NEUROLOGICAL INVESTIGATION AND TREATMENT OF POLIOENCEPHALOMALACIA AFFECTED GOATS

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"NEUROLOGICAL INVESTIGATION AND TREATMENT OF POLIOENCEPHALOMALACIA AFFECTED GOATS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that the thesis, entitled **"NEUROLOGICAL INVESTIGATION AND TREATMENT OF POLIOENCEPHALOMALACIA AFFECTED GOATS"** is a record of research work done independently by **Dr. Arun George** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to him.

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Dedicated to
My Beloved Parents

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LIST OF ABBREVIATIONS

BBB	-	Blood-brain-barrier
CCN	-	Cerebrocortical necrosis
CNS	-	Central nervous system
CSF	-	Cerebrospinal fluid
dl	-	Decilitre
DLC	-	Differential leucocyte count
EEG	-	Electroencephalography
g	-	Gram
mg	-	Milligram
PEM	-	Polioencephalomalacia
RAS	-	Reticular activating system
TLC	-	Total leucocyte count

Introduction

1. INTRODUCTION

Domesticated goat descended from the wild ancestor *Capra hircus*. Archeological studies indicated that goats were reared in Egypt in the fourth millennium B.C., whereas carbon dating showed that goats were maintained in Jerico in 7000 B.C (Zeuner, 1963).

Goats because of its high prolificacy, less floor space requirement and ability to browse plants that are not consumed by large ruminants, remained in the paramount position among small ruminants. They still retain their popularity as a source of animal protein through their milk and meat. The milk of a goat is similar to that of a cow with an additional medicinal value and easy digestibility because of its smaller fat globules. Goats' meat called chevon is usually low in fat. All these factors might have gained it the title "poor man's cow".

In India, goat population increased from 47.08 million in 1951 to 123 million in 2000 (FAO, 2000), and ranks first in the world. Goat meat or chevon constitutes nearly 35 per cent of the total meat production in the country from livestock other than poultry.

In Kerala, there were about 18.6 lakh goats as per livestock census, 1996. Of these 13.7 lakhs were females and 4.9 lakhs were males. Goats constituted around 33.36 per cent of the total livestock in the state.

Intensive goat production has resulted in an increase in the number of high producing animals and these animals are more prone to stress and thereby diseases.

As any other animal, goats too suffer from a variety of diseases. Infectious diseases bring out their effects through severe morbidities and fatalities. Non-infectious diseases like parasitic diseases and nutritional deficiencies results in under-nutrition, decreased weight-gain and decreased production, and thereby contribute to economic loss. In addition to this, there are certain emerging diseases in goats. One such condition is polioencephalomalacia (PEM) or cerebrocortical necrosis (CCN), which can lead to high mortality and serious economic losses.

Polioencephalomalacia occurs sporadically in young cattle, sheep and goats, and other ruminants, and is characterized clinically by sudden onset of blindness, head-pressing, opisthotonus and convulsions, a rapid response following thiamin therapy in the early stages and pathologically by acute cerebral oedema and laminar necrosis of cerebral cortex (Radostits *et al.*, 1994).

Diseases characterized by neurological disorders are often neglected in the case of ruminants. Neurological examination and proper differential diagnosis is necessary to rule out deadly diseases like rabies, which has got lot of public health importance, and to formulate an effective therapeutic

regimen. Therefore a study on polioencephalomalacia was proposed with the following objectives.

1. To study the neurological manifestations, physico-chemical changes in CSF, electroencephalogram (EEG), radiographic changes and histopathology of brain in goats affected with polioencephalomalacia (PEM).

2. To assess the therapeutic efficacy of parenteral administration of thiamine hydrochloride along with bismuth carbonate orally in PEM cases.

Review of Literature

2. REVIEW OF LITERATURE

Polioencephalomalacia (PEM) was a common central nervous system (CNS) derangement of ruminants that resulted from necrosis of grey matter (Smith, 1996). PEM occurred sporadically in young cattle, sheep and goats and other ruminants, and was characterized clinically by sudden onset of blindness, head pressing, opisthotonus, convulsions and a rapid response following thiamine therapy in the early stages (Radostits *et al.*, 1994).

2.1 Etiology

Polioencephalomalacia was first reported by Jensen *et al.* (1956) in cattle and sheep and he observed that this disease was characterized by focal necrosis disseminated throughout the cerebral cortex. The disease occurred in both feedlot and pastured cattle and sheep.

Intraruminal administration of amprolium, a thiamine antagonist, which interfered with the phosphorylation of thiamine, resulted in the development of cerebrocortical necrosis in pre-ruminant calves (Markson *et al.*, 1974).

A search for the source of thiaminase in the rumen contents and faeces of sheep affected with PEM resulted in isolation of bacteria, which produced thiaminase type-I activity *in vitro*. After taxonomic studies they were

considered to be strains of *Bacillus thiaminolyticus*. Concurrent studies by other workers revealed the presence of strains of *Clostridium sporogenes* in sheep affected with PEM (Morgan and Lawson, 1974).

Rumen acidosis established rumen conditions congenial for the development of PEM due to the decreased pH, which was optimum for the thiaminase activity or by proliferation of thiaminase producing bacteria (Brent, 1976).

Dickie and Berryman (1979) observed that cattle grazing on *Kochia scoparia* (Mexican fire weed) developed signs of PEM and the problem did stop when they were moved from the pasture containing Kochia

Range cattle, which had access to *Kochia scoparia* developed cerebral edema and PEM (Dickie *et al.*, 1979).

Bakker *et al.* (1980) induced PEM in sheep, using local bracken fern (*Pteridium escuelinum*) rhizomes as a source of thiaminase type I and pyridine as a basic co-substrate. The condition was thiamine responsive and PEM was confirmed by histopathological examination.

Deprivation of water for approximately 48 hours in cattle produced neurological signs and necropsy of brain revealed deep laminar cerebrocortical necrosis (Padovan, 1980).

Edwin *et al.* (1982) reared calves on thiamine free synthetic milk and suggested that CCN might result from profound thiamine deprivation alone.

The cumulative thiamine metabolism of the predominant bacteria associated with the rumen of PEM-affected heifers led to substantial net thiamine destruction, whereas metabolism associated with the rumen of a normal steer led to thiamine production. PEM might occur as a consequence of alteration of the metabolic activities of the predominant resident ruminal bacteria associated with diseased cattle (Haven *et al.*, 1983).

Raisbeck (1982) in a retrospective study demonstrated that a statistically significant and possible relationship existed between PEM and high sulfate rations.

The level of thiamine in serum and rumen contents of PEM affected calves was significantly low. These affected animals became almost normal with parenteral thiamine therapy for three days (Fakhruddin *et al.*, 1987b).

Gooneratne *et al.* (1989) reported that inadequate microbial synthesis, impaired absorption and utilization of vitamin B1, presence of vitamin B1 antimetabolites, increased demand for vitamin B1 or increased rate of B1 excretion, all these could lead to thiamine inadequacy. Diets high in sulfur decreased the amount of vitamin B1 entering the duodenum probably due to reduced synthesis of vitamin B1.

Calves fed on experimental diet with added sodium sulfate induced polioencephalomalacia (Gould *et al.*, 1991).

McAllister *et al.* (1992) induced PEM in lambs by the administration of a sulfide solution and the neurological signs occurred included stupor, coma, visual impairment and seizures

PEM developed in mature cattle on pasture, when the primary water source contained a markedly elevated level of sodium sulfate (Hamlen *et al.*, 1993).

According to Jeffrey *et al.* (1994) calves and lambs fed ammonium sulfate as urinary acidifier developed signs of CCN and were non-responsive to thiamine therapy with normal erythrocyte transketolase levels.

Role of thiamine deficiency in the etiopathogenesis of PEM was further supported by the recovery of spontaneously affected animals following thiamine therapy, but the exact mechanism involved remained to be further studied (Lonkar and Prasad, 1994a).

Tanwar *et al.* (1994) concluded that oral administration of amprolium (300 mg/kg body weight orally) for 4-6 weeks produced biochemical changes characteristic of PEM in buffalo calves.

Steers fed with high-sulfate diet developed PEM and the onset of signs was associated with increased sulfide concentration in the rumen fluid (Cummings *et al.*, 1995).

Elemental sulfur toxicosis in a flock of sheep precipitated signs of PEM and treatment with thiamine was not of value in most animals (Bulgin *et al.*, 1996).

Hill and Ebbet (1997) diagnosed PEM clinically in heifers grazing *Chou moellier* (*Brassica oleracea*). Clinical signs included ataxia, recumbency, blindness and aimless walking. High sulfur content in the *Chou moellier* and a recent change in grazing management were the most likely causes of the outbreak.

According to McAllister *et al.* (1997), outbreaks of PEM in feedlots were associated with the introduction of high sulfate water during hot weather, which increased water consumption. Cattle had significantly high sulfide concentration in ruminal fluid after entering the feedlot.

PEM was a neuropathologic condition of ruminants that could be induced by altered thiamine status, water deprivation-sodium ion toxicosis, lead poisoning and high sulfur intake. Investigations on sulfur related PEM demonstrated that the onset of clinical signs coincided with excessive ruminal sulfide production (Gould, 1998).

Loneragan *et al.* (1998) reported that the pathologic concentrations of ruminal hydrogen sulfide appeared to be a central component in the pathogenesis of sulfur associated PEM.

Radostits *et al.* (2000) reported molasses toxicity in Cuba in cattle fed on a liquid molasses-urea feeding system with limited forage. The clinical and necropsy findings were identical to PEM

Experimental reproduction of clinical signs simulating PEM could be the result of 'amprolium poisoning encephalopathy' since, amprolium was found in the brain tissue of animals fed with amprolium (Radostits *et al.*, 2000).

The dietary content of copper, zinc, iron and molybdenum might also have important modifying influences on sulfur toxicosis. Molybdenum and copper could combine with sulfur to form insoluble copper-trithiomolybdate. Copper, zinc and iron form insoluble salts with sulfide and their low level would increase the bioavailability of sulfide in the rumen (Radostits *et al.*, 2000).

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 a decrease in ruminal thiamine concentration (Radostits *et al.*, 2000).

2.2 Epidemiology

2.2.1 Species

PEM was reported in sheep and cattle (Jensen *et al.*, 1956), horses (Cymbaluk *et al.*, 1978), goats (Smith, 1979), white tailed deer (Wobester and Runge, 1979), antelope (Blood *et al.*, 1979) and dogs (Breud and Vendevelda, 1979).

2.2.2 Incidence

Morbidity rate in sheep and cattle were generally less than 10 percent (Spence *et al.*, 1961). PEM was first reported in India by Tanwar (1987), from the arid zone of Rajasthan.

Though the disease was prevalent throughout the year in Bikaner, Rajasthan, the occurrence was higher in late winter (January / February), followed by midsummer (April), rainy season (August to October), and early winter (November) (Lonkar *et al.*, 1993).

In Kerala, the disease occurred throughout the year with maximum incidence in the month of April, followed by May, March and February. Reduced incidence in January, August, September and October (Nair, 1999).

Maliekal (2000) reported that the disease occurred throughout the year with maximum occurrence in the monsoon and post-monsoon period.

2.2.3 Age

Animals in the age group of 12-18 months showed maximum incidence (Jensen *et al.*, 1956). Terlecki and Markson (1961) reported that the incidence of PEM ranged from 2 to 7 months of age in sheep and 10 weeks to 11 months in cattle. The disease occurred most commonly in well-nourished thrifty cattle 6-18 months of age (peak incidence in 9-12 months of age), which had been in the feedlot for several weeks. The disease might affect goats from 2 months to 3 years of age (Radostits *et al.*, 2000).

2.2.4 Sex

Incidence of PEM in sheep was 38 percent in males and 62 percent in females (Jensen *et al.*, 1956). Sobhanan (1981) concluded that the incidence was more in females because male goats were disposed off at an early age and only females were grown to the adult age. Occurrence was more in females and in the age group of 6 months to 5 years (Maliekal, 2000).

2.2.5 Diet

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, rumen pH became acidic and bacterial flora changed to produce potent thiaminase precursors responsible for the destruction of ruminal thiamine.

In Kerala, rice (a carbohydrate rich diet) formed the main component of concentrate ration which possibly reduced the rumen pH to produce a congenial environment for increased thiaminase production (Sobhanan, 1981). Water source containing markedly elevated levels of sodium sulfate resulted in the development of PEM (Hamlen *et al.*, 1993).

History of PEM affected animals suggested that carbohydrate rich diet was one of the predisposing factors for the development of the disease in Kerala (Maliekal, 2000).

PEM was commonly associated with milk replacer diets in kids or concentrate feeding in older goats (Radostits *et al.*, 2000).

2.3 Clinical signs

Pierson and Jensen (1975) observed that PEM manifested in two forms in lambs – acute and subacute. In acute form, affected lambs were found dead or prostrate. Those that were alive had hyperesthesia, involuntary contraction of some muscles, limb movements and convulsions. In subacute form, lambs became blind, moved aimlessly in circles with incoordination, appeared disoriented and developed muscle tremors and fell down. Characteristic stances included lowering the head to ground level, staring above the horizon and extended head over the back.

Temporary blindness, partial protrusion of tongue as though partially paralysed, depression, ruminal atony, anorexia and nystagmus were noticed (Dickie *et al.*, 1979).

Characteristic neurological signs exhibited by goats were tremors of head, tonic-clonic convulsions, marked opisthotonus, stiffness of fore and hind legs, hyperesthesia to touch and sound, lack of menace reflex, nystagmus and circling (Sobhanan, 1981, Tanwar *et al.*, 1983 and Tanwar, 1987).

