidence of deficiency of that vitamin.

A statistically significant increase in L : P ratio was observed in animals of group III only. In group I and II the increase in L : P ratio was not statistically significant. Lonkar and Prasad (1993) observed wide fluctuation in L : P ratio at various intervals. Lactate pyruvate ratio of group III animals became comparable to normal values within 48 h. This suggested that metabolism of pyruvate became normal within two days of treatment with thiamine.

5.5.7 Thiamine

Mean serum thiamine level in the healthy group was 4.05 ± 0.39 μg/dl. This was comparable to the value reported by Tanwar, (1987) Gauri and Vashistha, (1988) in goats and Gupta *et al.* (2000) in calves. A statistically significant decrease was observed in serum thiamine level of diseased groups at

zero hour. Similar observations were made by Hill *et al.* (1988); Rammell and Hill (1988); Lonkar and Prasad (1993) in PEM affected animals. After therapy, the level again increased and became comparable to normal values. Benevenge *et al.* (1990); Fakkruddin *et al.* (1987b) reported similar findings in cattle and goats respectively. Ruminants meet their thiamine requirement mainly through intraruminal microbial synthesis and its deficiency accounts for prominent neurologic signs Edwin and Jackman (1973) detected adequate amount of thiaminase to cause destruction of ruminal thiamine in the rumen of PEM affected cattle and sheep. Hamlen *et al.* (1993) stated that direct measurement of blood thiamine was unreliable as most of it was stored in erythrocytes. Hence measurement of erythrocyte or tissue thiamine level would be more indicative of thiamine status of animal. Normal level of thiamine was detected in clinical cases of PEM by Loew and Dunlop (1972).

5.5.8 Erythrocyte transketolase and per cent TPP effect

The activities of thiamine – requiring enzymes were substantially reduced at the time of deficiency. This leads to inhibition of carbohydrates metabolism and has general effects on metabolic activity of brain (Brent and Bartley, 1984). Morgan (1973) proposed that impaired energy metabolism could lead to defects in cell volume regulation of astrocytes resulting in astroglial swelling and ultimately cerebral oedema. Many of the clinical signs of PEM could be attributed to cerebral oedema.

Erythrocyte transketolase level and per cent TPP effect in healthy animals were 54.14 ± 6.45 IU/l and 17.40 ± 4.10 respectively. Statistically significant decrease in TK level with massive elevation in TPP effect was observed at zero hour in all diseased animals. The values decreased and became normal within 48 h of treatment.

Transketolase (TK), an enzyme of the hexose monophosphate shunt, is present in mammalian tissues including heart, liver and erythrocytes. TK uses thiamine pyrophosphate (TPP) as coenzyme and magnesium as cofactor to

catalyse transfer of a ketol group (CoCH$_2$OH). The progressive decrease in erythrocyte TK activity and increase in per cent TPP effect in the present study could be correlated with the level of thiamine in serum. Brin *et al.* (1960); Syamasundar *et al.* (1993); Tanwar (1994) stated that combination of reduced TK activity and increased TPP effect as specific indications of thiamine deficiency in human beings and goats. Thiamine therapy resulted in marked depression of TPP effect and elevation of TK activity. Spicer and Horten (1981) also observed similar findings in PEM affected sheep.

5.6 RESPONSE TO THERAPY

Animals placed in group I were treated with thiamine hydrochloride @ 50 mg/kg body weight daily for four days. Half the dose was administered intramuscularly and half intravenously. In group II, a modified treatment, thiamine as in group I and oxytetracycline orally (to suppress the growth of thiaminase producing bacteria) @ 500 mg/goat once (on the day of admission) was adopted. Group III animals were treated with oral dose of thiamine (to repress the action of thiaminase and to inhibit the proliferation of thiaminase producing bacteria) @ 1g/animal once (on the day of admission) along with parentral administration as in group I.