In PEM, animals after a short period of dullness wandered aimlessly in wide circles, became ataxic and collapsed with intermittent convulsions and evident hyperesthesia, nystagmus, opisthotonus, trismus and clonic extensor spasms (Jackman, 1985).

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 partially in both hind limbs, opisthotonus, paddling, convulsions, temporary blindness, nystagmus, absence of menace reflex and normal pupillary response.

Clinical signs of amprolium induced CCN in goats included depression, staggering gait, inco-ordination, blindness, head pressing and later upward turning of head, twitching of facial muscles and absence of menace

response. In terminal stages, paddling, grinding of teeth, dyspnea, coma and death were recorded (Lonkar and Prasad, 1992a).

Chahar *et al.* (1993) observed, in amprolium-induced sheep, depression, progressive ataxia of hind limbs, staggering gait, loss of vision, absence of menace response, grinding of teeth and champing of jaws. In addition to these, circling, knuckling of fetlock, jumping and bounding gait, opisthotonus, lock-jaw condition and clonic-tonic convulsions were observed.

Major clinical signs in PEM affected buffalo calves were ataxia, kyphosis, lacrymation, opisthotonus, torticollis, sternal/lateral recumbency, coma and death (Syamasundar and Malik, 1993).

Clinical symptoms observed by Tanwar (1995) in buffaloes affected with CCN were anorexia, weakness, loss of balance, blindness, lateral recumbency, convulsions, opisthotonus, paddling movements, hyperesthesia, nystagmus, dorso-medial strabismus and absence of menace reflex.

Tanwar and Malik (1995) observed the clinical symptoms of amprolium-induced PEM in buffalo calves and reported dullness, rigid stance, muscle tremors, ataxia, kyphosis, broad based stance, blindness, nystagmus, hyperesthesia, convulsions, extensor rigidity of thoracic limbs, paddling and opisthotonus.

2.4 Pathogenesis

2.4.1 Thiamine

Markson *et al.* (1974) stipulated that pyruvate was the substrate for pyruvate decarboxylase system of enzymes, which were Thiamine Pyro Phosphate (TPP) dependent. Hence in thiamine deficiency there was rise of blood pyruvate level; lactate was readily formed from pyruvate by lactate dehydrogenase and vice-versa.

Morgan (1973,1974) reported that cerebral edema was secondary to a primary neuronal defect at the biochemical level and periaxonal accumulation of fluids led to increased intracranial pressure.

CCN was not considered to be due to failure of thiamine synthesis in the rumen nor due to any defect in its absorption from the intestines. In the rumen, thiaminase, in the presence of a co-substrate, not only destroyed thiamine but also created thiamine analogues that inhibited one or more thiamine-requiring reactions necessary for energy metabolism in the central nervous system (Brent and Bartley, 1984).

There was significant elevation of blood lactate and blood pyruvate levels in PEM affected animals (Syamasundar and Malik, 1993).

Thiamine diphosphate, a coenzyme for the activation of transketolase was produced from thiamine. Transketolase found in the glial cells and

erythrocytes, was an important enzyme involved in the glucose metabolism. Since brain was glucose dependent, glial cell transketolase played an important role in metabolic activities of brain (Hamlen *et al.*, 1993).

Lonkar and Prasad (1994a) suggested that hypoxia might be the result of poor energy utilization because, for energy, brain depended on glucose through the pentose phosphate pathway. In this pathway transketolase was a rate-limiting enzyme, which needed thiamine.

Thiamine was an essential component of several enzymes involved in intermediary metabolism and a state of deficiency resulted in increased blood concentration of pyruvate, a reduction in the lactate-pyruvate ratio and depression of erythrocyte transketolase. The brain of calf had a greater dependence on the pentose phosphate pathway for glucose metabolism, in which pathway the transketolase enzyme was a rate-limiting enzyme. The first change, which occurred, was an edema of the intracellular compartment, principally involving the astrocytes and satellite cells. This was followed by neuronal degeneration, which was considered secondary. It was suggested that the edema might be due to a reduction in adenosine triphosphate (ATP) production following a defect of carbohydrate metabolism in the astrocytes. The three basic lesions- compact necrosis, edema necrosis and edema alone – suggested a multifactor etiology for PEM (Radostits *et al.*, 2000).

2.4.2 Sulfur

Excessive sulfur in the diet resulted in an increased production of hydrogen sulfide in the rumen gas cap. Because as much as 60 percent of eructated gas could be inhaled by ruminants, affected animals probably inhaled various amounts of hydrogen sulfide gas as well. The sulfide crossed the respiratory epithelial barrier and could cause severe respiratory distress (Dougherty and Cook, 1962 and Kandylis, 1984).

Raisbeck (1982) reported that sulfite produced from sulfates by rumen microflora might destroy ruminal thiamine as it was produced, causing PEM by a mechanism similar to that attributed to ruminal thiaminase.

Durand and Komisarczuk (1988) found that in the rumen, sulfur changed to hydrogen sulfide by the action of rumen microorganisms. Sulfide was absorbed across the rumen wall at a rate much faster than ammonia, and absorption rate was a function of the sulfide concentration and rumen pH. Sulfide also had a direct paralytic effect on the carotid body and, thus, could also inhibit respiration.

Short and Edwards (1989) noted that sulfide affected oxidative metabolism by inhibiting the action of catalases, peroxidases, dopa oxidases, dehydrogenases, carbonic anhydrase and dipeptidases

The toxic effects of excess dietary sulfur were unique in ruminants. Brain tissue, because of its high lipid content would be extremely vulnerable to lipid peroxidation by sulfate-derived free radicals. It was shown that a deficit of thiamine increased the intensity of lipid peroxidation. Free thiamine was thought to protect the cell by scavenging potentially toxic intermediates generated by the myeloperoxidase hydrogen peroxide halide system (Olkowski *et al.*, 1992). In addition, sulfide combined with hemoglobin to create sulfhemoglobin, reducing the oxygen carrying capacity of the blood (Bulgin *et al.*, 1996).

Decreased rumen pH favoured increased production of hydrogen sulfide by the rumen microbial population (Bulgin *et al.*, 1996), relating it to the rapid onset of clinical signs and contributed to the toxicity of the sulfur.

Ruminal sulfide after systemic absorption, might cause decreased ATP production and necrosis of grey matter, because neurons were highly susceptible to ATP depletion (McAllister *et al.*, 1997).

Loneragan *et al.* (1998) suggested the possibility of blood vessel degeneration in the brain stem as well as generalized vasculopathy with increased absorption of sulfide into the system in ruminants.

Diets high in sulfur resulted in the production of potentially toxic hydrogen sulfide in the rumen as rumen microflora required 10-12 days for getting adapted to higher dietary sulfate content. In experimental sulfate

diets, which induced PEM, the rumen pH decreased during the transition to the experimental diet and the acidic condition in the rumen favored increased rumen gas concentration of hydrogen sulfide. If ruminants inhaled 60 percent of eructated gases, inhalation of hydrogen sulfide could be a route of systemic sulfide absorption, in addition to the gastrointestinal absorption. Sulfide inhibited cellular respiration leading to hypoxia, which might be sufficient to create neuronal necrosis in PEM. The nervous system lesions of sulfur toxicosis were indistinguishable from lesions in the naturally occurring disease (Radostits *et al.*, 2000).

2.5 Cerebrospinal fluid

Sorjonen (1987) reported that the cerebrospinal fluid (CSF) albumin values could be used as indicators of blood-brain barrier disturbance.

CSF of goats of either sex affected with experimentally induced PEM with amprolium was colorless, without any turbidity or flakes, with an increase in protein content than normal (Lonkar and Prasad, 1992b).

Scott (1992) collected CSF samples from sheep with neurological disorders and observed a significant increase in the group mean CSF protein and white blood cell (WBC) count in meningitis, listeriosis and spinal abscess, but not in PEM.

Syamasundar *et al.* (1992) observed a significant increase in glucose, protein, pyruvate, lactate and total WBC count, in CSF, in PEM affected calves.

Braund *et al.* (1993) observed an increased protein concentration in CSF, sampled from the atlanto-occipital and lumbosacral spaces in lambs with congenital hypomyelination neuropathy.

Sargison *et al.* (1994) observed normal CSF values in lambs with PEM.

CSF collected from buffalo calves with PEM was clear, colorless and negative to Pandy's test. The level of electrolytes sodium, potassium, calcium, phosphorus and magnesium were not altered (Tanwar *et al.*, 1994).

Disorders in which neuronal necrosis was a primary feature, such as thiamine responsive PEM, typically had pleocytosis and increased total protein (George, 1996).

Brain edema severe enough to result in ischemia, infarction, or herniation resulted in dysfunctional blood-brain-CSF barrier, vasogenic edema and an elevated CSF protein. If neuronal necrosis ensued, the WBC count might also increase. Even in the absence of vasogenic edema, BBB leakage might occur, perhaps because of the biochemical effects of the disorder on the barrier cells (Bailey and Vernau, 1997).

CSF was clear and the CSF protein evaluation revealed high protein level in spontaneous PEM cases in goats, whereas in experimental cases, the concentration remained within the normal range indicating that it had no diagnostic value (Nair, 1999).

The level of CSF protein in PEM affected cattle might be normal to slightly or extremely elevated. A range from 15 to 540 mg/dl with a mean value of 90 mg/dl in affected cattle was recorded. There might also be a slight to severe pleocytosis in which monocytes or phagocytes predominate (Radostits *et al.*, 2000).

2.6 Electroencephalography

2.6.1 Normal Animals

According to Merrick and Scharp (1971) electroencephalograms of cattle, 'Standing with eyes open', displayed a mixture of frequencies, with intermittent episodes of low-voltage fast activity (25 to 27 cycles/second). Animals, 'lying down with head erect and eyes open' showed a pattern similar to that obtained in 'standing with eyes open' with comparable fast (24 c/s) waves of moderate amplitude. 'Lying down animals with head supported and eyes partially closed' revealed diffuse, random and slow waves and had considerable amplitude with some, superimposed, low-voltage fast activity.

Strain *et al.* (1986) recorded the low-voltage, high frequency pattern typical of the alert state in adult sheep.

Electroencephalograms recorded from Japanese Black calves, 1-10 weeks of age, were of dominant low-frequency activity, superimposed by low-voltage high frequency activity in the alert state (Takeuchi *et al.*, 1993).

EEG studies in calves aged three to six weeks of age, showed an alpha rhythm-like pattern. These waves appeared in the relaxed state and were localized in the occipital and vertex areas. In the sleep state, EEG pattern consisted of slow waves with high amplitude (150 microvolt). Calves between seventh to tenth week of age exhibited an increase in amplitude of theta and alpha waves, and an increase in appearance of fast activities in the relaxed state. The frequency of fast activity exceeded 25 Hz, especially in the frontal area. In the sleep state, the amplitude of slow waves decreased to less than 100 microvolt (Takeuchi *et al.*, 1998).

2.6.2 PEM affected animals

Electroencephalographic recordings in lambs given amprolium showed spindles with a frequency of 3 to 7 Hz without obvious functional changes (Dunlop *et al.*, 1981).

Itabisashi *et al.* (1990) observed that EEG changes were almost similar in amprolium dosed cattle and sheep. The changes found in the EEG included

continuous slow waves and long lasting spindles, both of which appeared diffusely. Alert states such as feeding and movement of head with blinks were normally associated with low voltage EEG. The amplitude of the abnormal slow waves was low during the earlier stage of EEG slowing period and changed gradually as high as that of normal irregular waves of high amplitude and slow frequency. In some cases convulsions occurred with no distinct pattern of EEG but with continuous diffuse slow waves of already attenuated amplitude or of high amplitude. This pattern of high amplitude was interrupted for a few seconds of convulsions by low amplitude activity, similar to the normal alert pattern. Long lasting spindles appeared mainly at a rate of 2 to 4 episodes/ 10 minutes with 600 to 900 microvolts, maximum peak-to-peak amplitude, and with 30 to 120 seconds duration. Spindle episode consisted of slow wave trains or sharp-(or spike-) and –slow- wave complexes.

EEG examination was carried out in CCN affected calves with and without clinical signs. EEG obtained showed high amplitude (50-160 microvolt) and slow (1-4Hz) activity (HASA). Other abnormalities observed were asymmetric sharp waves and diffuse lowered activity, which was almost flat in certain leads. It was suggested that one of the characteristic EEG patterns of a CCN patient might be HASA and decreased fast activity. The degrees of abnormalities of the EEGs coincided with the seriousness of the lesions. As the EEG obtained from the calf having no neurological signs

showed an abnormal pattern, it was considered that EEG examination in the early stages of the disorder was valuable to diagnose CCN (Suzuki *et al.*, 1990).

Syamasundar *et al.* (1991) conducted studies on amprolium induced PEM in buffalo calves and reported abnormalities in the EEG. EEG changes included a decrease in frequency patterns, occasional spindles and decreased voltage patterns during the onset of clinical signs. This was followed by abnormal slow waves and a high voltage slow frequency pattern. In the comatose stage, there was little evidence of electrical activity (Syamasundar *et al.*, 1996).

2.7 Radiography

Fractures of the skull were very difficult to diagnose radiographically. Fractures involving the frontal, parietal or occipital bones might be hardly visible because of the overlying structures of the skull. Maxillary and nasal bone fractures were easily demonstrated radiographically. The different types of skull fractures included depression, stellate, linear and comminuted fractures (Carlson, 1967).

Brain damage from direct trauma (maceration or compression) or indirect trauma (hematoma of the epidural, subdural, subarachnoid or intra cerebral type) could be sequelae to skull fractures. The diagnosis was based

on history of trauma, radiographic examination, presence of any abnormal neurological signs and clinical signs (Whittick, 1974).

Radiographic examination of the skull of smaller animals, such as sheep, pigs, and young horses and cattle, to detect the abnormalities, which were affecting the nervous system, was performed as an aid to diagnosis (Radostits *et al.*, 1994).

2.8 Treatment

Thiamine administration was effective for PEM in goats (Pierson and Jensen, 1975; Tanwar, 1987).

Tanwar *et al.* (1983) suggested that goats showing typical signs of PEM should be treated with thiamine, parenterally, as early as possible and advanced cases of it might not respond because of the death of cerebral neurons.

In PEM, administration of thiamine parenterally was advised for the alleviation of symptoms and thiamine deficiency could be best depicted by analyzing the erythrocytes for transketolase (Jackman, 1985).

Fakhruddin *et al.* (1987b) observed that goats appeared to feel comfortable with gain of vision and started to take their feed after the course of treatment with thiamine. The results clearly revealed that thiamine was the drug of choice for treatment of animals affected with PEM, preferably, half

the dose intravenously and the remaining half intramuscularly, to be repeated after an interval of 24 hours for three days for complete recovery.

Thiamine administered parenterally half the dose intramuscularly and half intravenously was very effective in naturally occurring cases of PEM in goats. In advanced cases supportive therapy with dexamethasone and mannitol was also rewarding (Gauri and Vashistha, 1988).

Chahar *et al.* (1993) determined the efficacy of thiamine hydrochloride at different stages of the disease and reported that in early stages of the disease thiamine was found as an efficient drug for the treatment of PEM.

Tanwar (1995) concluded that thiamine deficiency occurred in buffaloes and successful treatment of thiamine deficiency depended upon early recognition of the disease when the animal responded rapidly to a single dose of thiamine. If treatment was delayed the cerebral neurons became irreversibly damaged, and prognosis was grave.

Oral administration of bismuth carbonate had beneficial effects in PEM due to sulfur toxicosis (Bulgin *et al.*, 1996; White, 1964). The dissociation rate of sulfide might be reduced by administering bismuth carbonate, which would increase rumen pH, thus, limiting absorption of sulfur from the rumen (Kandylis, 1984). Oral administration of a broad-spectrum antibiotic to kill sulfide-producing microflora would be beneficial (Bulgin *et al.*, 1996).

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

In PEM the drug of choice was thiamine hydrochloride at the rate of 10 mg/kg body weight intravenously every 3 hours for a total of 5 treatments. When treatment was given within a few hours of onset of signs, a beneficial response within 1-6 hours was common and recovery occurred in 24 hours. Animals, which responded to treatment, might do so within the first 48 hours and those, which were still clinically subnormal, and anorectic by the end of the third day should be slaughtered for salvage. Oral administration of thiamine or thiamine derivatives was indicated when thiaminases were thought to be in the alimentary tract. Thiamine hydrochloride at a rate of 1g for goats and 5g for calves, in a drench, was recommended. Thiamine propyldisulfide could depress the thiaminase activities in the ruminal fluid within two hours after oral administration (Radostits *et al.*, 2000).

Molasses toxicity was not thiamine responsive and could be reversed by feeding forage. Molasses had high inorganic sulfur content and the thiamine concentrations in the brain and liver of PEM affected animals, which were fed molasses, did not differ from those of normal cattle (Radostits *et al.*, 2000).

Singh *et al.* (2000) reported that thiamine hydrochloride was the drug of choice preferably half dose by intravenous route and half dose by intramuscular route for 3 days in the early stages of the disease.

2.9 Gross and histopathology

Morgan in 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 range cattle died of PEM was moist, soft and swollen, with flattened gyri. On histopathology laminar cortical necrosis was observed (Dickie *et al.*, 1979).

Thornber *et al.* (1979) observed severe brain swelling and uniform yellow discoloration of the dorsal and lateral aspects of the cerebral cortex. Histologically, degenerate and necrotic grey matter featured shrinkage and marked dilatation of perivascular spaces. All the experimental lambs showed pachy leptomeningeal infiltration of neutrophils and small and large mononuclear cells.

Histopathology of brains of animals, which showed neurological signs when fed with bracken fern rhizome, was consistent with PEM and was also indistinguishable from field cases of the disease (Bakker *et al.*, 1980).

According to Jackman (1985) gross appearance of brain, in PEM, revealed irregular areas of yellowish discoloration of cerebral gyri.

The brain of PEM affected goats was grossly soft and spongy in nature with yellowish discoloration particularly of cerebral hemispheres. Histopathology of brain tissue revealed focal areas of microcavitation, loose matrix and edematous brain tissue with a few prominent and congested vessels. The neurons in affected areas were shrunken with strong acidophilic cytoplasm. The nuclei of neuronal cells were also shrunken and pyknotic. A few lymphocytes and monocytes were also seen at the perivascular spaces (Fakhruddin *et al.*, 1987a).

Tanwar (1987) noted that in PEM affected goats, the entire dorsal aspect of the cerebrum was soft with a yellowish discoloration of cerebral cortex especially the occipital lobes. On histology there was segmental focal laminar necrosis of the cerebral cortex with shrunken neurons and perineuronal vacuolation. There was also marked dilatation of perivascular spaces.

In sulfide induced PEM, fresh brains were soft and had flattened cortical gyri with yellowish discoloration. Histological examination revealed neuronal necrosis within the cerebral cortex with shrunken, densely eosinophilic cytoplasm and pyknotic or absent nuclei, granular or vacuolated neuropil without identifiable dendritic architecture, endothelial hypertrophy

and hyperplasia, astrocytosis and infiltrating gitter cells. Nuclei within necrotic neurons were dark or normal in appearance. Middle laminae were consistently affected with cortical lesions (McAllister *et al.*, 1992).

In sulfur induced PEM, extensive multiple white-yellowish foci of necrosis, some in a laminar pattern, were seen in the cortex, midbrain and brainstem. The necrotic lesions of cerebral cortex involved gyri and sulci extending, in some areas, to the sides and depth of the sulci (Olkowski *et al.*, 1992).

Chahar *et al.* (1993) noticed that in PEM affected sheep, brain was grossly softened and yellowishly discolored. Histopathology depicted chromatolysis of some of the neurons in cerebral cortex. Cytoplasm was eosinophilic and presented occasional vacuolation. Pericellular edema of neurons, swelling and proliferation of endothelial cells were also seen. Cerebellar cortex revealed alteration in the purkinjee cells and involved cells appeared necrotic, losing their nuclear and cytoplasmic details.

Hamlen *et al.* (1993) reported that in sulfide toxicity in cattle, brain grossly appeared soft, multifocally flattened and yellowish. Petechial haemorrhagic foci also extended cranially from the thalamus into the basal ganglia and internal capsules. Histopathology of brain showed bilateral and multifocal, acute necrosis of cortical grey matter, most consistently involving outer cortical laminae. Sections of thalamus, midbrain and medulla showed

similar severe acute parenchymal necrosis. These regions tended to show more edema, with prominent perineuronal and perivascular vacuolations.

Gross and histopathological changes in amprolium fed buffalo calves were studied by Tanwar *et al.* (1993). He found that gross lesions included congestion and haemorrhages in the meninges. The cerebral gyri were swollen with yellowish discoloration of cerebral cortex. There was shrinkage of neurons, perivascular and pericellular edema, necrosis of neurons, satellitosis, glial nodule and gliosis. Blood vessel walls were thickened due to hypertrophy and hyperplasia of endothelial and adventitial cells. Purkinjee cells of cerebellar cortex were degenerated. In the necrotic areas neuropils were fragmented and edematous. Subcortical greymatter of the thalamus showed necrosis of neurons and gliosis.

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/triangular neurons, malacic foci, extravasation of erythrocytes, gliosis, satellitosis, perivascular cuffing and prominence of capillary epithelium confined to cerebral cortex. The lesions of cerebellum were comparatively mild and

included engorgement of blood vessels, vacuolations around neurons in grey matter, vacuoles in degenerating purkinjee cells and their disappearance.

Multifocal sections of the cerebral cortex and brain stem of beef calves with sulfur toxicosis were examined histologically. Segmental areas of cerebral cortex had pale staining, vacuolated neuropil and shrunken angular necrotic neurons with homogenous eosinophilic cytoplasm and pyknotic nuclei. Cortical areas without overt neuronal necrosis had large pericellular and perivascular spaces indicative of intracellular edema. Focal areas of haemorrhages in the thalamus and midbrain were associated with degeneration of veins and venules. Vessel walls were segmentally homogenous, eosinophilic and sometimes had karyorhectic debris. A low number of macrophages, lymphocytes and polymorphonuclear cells were evident in the perivascular space (Loneragan *et al.*, 1998).

Nair (1999) studied the histological lesions of the brain in experimental and natural cases of PEM in goats and found that they were comparable in different segments of the brain. Mostly it was characterized by diffuse laminar cortical degeneration and necrosis, occasional neuronal swelling, glial cell reaction and white matter vacuolation. Vascular changes predominated in the sodium sulfate group and also in the natural cases. There was glial cell response in the form of nodules in sodium sulfate group and natural cases. A

predominant perivascular accumulation of lymphocytes, gitter cells and monocytes were seen in the natural cases.

Diffuse cerebral edema with compression and yellow discoloration of the dorsal cortical gyri was evident and the cerebellum was pushed back into the foramen magnum with distortion of its posterior aspect. Histologically, the lesions were bilateral laminar necrosis and necrosis of deeper cerebral areas. The necrosis was prominent in the dorsal occipital and parietal cortex, but bilateral areas of necrosis were also seen less frequently in the thalamus, lateral geniculate bodies, basal ganglia and mesencephalic nuclei. Lesions of the cerebellum were also present. The severity and distribution of the lesions probably depended on the inter-relationship between clinical severity, age of the affected animal and length of illness before death (Radostits *et al.*, 2000).

Materials and Methods

3. MATERIALS AND METHODS

Goats brought to the Veterinary Hospital, College of Veterinary and Animal Sciences, Mannuthy, between November 2000 and August 2001 with clinical symptoms suggestive of PEM were used for the study. After detailed anamnesis and clinical examination, seven cases were diagnosed to be suffering from PEM. These animals were treated with thiamine hydrochloride at the dose rate of 50 mg/kg body weight twice daily intravenously and bismuth carbonate at 15 g/animal orally for four days.

3.1 Parameters studied

Detailed clinical and neurological examinations were performed (Smith, 1996) as per the 'Proforma for Clinical Examination' given in Appendix. CSF collection and analysis, electroencephalographic recording, radiography of the skull and histopathology of brain were performed. Six apparently healthy goats, maintained under identical conditions, served as controls.

3.1.1 Clinical and neurological examination

Clinical examination included history taking and examination of the patient. Examined the patient to ascertain its general appearance, behaviour, posture, gait, urination and defeacation. Respiration, pulse, temperature and

nature of mucous membrane were recorded. Physical examination of head, neck, fore limbs, trunk, hind limbs, tail and anus were also carried out.

Neurological examination included behaviour, posture and gait, cranial nerve examination, postural reactions like wheelbarrowing and proprioceptive positioning, and spinal cord reflexes like patellar reflex, flexor reflex, panniculus reflex and perineal reflex.

3.1.2 Cerebrospinal fluid

The lumbo-sacral site was clipped and surgically prepared. The animal was positioned in lateral recumbency and three ml of CSF was collected from the lumbo-sacral site (Plate 1) as per the procedure of Scott (1995) using a 20 G, 1½ inch long needle.

3.1.2.1 Physical and chemical characters

CSF was examined visually for their physical characters like colour, transparency and consistency at the time of collection as per Scott (1995).

Total protein concentration of CSF was estimated by modified biuret method (Inchiosa, 1964) using total protein kit marketed by Boehringer Mannheim. Analysis was carried out by using Boehringer 5010 semi-automatic spectrophotometer, under standard conditions of operation as recommended by Boehringer Manheim.

3.1.2.2 Microscopical examination

Cytological examination of CSF was performed within 30 minutes of collection (Bailey and Vernau, 1997).

3.1.2.2a Total leucocyte count

Total leucocyte count was determined as per the procedure described by Benjamin (1998). 'CSF diluting fluid'(Marketed by New India Chemical Enterprises, Cochin) was used as the diluent.

3.1.2.2b Differential leucocyte count

Differential leucocyte count (DLC) of CSF was determined using the technique described by Bailey and Vernau (1997).