5.6.1 Clinical response

Improvement was recorded in all the groups the very next day itself, but the response was remarkable in the animals of group III. Parentral administration of massive doses of thiamine might have helped in restoring the blocked energy metabolism in the brain thereby leading to reduction in cerebral oedema and associated clinical signs. Among the 21 animals, seven animals (two in group I, two in group II and three in group III) were presented to the hospital in lateral recumbency with severe convulsions, paddling, and nystagmus. All the animals started consuming leaves by second or third day.

One recumbent animal of group I was able to stand when supported by fourth day and started walking by fifth day. The other severely affected animal in group I, developed radial paralysis.

The recumbent animals of group II, could stand and walk by third day. No complications developed in this group.

In group III, two recumbent animals were able to stand and walk by second day. One animal of this group was presented to the hospital in a very bad condition (left lateral recumbency, occasional stargazing posture, respiratory difficulty, paddling of limbs). This animal was on sternal recumbency on the third day of treatment and was able to get up without help on fourth day.

Around 50 per cent of the animals of this group recovered on third day of treatment when compared to 14.2 per cent in group II and none in group I. This condition did not recur in any of the animals studied. The clinical response of the treated animals suggested that modified treatments especially one dose of thiamine orally along with parentral administration aided faster recovery.

5.6.2 Biochemical response

Biochemical studies revealed low thiamine concentration in the rumen fluid of all the diseased animals at zero hour. Rumen liquor thiamine level increased to 3.45 ± 0.37 µg/dl in group I, 3.91 ± 0.42 µg/dl in group II and 4.23 ± 0.52 µg/dl in group III at 96 h. Even after four days of treatment, rumen fluid thiamine level of group I was still less than normal value. But in the other two groups it was above the normal value. In group III animals, rumen liquor thiamine level was comparable to control value by 48 h of study. Blood lactate, pyruvate, L:P ratio and thiamine level were restored to normal following treatment at faster rate in group II and III when compared to group I. Not much variation in TK level or per cent TPP effect was observed between the different groups. Pair wise comparison (ANOVA) did not show any significant variation between these groups.

In the oxytetracycline treated group, the destruction of thiaminase producing bacteria might be responsible for the higher level of thiamine in rumen at 96 h. In group III animals, orally administered thiamine might be responsible for higher concentration of ruminal thiamine and blood thiamine at 48 h of experiment. According to Pill (1967); Spicer and Horton (1981) parentrally administered thiamine restored tissue thiamine level, but it could not alter the situation in the rumen where ingested thiamine or thiamine of microbial origin would continue to be destroyed by the action of thiaminase.

According to Thomas (1989) an initial predisposing factor that leads to development of PEM might be a drop in thiamine concentration in the rumen due to factors other than the action of thiaminase. If this lower concentration of thiamine is insufficient to repress thiaminase production then proliferation of *B. thiaminolyticus* and production of increased levels of thiaminase may occur in the rumen which may ultimately be responsible for thiamine deficiency.

Polioencephalomalacia in goats is currently treated by intravenous or intramuscular administration of thiamine hydrochloride. Radostits *et al.* (2000) recommends parentral administration of thiamine @ 5 mg/kg body weight given every three hours. The initial dose is usually given I/V followed by I/M injection daily for 2 to 4 days. An oral sources of thiamine should be given daily for 10 days. The conventional treatment requires the animal to be admitted as inpatient and constant monitoring. The line of treatment adopted in the present study will be more convenient for the field veterinarians and farmers. The present study suggests oral thiamine therapy, as an appropriate and effective means to control the thiaminase activity in the rumen and at the same time supports the animal with thiamine both directly and indirectly.

5.7 AUTOFLUORESCENCE

The presence of autofluorescence is an indication of necrosis in the brain (McAllister *et al.*, 1992; Sagar *et al.*, 1996). The brain samples collected from animals that died during the study did not reveal autofluorescence. Nair (1999)

opined that autofluorescence was not consistently observed in all the affected animals. The cause of autofluorescence of necrotic tissue when illuminated with UV light was attributed to accumulation of lipid peroxidation products as a result of the degradation of the lipoidal material within the macrophages. This test was only a supportive one and has no primary diagnostic value (Lee and Little, 1980).