3.1.3 Electroencephalography

The animal was secured in lateral recumbency, on a wooden table and the hair over the scalp was clipped short, in order to facilitate proper contact with electrodes. Non-invasive disc electrodes were used in this study. Electrodes were glued bilaterally, over the left and right frontal, left and right occipital, and vertex areas. Ground electrode was attached at the caudal edge of the external occipital protuberance. Electrode connection was made intact with the help of kaolin paste (kaolin powder with normal saline). Placement

PLATE- 1

PLATE- 2

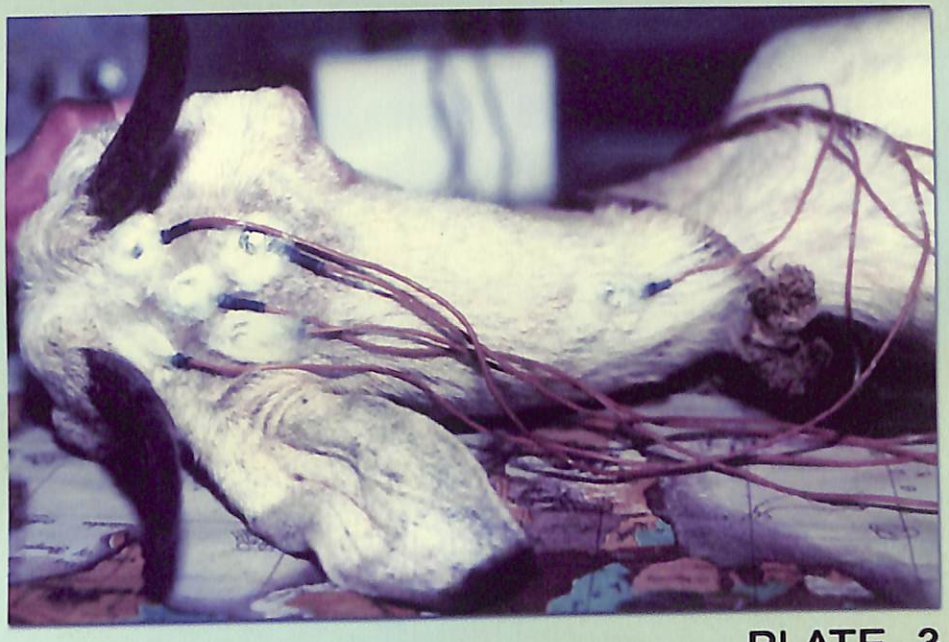
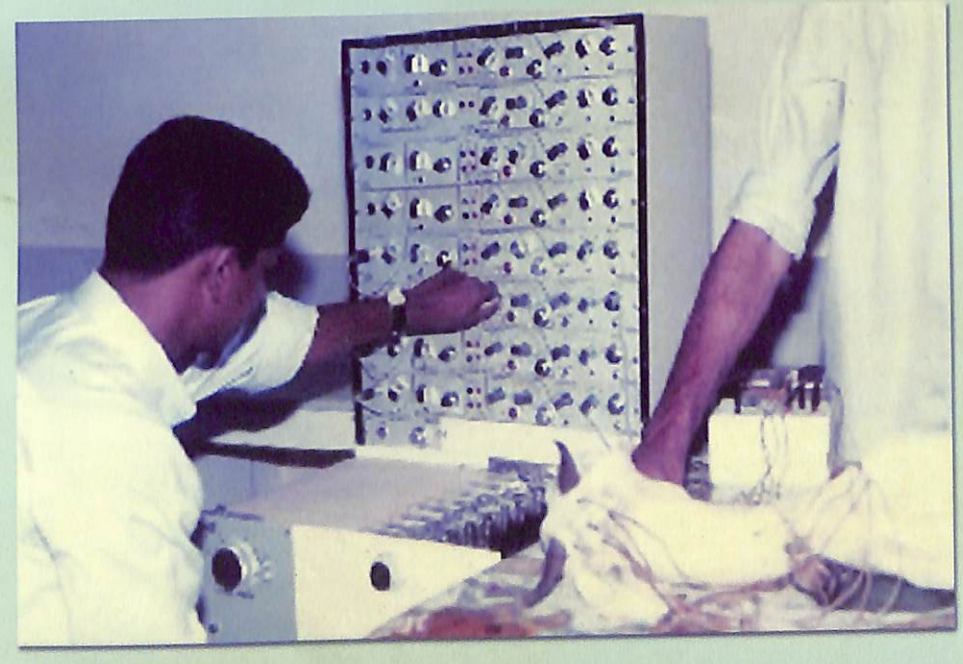


PLATE- 3



sites for electrodes (Plate 2) were identified as described by Takeuchi *et al.* (1998).

EEG recordings were made using a polygraph (Plate 3) with time constant at 0.3 seconds, amplification sensitivity of 50 microvolt/cm, half amplitude high frequency of the pen at 75, 50 Hz filter in ON position and paper speed at 25 mm/second. Precautions were taken to avoid visual and auditory stimulation of the patient and all other electrical equipments were unplugged from the AC power line. The amplitude was calculated on the basis of a calibration signal (50 microvolt = 1 cm) at the beginning of the record.

3.1.4. Radiographic examination

The lateral radiograph of skull, of the animal was taken in a 10" x 12" radiographic film as per the technique described by Singh and Peshin (1994). The radiographic exposure parameters were set at 60 Kvp and 16 mAs.

3.1.5 Treatment adopted

PEM affected animals were treated with thiamine hydrochloride (Marketed by SISCO Research Laboratories Private Ltd., Mumbai) parenterally along with bismuth carbonate orally for four days. Thiamine hydrochloride was given at the rate of 50 mg/kg body weight twice daily intravenously after dissolving in five to ten ml of distilled water. Bismuth

carbonate at the rate of 15 g per animal was administered orally as a drench with the help of a stomach tube. Recumbent animals were given proper care and physiotherapy.

3.1.6 Gross and Histopathology

One animal, which did not respond to the therapy, was sacrificed. Brain was collected at the time of post-mortem and examined grossly. Representative samples collected from different parts of the brain were preserved in 10 per cent buffered formalin.

Preserved tissues were processed, after proper fixation, by routine paraffin embedding technique (Sheehan and Hrapchak, 1980). Sections were cut at 3-5 microns, using a microtome and stained with Haematoxylin and Eosin (Bankroft and Cook, 1984) for evaluation of the histological changes.

3.2 Statistical analysis

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

Results

4. RESULTS

Results of the present study are presented in the Tables 1-2, Figures 1-6 and Plates 1-20. Seven clinical cases of polioencephalomalacia were subjected to detailed clinical and neurological investigation. Two cases were severe and of these, one did not respond to the treatment adopted (thiamine and bismuth carbonate therapy).

4.1 Clinical examination

4.1.1 Signalment and history

The age group of PEM affected animals ranged from one and a half months to two years. Of the seven goats, five were females and two were males. All of them were non-descript.

Five affected goats had the common history of being fed with rice gruel regularly. Initial symptoms reported were staggering gait, circling, deviation of head, falling down and broad based stance. Appetite was not markedly affected in the cases except two which were anorectic/inappetant. Animals, except the anorectic, consumed roughages and water when offered. Defecation and urination were normal in all the animals. Two of the cases were acute in onset and were found recumbent with opisthotonus posture and paddling of limbs. All the animals suffered from the disease for the first time.

4.1.2 Clinical findings

All the animals were dull and depressed in the initial stages of the disease. The important clinical signs noticed were staggering gait, frequent falling down, head pressing, jerking of head, broad based stance, head-tilt and lateral deviation of head (Plates 4-8). Out of the seven animals, one animal was anorectic and another inappetent. All the other five goats were normal in their feeding habits. The onset of the disease was sudden and severe in two cases, and were presented to the hospital in lateral recumbency. All the other animals could stand with a broad based stance or walk with a staggering gait. Hyperesthesia, low carriage of head, extension of head and neck, aimless wandering, ataxia and vertical or horizontal nystagmus were also noticed. Tonic-clonic convulsions, opisthotonus and star-gazing posture were seen. Affected animals often showed self mutilated injuries on eyelids due to convulsions and frequent falling down and rubbing on the ground. Froathiness at mouth, was noticed only in one case. Shivering, muscle tremors and grinding of teeth were also seen. Animals preferred to lie down to any one side only. Head was deviated to the left side in four cases and to the right side in three cases. Animals resisted any attempt to turn it to the opposite side and the attempts precipitated convulsions and violent movements to regain the previous position occupied by the animal. Most of the animals exhibited paddling of limbs when they were made to lie in lateral recumbency. Menace response was

Plate 4. Goat affected with PEM – before treatment

Plate 5. Goat affected with PEM – second day of treatment

Plate 6. Goat affected with PEM – third day of treatment

Plate 7. Goat affected with PEM – after treatment

Plate 8. Typical head-tilt in a diseased goat

PLATE- 4

PLATE- 5

PLATE- 6

PLATE- 7

PLATE- 8

present in all the cases except one and mydriasis was evident in three animals. Stiffness of limbs was a common feature and was more pronounced in the hind limbs. The limbs could be flexed only with application of force.

The rate of respiration recorded was 32.60 ± 3.50 per minute, at the time of presentation to the hospital. It gradually decreased during the course of treatment and the mean value of respiration at the time of discharge from the hospital was 27.16± 1.14 per minute (Table 1, Figure 1).

The pulse rate was 78.10 ± 6.92 per minute in the diseased animals and was found to be in the normal range. Pulse rate decreased steadily during the course of treatment and the mean value after recovery was 69.16 ± 4.29 per minute (Table 1, Figure 2).

The body temperature recorded before the treatment was found to be normal (102.53 ± 0.16°F). Mean value at the end of treatment period was 102.20 ± 0.08 °F (Table 1, Figure 3).

Mucous membrane of all the affected animals were normal (pale roseate) in colour except the cases in which mucous membrane along with the whole eye were reddened due to self mutilation and constant rubbing on the ground as a result of convulsion.

Rumen motility was normal in all the cases (5-8/5 minutes) except in the two severe cases in which its was three per five minutes.

4.2 Neurological examination

4.2.1 Behaviour

All the animals were dull and depressed when presented to the hospital and one of them wandered aimlessly.

Following the treatment four of the affected animals became alert and active by the third day and one by fourth day. Of the two severe cases, one regained normal sensorium by the sixth day and the other remained in the lateral recumbent stage, without any improvement until death.

4.2.2 Posture and gait

Gait of the affected animals except the two severe cases were characterized by ataxia, circling and stiffness or spasticity of limbs. Spasticity was present in all the limbs or only in hind limbs. Spasticity of hind limbs were more pronounced. Both the severe cases were in lateral recumbency. Lateral deviation of head and neck was seen in less severe cases.

In all the recovered cases posture and gait became normal within four days of treatment except one severe case, which took eight days for complete recovery.

4.2.3 Cranial nerve examination

Hyposmia was noticed in three cases including the two severe cases. In all the other cases olfactory nerve was found to be normal. Two of the animals became normal by second or third day. One of the severe cases remained hyposmic for six days.

Menace response was present in all the cases except one acute case in which it was absent. Menace response reappeared on the second day itself in that animal.

Pupillary reflex was sluggish in six cases and normal in one when they were presented to the hospital. Reflex became normal in three of the affected animals by the third day of treatment and one by second day itself. In both the severe cases pupillary reflex became normal by the fifth day.

Ventro-lateral, dorso-medial and medial strabismus were not observed in any of the cases studied.

Palpebral and corneal reflex were normal in all the diseased animals.

All the affected animals resisted stimulation of nasal meatus by foreign material (a piece of straw) by withdrawing its head. This reflex was positive even in the three animals which showed hyposmia.

All the diseased animals irrespective of the severity of the condition resisted attempts to open the mouth.

No abnormality could be detected on palpation of ears, lips, eyelids and muzzle. Normal tonicity was detected in all the cases.

Physiological nystagmus was present in only one affected animal. Bilateral vertical nystagmus was noticed in four cases and a combination of horizontal of the right and vertical of the left was observed in the other two cases. Physiological nystagmus reappeared by third (two cases), fourth (one case) and fifth day (two cases) and only by seventh day in the severe case that responded to treatment.

Gag and laryngeal reflexes were present in all the affected cases.

Atrophy of neck muscles – trapezius, sternocephalicus and brachiocephalicus were not seen in any of the cases. Animals with lateral deviation of head and neck flexion exhibited stiffness of the neck.

All the affected animals resisted pulling out the tongue from the mouth and the tongue was retracted as soon as the pressure on the tongue was relieved.

4.2.4 Postural reaction

All the diseased animals showed asymmetric limb movements and stumbling or knuckling during wheelbarrowing test at the time of presentation to the hospital, except one case in which the animal could use its limbs symmetrically forward and backward but could not keep its head in the same line as that of the body. All the animals except the two severe cases, used its limbs symmetrically towards the end of the treatment i.e., third or fourth day.

Lateral deviation of head disappeared by fourth or fifth day. One severe case responded by the eighth day.

Proprioceptive positioning was normal in two of the seven cases. Those cases that responded to the treatment became normal between days three and five. The positioning response in the severe case that responded to treatment improved only by the eighth day.

4.2.5 Spinal cord reflexes

The patellar reflex was normal in two diseased animals and responded by an extension of the stifle joint. Three of the affected animals exhibited exaggerated response and the other two were sluggish to respond. By the third day all the animals with exaggerated/sluggish movement responded and the reflexes became normal.

Flexor reflex was normal in one of the affected cases. Five of the animals showed exaggerated limb flexion and one was sluggish to respond. All the animals responded between second and fourth day.

Three of the cases responded to panniculus reflex normally. All the other animals exhibited hyperesthesia. Panniculus reflex became normal by second or third day in all the cases, which evinced hyperesthesia.

Three of the cases had normal perineal reflex, and the other four exhibited exaggerated contraction of anal sphincter. These four animals responded normally by second or third day.

4.3 Cerebrospinal fluid

Cerebrospinal fluid collection from the lumbo-sacral site was a simple and safe procedure (Plate 1). CSF could be collected from all the animals except one which was only one and a half months old. In three cases first few drops of CSF were mixed with traces of blood and this portion was discarded. Subcutaneous anaesthesia at the lumbo-sacral site was not necessary since all the animals co-operated under proper restraint.