5.8 PATHOLOGY

Brain was grossly soft and swollen with yellowish discolouration of cerebral hemispheres and had haemorrhages on meninges. This was consistent with the findings of Dickie et al. (1979); Jackman (1985); Fakhurddin et al. (1987a); Tanwar et al. (1993); Nair (1999).

Histopathologic changes observed in the cerebral grey matter were varying degrees of degeneration and necrosis, predominant gliosis and neovascularisation, perineuronal oedema, perivascular cuffing of mononuclear cells and accumulation of neutrophils at cortico-medullary junction. Thornber et al. (1979); McAllister et al. (1992); Chahar et al. (1993); Nair (1999) also observed similar findings in PEM affected animals. Cerebellum showed extensive perkinje cell degeneration. The pons and medulla showed no characteristic histological lesions.

Lesions of spinal cord included varying degrees of capillary congestion, thrombosis and pericapillary and intracapillary accumulation of neutrophils and mononuclear cells. No characteristic lesion could be observed in the plexus. More or less similar lesions were recorded in amprolium induced PEM by Spicer and Horton (1981); Nair (1999) in goats and they attributed the lesions to thiamine deficiency and subsequent metabolic derangement.

Electronmicroscopically, structural changes were more pronounced in the neuropil and in the cell body of neurons. Spongy changes were predominant in the neurophil. Nuclear membrane lysis, condensation of nucleolus, mitochondrial pleomorphism and ballooning and splitting of myelin were the ultrastructural lesions. Nair (1999) also reported same type of lesions in natural cases of PEM.

The histopathology and ultrastructural changes observed in the present study pointed to a primary biochemical insult which resulted in the derangement of volume control mechanism of the neurons possibly due to derangement of ATPase dependent sodium pump mechanism.

5.9 CONCLUSION

The present study revealed the prevalence of polioencephalomalacia as a disease condition with characteristic clinical signs in Kerala. The disease occurred throughout the year. The feeding practices in Kerala might predispose to PEM in goats. Nystagmus and the animals lying on one side were the most important and characteristic clinical signs. The physical character and microbial activity of rumen fluid were apparently normal. Detailed microbiological studies are required. Important biochemical changes in the blood viz. decreased thiamine, increased lactate and pyruvate, decreased TK with progressive increase in per cent TPP effect and response to thiamine therapy confirmed the role played by thiamine in the etiopathogenesis of PEM. Sulphates, magnesium, copper and cobalt do not seem to play a role in the etiopathogenesis of PEM in Kerala.. Neuropathological studies revealed swollen brain and with varying grades of focal degenerative and necrotic changes. Oedema observed in present study may be a basic factor in the causation of early neuropathological symptoms. When the disease progressed there was focal necrosis which further augmented the clinical symptoms. Affected animals responded often dramatically to thiamine administration. Oral administration of thiamine along with parentral administration led to speedy recovery. The quick response to the treatment might be due to restoring the fluid mechanism in the cell causing a quick reversal of the symptoms.

Further studies on the following lines are suggested

- Screening of common roughages consumed by goats for the presence of thiaminase mimicking/polyphenolic compounds.

- Rumen microbial changes in polioencephalomalacia.

- Role of antimetabolites/ other blocking factors if any in cerebrocortical necrosis.

- Basic biochemical mechanism in the neurons which might be responsible for the inflicting damage to the cell body and also myelin sheath in the axons.