4.3.1 Physical and chemical characters

CSF collected from all the affected animals were clear and colourless. The consistency of CSF was watery (Table 2). No difference could be appreciated from the CSF of healthy animals.

The mean total protein content of CSF of normal and diseased animals were 14.50 ± 0.99 mg/dl and 35.17 ± 3.15 mg/dl respectively (Table 2, Figure 4). Statistically significant increase ($P \leq 0.01$) was noted in diseased animals when compared with normal animals.

4.3.2 Microscopical examination

4.3.2.1 Total leucocyte count

Mean value of total leucocyte count was 4.70 ± 1.43 cells/µl in normal healthy animals and 9.70 ± 1.84 cells/µl in the diseased animals (Table 2,

Figure 5). No statistically significant difference was noted in total leucocyte count between the diseased group and the control group of goats.

4.3.2.2 Differential leucocyte count

Differential leucocyte count revealed lymphocytes (80%) and monocytes (20%) in normal animals. Corresponding values obtained from the diseased animals were 84 per cent and 16 per cent (Figure 6).

4.4 Electroencephalography

Recording of EEG using disc electrodes was a simple and safe procedure (Plates 2 and 3). Restraining the animal in the lateral recumbent position for prolonged periods was the only difficult exercise. Disc electrodes glued to the skull with the help of kaolin paste and a small piece of cotton was sufficient to withstand minor movements of head. Plugging of ear with cotton, in order to avoid auditory stimulation, precipitated shaking of head and uneasiness. Hence this practice was not adopted and maximum care was taken to create a comfortable environment free of noise. Electroencephalographic recordings could be taken for sufficiently long periods (15 to 30 minutes), without much disturbance, with the animal in lateral recumbent position.

4.4.1 EEG recordings

In control animals, the electroencephalographic pattern revealed normal low voltage fast activity (LVFA) of 10-50 microvolts and 20-30 cycles/second

resembling beta rhythms. Some of the animals depicted high amplitude slow waves or a mixture of these two (Plates 9 and 10).

The abnormal patterns detected in the EEG of diseased goats included slow waves and asymmetric sharp waves (Plate 11). The abnormal slow waves were not always continuous and were seen along with normal waves. Most of the animals showed slow waves of high or low amplitude. Other abnormalities obtained were high amplitude slow activity (HASA) (Plate 12) and diffuse slow activity.

Slow wave activity was seen in the occipital area in four cases. In two cases, the Right Frontal-Vertex areas showed slow wave activity. In another case, the left half of the brain showed slow waves and EEG recordings from other parts of the brain showed normal EEG pattern or occasional high amplitude slow activity (HASA) with a normal background. In two of the affected cases HASA was noticed prominently in all the montages with amplitudes ranging from 50-180 microvolts and a slow activity of 1-4 Hz.

4.5 Radiographic examination

Radiographic examination of the skull of diseased goats did not reveal any fracture or change in the density of the cranium (Plate 13). There was no apparent difference between the cranial radiographs taken from normal animals and affected goats.

Plate 9. Normal EEG - goat (a)

Plate 10. Normal EEG - goat (b)

PLATE-9

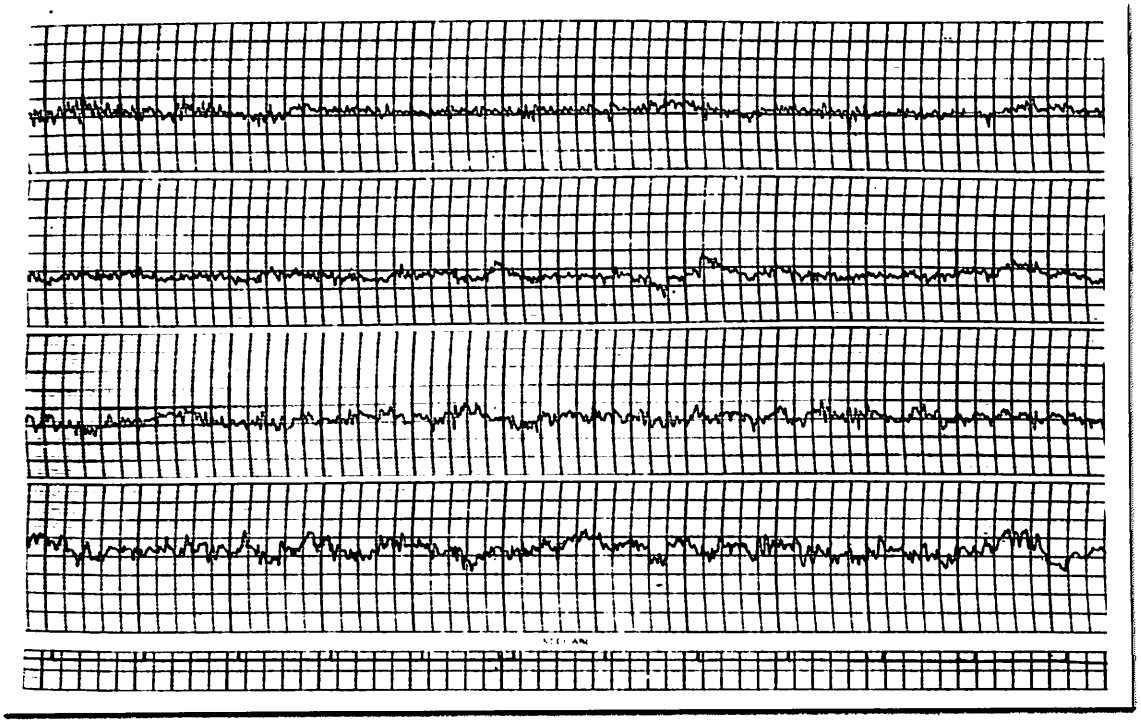


PLATE-10

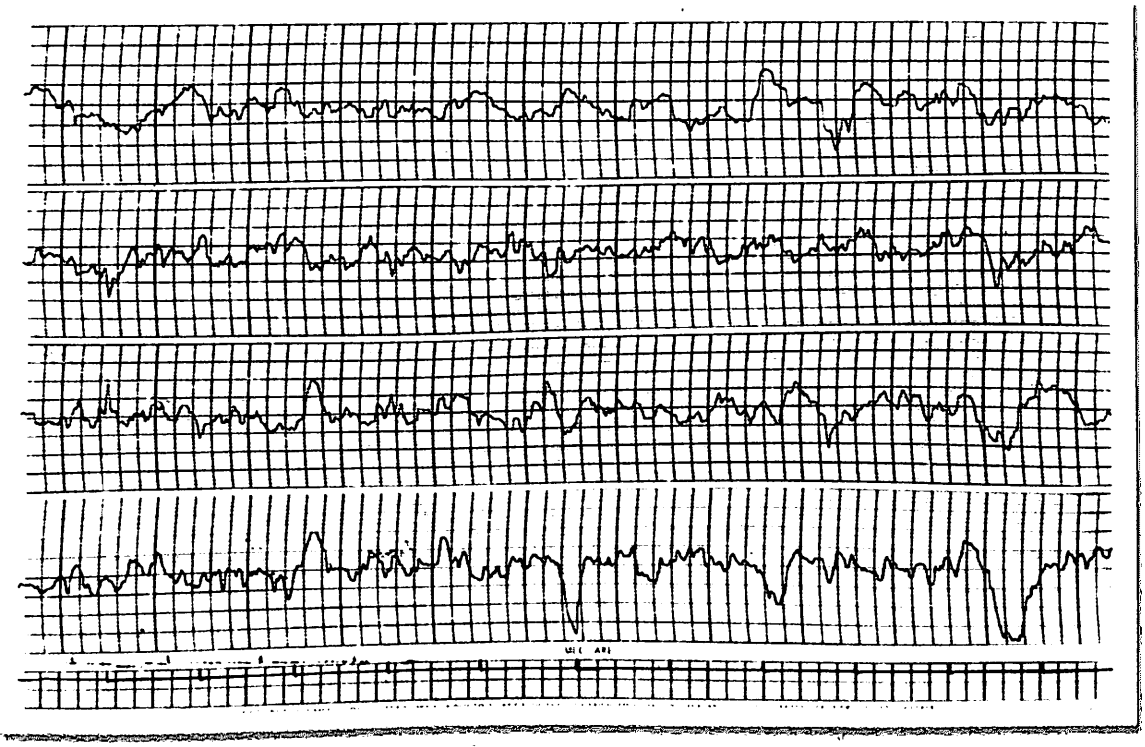


PLATE-11

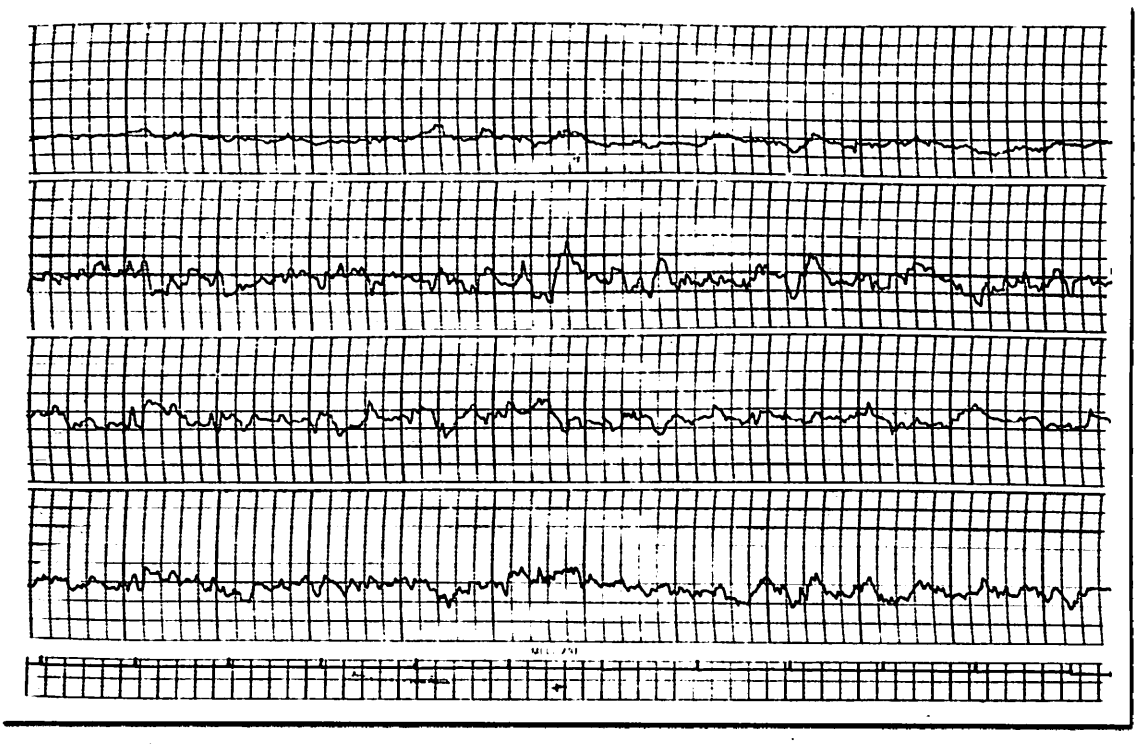
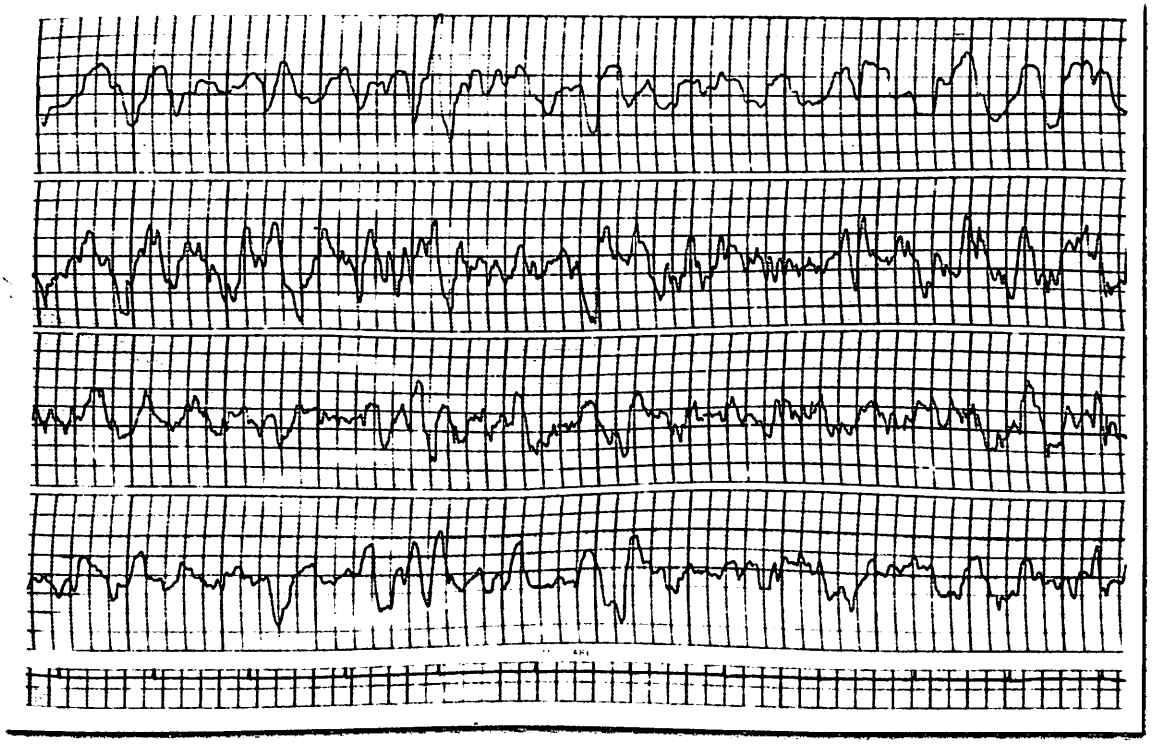


PLATE-12



4.6 Response to treatment

All the affected animals except one severe case responded to the treatment. The recovered animals started showing improvement the very next day itself. Two animals became almost normal by fourth day and three on fifth day (Plates 4 to 7). One animal that was severely affected could stand up when supported on the fourth day. Between fourth and eighth day it was given good nursing, care and physiotherapy. The animal became normal by the eighth day.

4.7 Gross and histopathology

The brain was soft, swollen and spongy in appearance with yellowish discolouration of the cerebral hemispheres. Cortical gyri was flattened and meninges was congested and haemorrhagic.

Histopathology of cerebrum revealed subpial congestion, necrosis of the neurons of the pyramidal and fusiform cell layers of the cerebral cortex with neuronophagia (Plate 14), focal accumulation of neutrophils in cortico-medullary area (Plate 15), vascular collapse, perivascular cuffing of lymphocytes (Plate 16), neovascularisation of the grey matter (Plate 17) in certain gyri in the cerebral hemispheres and perivascular oedema and satellitosis (Plate 18). White matter of the cerebrum appeared intact, but occasional congestion of the capillaries and oligodendroglia were seen.

In cerebellum interfolial pia was thickened and the vessels were highly congested with perivascular cuffing (Plate 19) and perivascular oedema (Plate

20). Neovascularisation of the granular cell layer in certain folia was also seen. Purkinjee cells in most of the fibres appeared intact and molecular layer was normal. Occasional clumps of infiltrating inflammatory cells, predominantly neutrophils, were seen in the white matter. Some of the astrocytes had become gamastocytes at these places having prominent cytoplasm and eccentrically located pyknotic nuclei.

Hippocampus also did not reveal any histological abnormalities. No histopathological lesions were detected in pons and medulla.

Plate 13. Radiograph of skull – PEM affected goat

Plate 14. Cerebrum – necrosis and neuronophagia

Plate 15. Cerebrum – focal accumulation of neutrophils in cortico-medullary area

Plate 16. Cerebrum – perivascular cuffing

PLATE-13

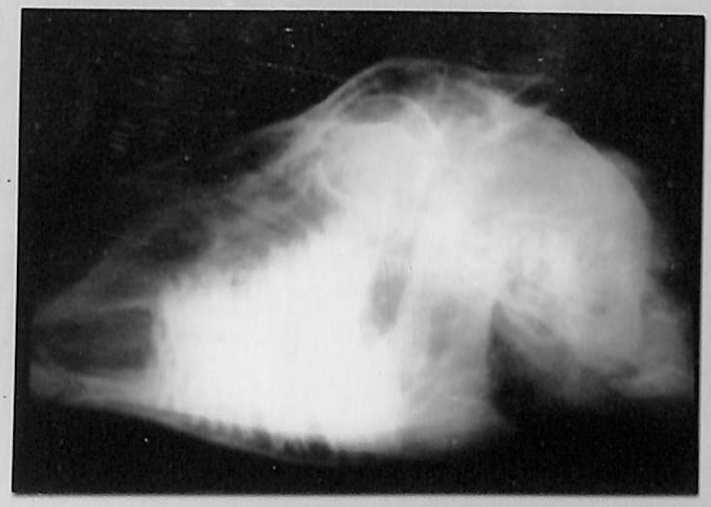


PLATE-14

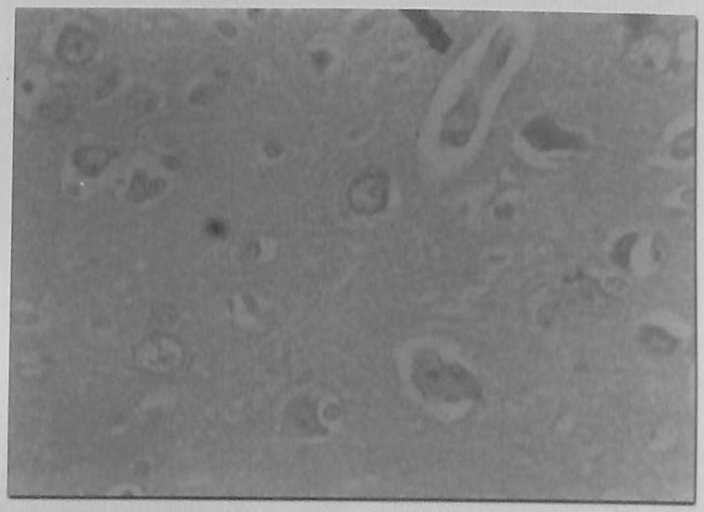


PLATE-15

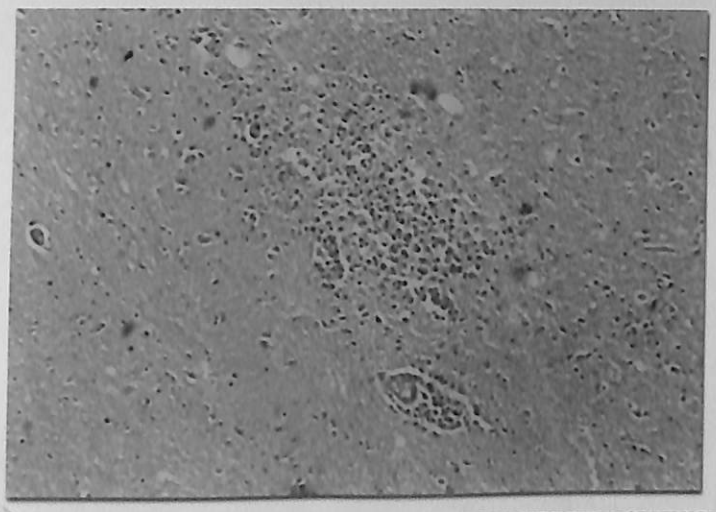


PLATE-16

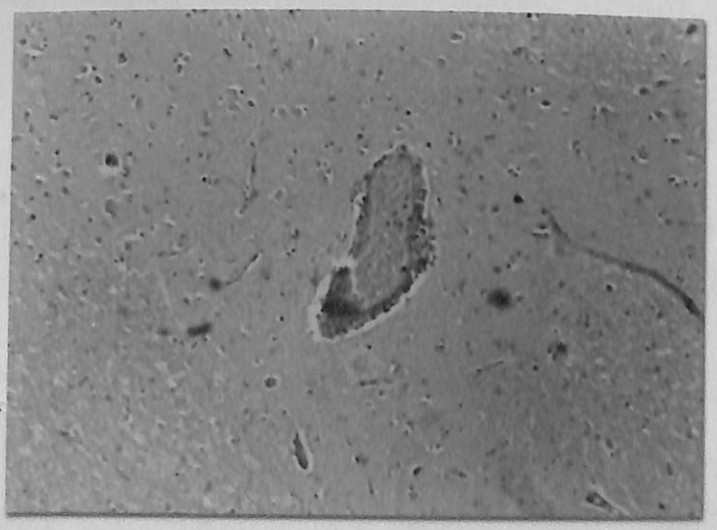


Plate 17. Cerebrum – neovascularisation

Plate 18. Cerebrum – perivascular oedema and satellitosis

Plate 19. Cerebellum – perivascular cuffing

Plate 20. Cerebellum – perivascular oedema

PLATE-17

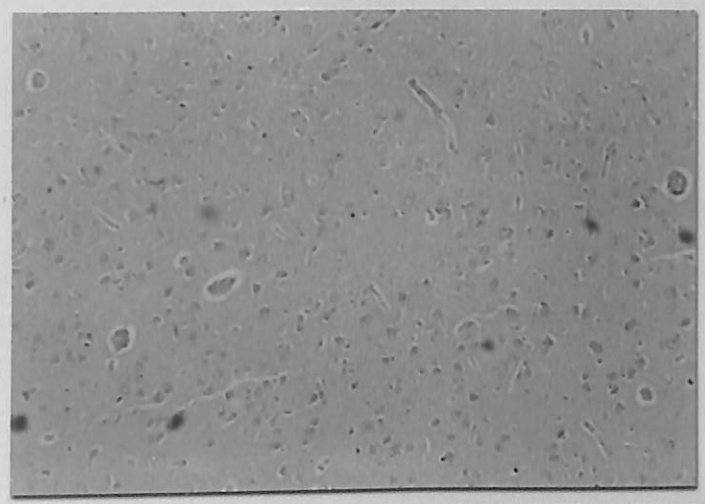


PLATE-18

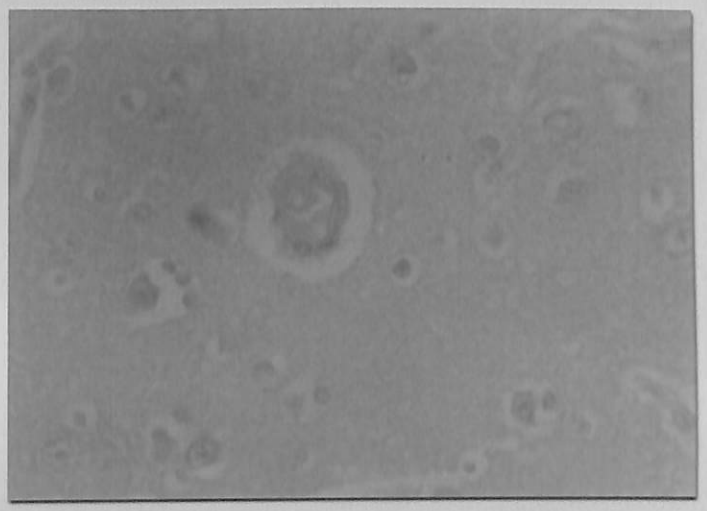


PLATE-19

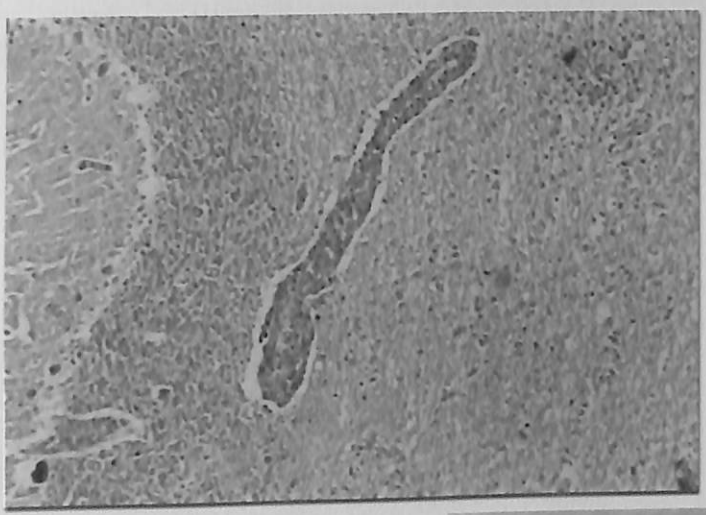


PLATE-20

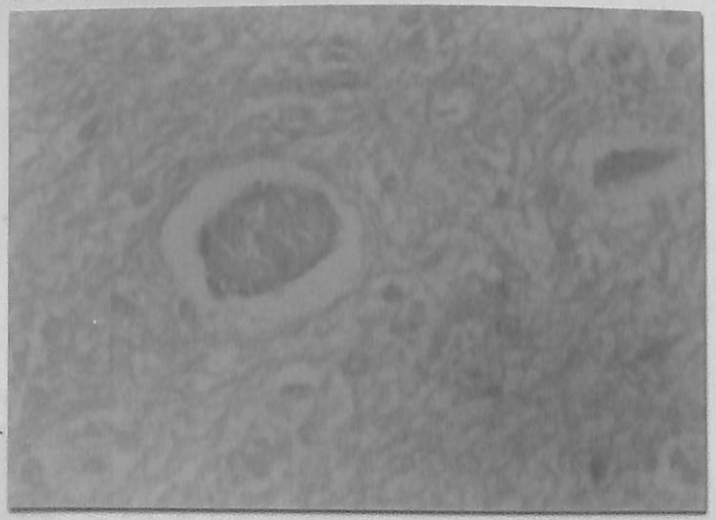


Table 1. Mean clinical data of PEM affected goats before and after treatment

Parameters	Before treatment Mean ± SE	After treatment Mean ± SE
Respiration (per minute)	32.60 ± 3.50	27.16 ± 1.14
Pulse (per minute)	78.10 ± 6.92	69.16 ± 4.29
Temperature (°F)	102.53 ± 0.16	102.20 ± 0.08

Table 2. CSF – Physical, chemical and microscopical parameters in healthy and diseased animals

Parameters	Healthy Animals	Diseased Animals
Colour	Colourless	Colourless
Transparency	Clear	Clear
Consistency	Watery	Watery
Total protein (mg/dl)	14.50 ± 0.99	35.17 ± 3.15**
Total leucocyte count (cells/µl)	4.70 ± 1.43	9.70 ± 1.84