Summary

SUMMARY

Polioencephalomalacia (PEM) is an important disease in goats and its pathogenesis, symptomatology, and therapeutic approaches are not well defined and documented. Hence an investigation was undertaken to assess the occurrence of the disease based on the data collected from 20 veterinary hospitals covering all the fourteen districts of Kerala state and also by personally interviewing the field veterinarians. Twenty one cases of PEM were divided into three groups of seven each (group I, II and III). Samples collected from six apparently healthy goats maintained under identical conditions were utilized for working out the normal values of the parameters studied. Detailed clinical examination was carried out in all the animals. Samples of rumen liquor and blood were collected before the start of therapy, 48 and 96 h of study and analysed. Studies on rumen liquor included evaluation of pH, physical characters, total volatile fatty acids, copper, cobalt, sulphate and thiamine levels. Biochemical parameters such as glucose, pyruvate, lactate, lactate pyruvate ratio, copper, cobalt, sulphate, magnesium, thiamine, erythrocyte transketolase and per cent TPP effect were estimated in the serum. Animals of group I was treated with thiamine hydrochloride @ 50 mg/kg body weight/day half of the dose intravenously and other half intramuscularly for four days. Group II animals were treated as in group I along with oral administration of 500 mg oxytetracycline per animal on the first day of treatment. Group III animals were treated as in group I along with 1 g of thiamine hydrochloride orally per goat on the first day.

Epidemiological data revealed that the disease occurred throughout Kerala with highest prevalence (3.61 per cent) in Malappuram district. The disease was prevalent throughout the year with maximum occurrence in the post-monsoon period. The age group of affected animals ranged from six months to two years. The feeding of rice gruel (carbohydrate rich diet) was considered to be one of the predisposing causes for the development of polioencephalomalacia of goats in Kerala. In the initial stages of the disease excitement, staggering gait, frequent bleating followed by low carriage of head, leaning on to the wall, wandering and

upward turning of head were reported by the owners. The characteristic clinical symptoms were nystagmus, lateral deviation of head, circling, lying only on one side and convulsions. The body temperature and pulse were normal in most of the affected animals.

Evaluation of rumen liquor revealed no significant changes in pH, physical characters, total volatile fatty acids, copper, cobalt and sulphate levels. All the diseased animals showed a low level of thiamine in rumen liquor on the day of admission with values of 2.11 ± 0.23 μg/dl in group I, 2.27 ± 0.49 μg/dl in group II and 2.09 ± 4.42 μg/dl in group III when compared to the normal value of 5.55 ± 0.24 μg/dl. The thiamine level returned to the normal by 48 h in group III and by 96 h in other groups.

Serum glucose level was significantly high before therapy in all the groups when compared to the normal value and returning to normal level by 48 h of treatment.

The serum lactate level was significantly high on the first day and comparable to the normal values by 96 h of treatment. Serum pyruvate was significantly high at zero hour in group I, II and III and became comparable to normal value by 48 h in group II and III and only by 96 h in the group I. Lactate pyruvate ratio was significantly high only in group III before treatment which returned to the normal level by 48 h.

Serum thiamine level was significantly low before treatment in all the groups with values of 2.01 ± 0.38 μg/dl in group I, 2.19 ± 0.23 μg/dl in group II, 2.29 ± 0.39 μg/dl in group III. Ninety-six hours after therapy, the serum thiamine level was significantly high in all the groups. Significant reduction in erythrocyte transketolase level with values of 33.89 ± 4.21 IU/l in group I, 43.57 ± 5.44 IU/l in group II, 32.26 ± 3.94 IU/l in group III were observed on first day before treatment. Significant increase in per cent TPP effect was observed in all the groups prior to treatment, and was comparable to normal value by 48 h. Evidences

presented here indicates that a state of thiamine deficiency exist in polioencephalomalacia.

The normal levels of serum copper, cobalt, magnesium and sulphate suggested that these electrolytes were not involved in the etiopathogenesis of PEM in Kerala.

Two cases were in an advanced stage and died on the second day of treatment. Post-mortem examination was conducted and different parts of CNS were collected for pathological examination.