** Significant statistically ($P \leq 0.01$)

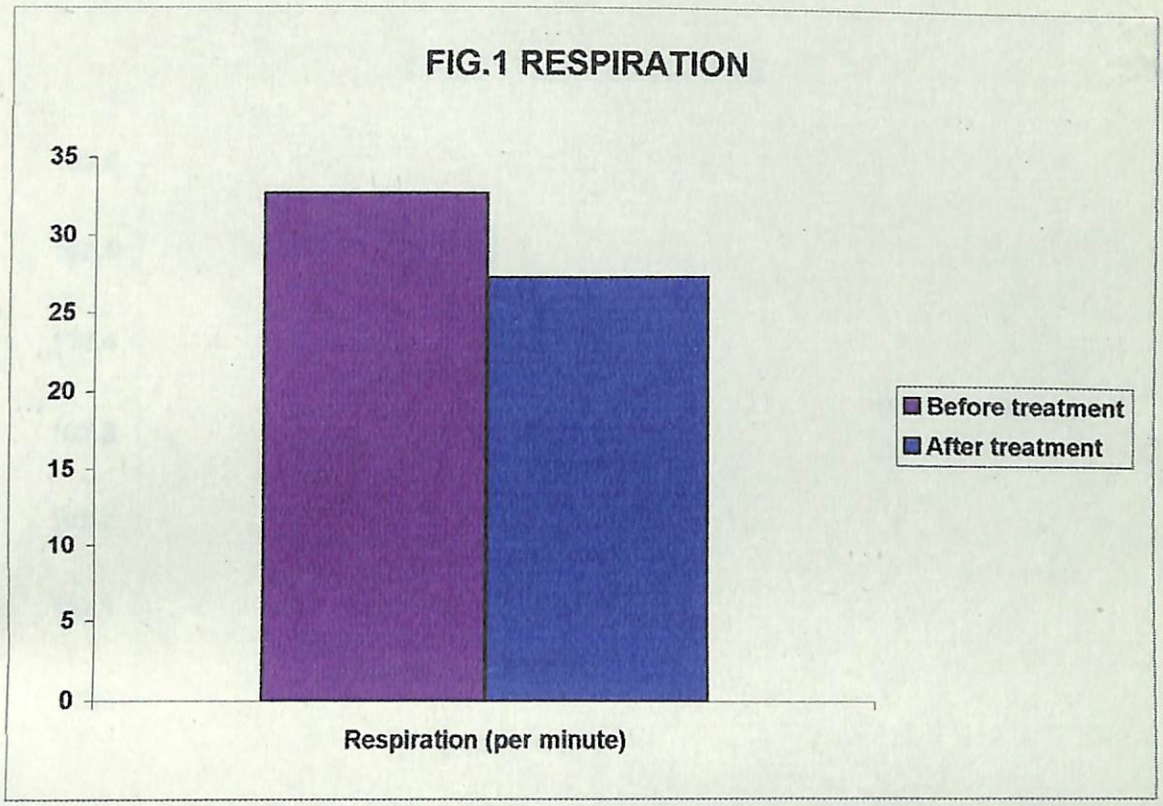


FIG.1 RESPIRATION
35
30
25
20
15
10
5
0
Before treatment
After treatment
Respiration (per minute)

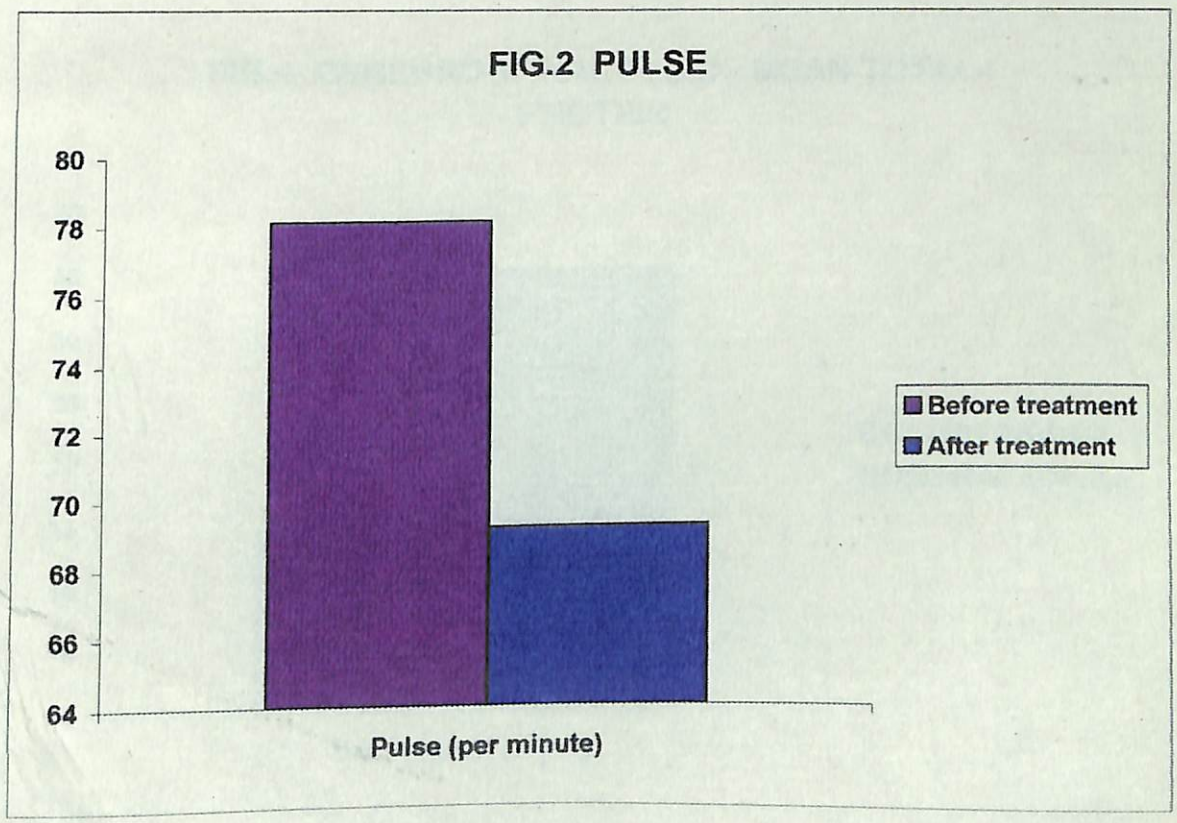


FIG.2 PULSE
80
78
76
74
72
70
68
66
64
Before treatment
After treatment
Pulse (per minute)

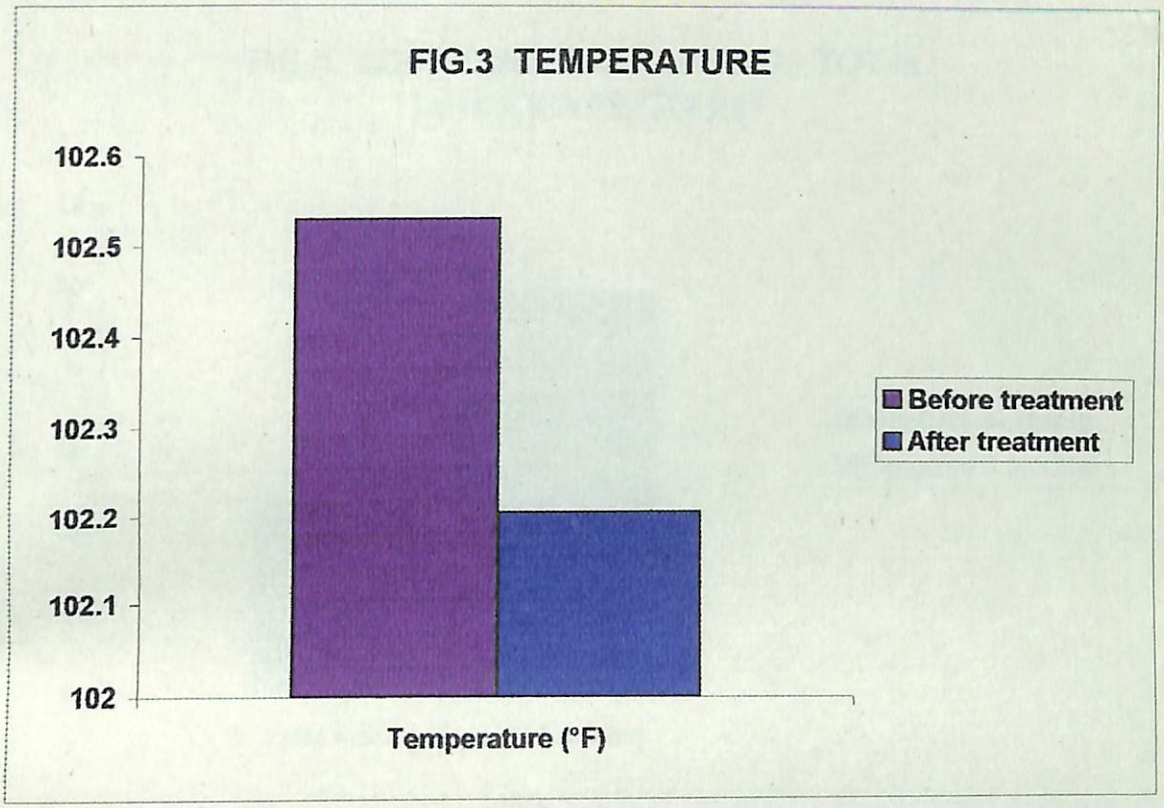


FIG.3 TEMPERATURE
102.6
102.5
102.4
102.3
102.2
102.1
102
Temperature (°F)
Before treatment
After treatment

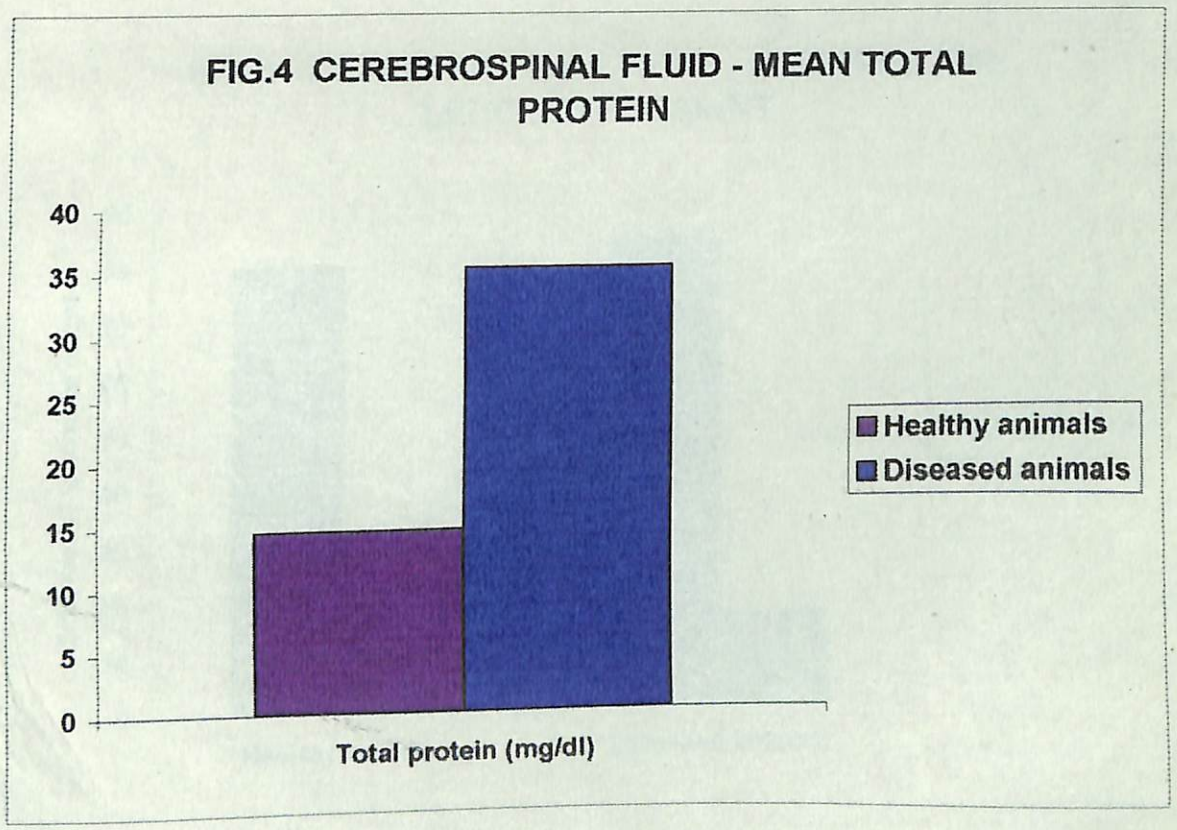


FIG.4 CEREBROSPINAL FLUID - MEAN TOTAL PROTEIN
40
35
30
25
20
15
10
5
0
Total protein (mg/dl)
Healthy animals
Diseased animals