Brain on gross examination showed extensive meningeal congestion. Whole brain was soft, swollen and there was diffuse yellowish discolouration of the cerebral grey matter. Neurons of the pyramidal and fusiform cell layers of the cerebral cortex with neuronophagia, satellitosis, perivascular edema, perivasular cuffing and neovasularisation of grey matter in certain gyri. These findings explained the need for initiation of treatment at the earliest. Electronmicroscopy revealed structural changes in the neuropil and in the cell body of neurons. Nuclear membrane lysis, condensation of component of nucleolus, mitochondrial pleomorphism, ballooning and splitting of myelin were the ultrastructural lesions.

All the animals of group I, II and III recovered after treatment. More than 50 per cent of the animals in group III recovered within three days of treatment when compared to 14.30 per cent in group II and none in group I. Although all the three lines of treatment were effective, parentral administration of thiamine @ 50 mg/kg body weight along with oral administration of thiamine @ 1g/goat was found to be more effective and helped in the speedy recovery of the PEM affected goats.

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ETIOPATHOGENESIS AND THERAPEUTIC MANAGEMENT OF THIAMINE RESPONSIVE CAPRINE POLIOENCEPHALOMALACIA

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ABSTRACT OF A THESIS
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ABSTRACT

A study on polioencephalomalacia in goats was conducted in the department of Clinical Medicine College of Veterinary and Animal Sciences during the period 1999 to 2002. The occurrence of the disease in Kerala was studied. The study revealed that the disease was reported throughout Kerala with maximum occurrence in the post-monsoon period. The disease was prevalent in all the age groups. Feeding of carbohydrate rich diet was one of the probable predisposing causes for the development of PEM.

Six apparently healthy goats maintained under identical conditions were utilized for recording normal values. Twenty one confirmed cases of PEM were equally divided into three groups at random. Detailed clinical examination, sampling and analysis of rumen liquor and blood were done before initiation of therapy, 48 and 96 h of study. Therapeutic management of group I was by administering thiamine hydrochloride for four days @ 50 mg/kg body weight. (half of the dose intravenously and the other half intra muscularly) whereas in group II and III it was supported with oral administration of 500 mg oxytetracycline/animal once (on the day of admission) and 1 gm of thiamine hydrochloride / animal once (on the day of admission) respectively.

The characteristic clinical signs were head tilt, lateral deviation of head, nystagmus, circling, staggering gait, pressing of head and lying only on one side. All the symptoms mentioned above were not present in all the animals. Rumen liquor study did not reveal any significant change in pH, physical characters, total volatile fatty acids, copper, cobalt and sulphate levels. Rumen liquor thiamine level was significantly low on the day of admission in all the groups, but became normal by 48 h in group III and by 96 h in other groups.

Normal level of serum copper, cobalt, magnesium and sulphate confirmed that these electrolytes were not involved in the etiopathogenesis of PEM in Kerala. Significant increase in glucose, lactate, and pyruvate levels were observed on the first day in all the diseased animals. The values decreased steadily after the

initiation of the treatment and the decrease was more marked in group III. Decreased level of serum thiamine, TK value with increase in per cent TPP effect were observed at zero hour in all the groups and were comparable to normal value after 48 h of treatment.

Two severely affected animals were utilized for the post-mortem studies. Brain on gross examination showed extensive meningeal congestion. Whole brain was soft, swollen with yellowish discolouration of cerebral grey matter. Histopathology of the brain of succumbed goats revealed necrosis of neurons of the pyramidal and fusiform cell layers of cerebral cortex with neuronophagia, satellitosis, perivascular edema, perivascular cuffing and neovascularisation of grey matter in certain gyri.

Ultrastructural lesions were characterized by neuronal swelling, membrane lysis, condensation of component of nucleolus, neuropil spongiosis, spiltting and ballooning of myelin at the intra-period line.

All the animals responded to the treatments adopted. More than 50 per cent of the animals in group III recovered by third day of treatment when compared to 14.28 per cent in group II and none in group I.

Therefore oral administration of 1 g of thiamine once on the day of admission along with parentral thiamine @ 50 mg/kg body weight is recommended for efficient and speedy recovery.