FIG.5 CEREBROSPINAL FLUID - TOTAL LEUCOCYTE COUNT

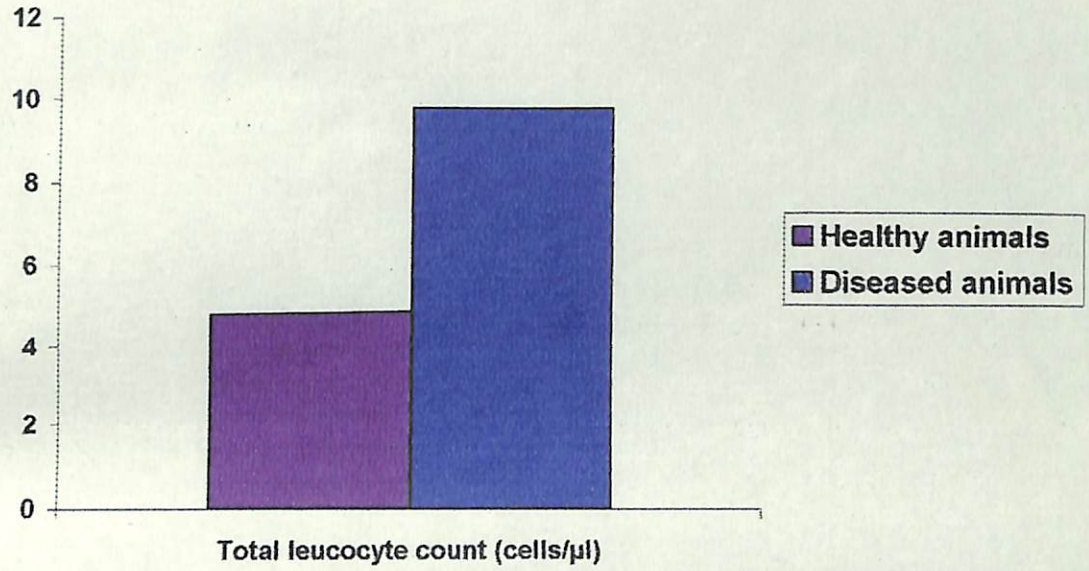
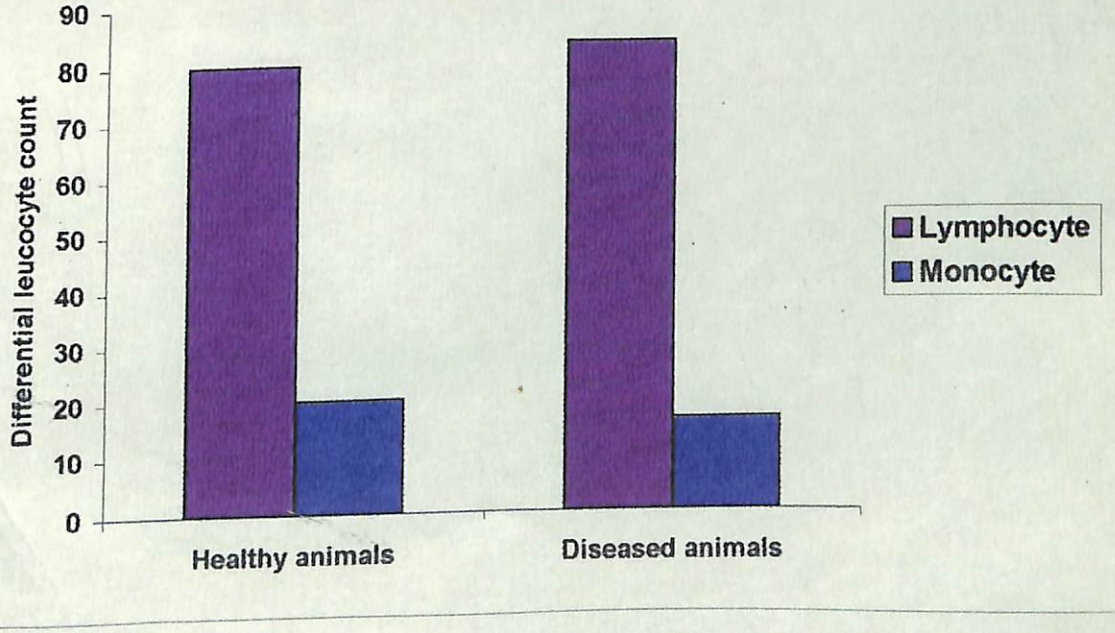


FIG.6 CEREBROSPINAL FLUID - DIFFERENTIAL LEUCOCYTE COUNT



Discussion

5. DISCUSSION

Diseases of CNS are difficult to be diagnosed and treated. Basically, the clinician is restricted to history taking and a detailed physical examination. Often these are not enough. PEM is a condition that can be successfully treated if diagnosed in the early stages (Mayhew, 1989).

5.1 Clinical examination

5.1.1 Signalment and history

The age group of the affected animals ranged from one and a half months to two years. Of these, five were females and two were males. Terlecki and Markson (1961) reported PEM in sheep aged two to seven months. Sobhanan (1981) recorded the disease in young goats of two months age and the age of the oldest goat affected was two and a half years. Similar observations were also made by Radostits *et al.* (2000). Maliekal (2000) stated that the age group of goats affected with PEM ranged from six months to five years. Occurrence of PEM in one and a half month old kid might be due to the environment in the rumen that favoured the growth of thiaminase producing bacteria (Radostits *et al.*, 2000).

Five cases had the common history of being fed with rice gruel regularly. Brent (1976) reported that cattle maintained on high concentrate and low forage diet had reduced ruminal thiamine synthesis. In Kerala, rice (a

carbohydrate rich diet), form the main component of concentrate ration. Irregular or excess feeding of rice often accounted for majority of ruminal dysfunctions with lowering of pH (Aleyas and Vijayan, 1981), which produced a congenial environment for increased thiaminase production (Sobhanan, 1981). Sapienza (1981) suggested that sudden changes from roughage to concentrate diet resulted in rapid multiplication of gram-positive bacteria such as clostridium sp., which inturn produced enzymes that destroyed thiamine in the rumen and caused CNS disorders.

History of PEM affected animals suggested that carbohydrate rich diet could be one of the predisposing factors for the development of the disease in Kerala. This finding was in accordance with the observations of Maliekal (2000).

5.1.2 Clinical findings

Early stages of the disease were characterized by dullness, depression, staggering gait, frequent falling down, head pressing, jerking of head, broad based stance, head tilt and lateral deviation of the head. Terlecki and Markson (1961) reported that the initial symptoms in sheep appeared to be aimless wandering, which within hours, progressed to circling and ataxia. Affected animals would stagger and sway while walking or stand with their foot wide apart. Lonkar and Prasad (1992a) observed varying degrees of anorexia, dullness, depression, weakness of hind limbs, staggering gait, incoordination,

lowering of head and partial blindness during the initial stages of PEM in goats. The manifestation of acute clinical signs was attributed to severe brain oedema (Olkowski *et al.*, 1992) and changes in the integrity of cranial nerves (Fakhruddin *et al.*, 1987a).

Appetite was not markedly affected in the diseased animals except the two severe cases which were anorectic/inappetent. Animals consumed roughages and water even when they were laterally recumbent. These signs were consistent with the findings of Dickie *et al.* (1979), Gauri and Vashistha (1988) and Tanwar (1995).

Hyperesthesia, low carriage of head, extension of head and neck, aimless wandering, vertical or horizontal nystagmus, tonic-clonic convulsions, opisthotonus and star-gazing posture, froathiness at mouth, shivering, muscle twitching, grinding of teeth, deviation of head and stiffness of limbs particularly hind limbs were the other predominant symptoms noticed during the subsequent stages of the disease. Neurological signs exhibited by goats were tremors of head, tonic-clonic convulsions, marked opisthotonus, stiffness of fore and hind legs, hyperesthesia to touch and sound, lack of menace reflex, nystagmus and circling (Tanwar *et al.*, 1983, Jackman, 1985 and Tanwar, 1987). Froathy salivation was noted in one case and was consistent with the findings of Fakhruddin *et al.* (1987a), Tanwar (1987) and Gauri and Vashistha (1988).

Another important and characteristic clinical sign noticed was that the animals preferred to lie down only on any one side. They resisted any attempt to turn it to the opposite side and the attempts precipitated convulsions and violent movements to regain the previous position. This was in accordance with the findings of Maliekal (2000). An animal with a cerebral lesion that compulsively circles often had the head and neck deviated to one side and tended to circle towards the side of the lesion (Braund, 1995).

Menace response was absent only in one of the affected cases. All the other six animals in this study depicted normal menace response. Blindness in PEM was recorded by Gauri and Vashistha (1988), Lonkar and Prasad (1992a), Chahar *et al.* (1993) and Tanwar (1995). Impairment of vision might be due to cortical injury in the region of calcarine fissure and permanent blindness resulted from necrosis of neurons, while recovery of vision followed non-lethal injury to neurons (Jensen *et al.*, 1956).

The rate of respiration recorded at the time of admission was 32.60 ± 3.50 per minute (Table 1, Figure 1). The pulse rate was 78.10 ± 6.92 per minute (Table 1, Figure 2). The initial increase in the rate of respiration and pulse could be due to excitement and occasional tremors. Body temperature remained normal throughout the clinical course (102.53 ± 0.16°F) (Table 1, Figure 3). 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), Fakhrudin *et al.* (1987b) and Maliekal (2000). The pulse and respiratory rate were 69.16 ± 4.29 per minute and 27.16 ± 1.14 per minute respectively after the recovery.

Mucous membrane of all the affected animals were pale roseate except the cases in which mucous membrane along with the whole eye was reddened due to constant rubbing 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 slight decrease in two severe cases. Loew and Dunlop (1972), Tanwar (1987) and Lonkar and Prasad (1994b) reported normal rumen pH in polioencephalomalacia affected goats. Slightly acidic rumen pH was reported in PEM cases that were fed high quantity of concentrates because rumen pH was influenced by the nature of feeds and fodders (Mella *et al.*, 1976). In Kerala, rice formed the main component of concentrate ration (Aleyas and Vijayan, 1981 and Maliekal, 2000). Slight decrease in the rate of rumen motility observed in two severe cases could be due to decreased rumen pH.

The clinical symptoms manifested by the animals suggested the involvement of cerebral cortex primarily and cerebellum to some extent. The signs also suggested that although PEM affected cerebrum diffusely, all the parts were not equally affected in all the cases and there were individual variations. Jackman (1985) reported that though clinical signs of CCN

reflected impairment of the CNS, all the symptoms might not necessarily be shown in all the cases. The variation in clinical picture could be due to the difference in the location and severity of the CNS lesion. Secondary changes in the brain due to cerebral oedema and increased intracranial pressure may result in cerebellar herniation into foramen magnum which might lead to other clinical signs as well (Radostits *et al.*, 1994).

5.2 Neurological examination

5.2.1 Behaviour

All the animals were dull and depressed and one of them wandered aimlessly. These findings were in accordance with the observations of Jackman (1985), Lonkar and Prasad (1992a) and Tanwar and Malik (1995). The level of responsiveness of animals to its internal and external environment was effected by the ascending reticular activating system in the brain stem and by the cerebral hemispheres (Mayhew, 1989). Consciousness and state of wakefulness were maintained by a continual flow of impulses from the thalamic portion of the reticular formation to the cerebral cortex (Redding, 1978a). Altered behaviour and mental status are frequently observed in cerebral syndrome (Braund, 1995).

5.2.2 Posture and gait

Gait of the diseased animals was characterized by ataxia, circling and stiffness and spasticity of limbs. Two severely affected animals were presented to the hospital in lateral recumbency. Lateral deviation of head and neck was seen in less severe cases. Singh *et al.* (2000) observed inability to stand, progressive ataxia, incoordination of movements with staggering gait and inability to walk in PEM affected animals. Similar symptoms along with circling were described by Gauri and Vashistha (1988) and Chahar *et al.* (1993). Lesions of cerebrum often resulted in deviation of the head and neck to the same side of the lesion and cerebellar diseases might be exhibited as jerky movements of head. In central vestibular disease there might be altered mental status, hemiparesis or ataxia, postural reaction deficit and a greater tendency to roll in one direction (Braund, 1995). Corticospinal motor pathways did not appear to be important in large animals. Large lesions destroying the cerebrocortical motor centres did not cause permanent abnormality in gait (Mayhew, 1989).

5.2.3 Examination of cranial nerves

Abnormalities detected during cranial nerve examination help in localizing a lesion near or within the brain stem as majority of the cranial nerves originated from mid brain/brain stem.

Hyposmia was noticed in three of the affected cases. Normal function of the olfactory nerve was equated with the patient's ability to smell the hand of the examiner or its feed (Mayhew, 1989). Hyposmia or anosmia might be due to conditions that affected the primary olfactory receptors in the nasal mucosa or neurons of the olfactory bulb and its tract (Redding and Braund, 1978). Hyposmia in the present study could be due to the pressure on the olfactory bulb or due to depressed activity of reticular activating system.

Menace response was absent only in one case and was present in all the other six animals. This contradicted the finding of Gauri and Vashistha (1988), Lonkar and Prasad (1992a), Chahar *et al.* (1993) and Tanwar (1995) who reported blindness as one of the key features in PEM. According to Mayhew (1989) lesions of the eye and optic nerve resulted in ipsilateral blindness, lesions of the optic tract and lateral geniculate nucleus caused contralateral blindness, space occupying lesions of the brain produced blindness and animals with diffuse cerebellar diseases had bilateral deficits in menace responses. Radostits *et al.* (1994) opined that in peripheral blindness both menace response and pupillary light reflexes were absent and in central blindness, with forebrain lesions, animals were blind while pupillary light reflexes were commonly intact. In cerebellar disorders a bilateral menace deficit might be noted although vision was not affected (Braund, 1995). Presence of eye sight and menace response in majority of the cases suggested that the occipital cortex was not seriously affected in all the cases of PEM.

Pupil was partially dilated in three cases. Pupillary light reflex was sluggish in six animals and normal in one. Jensen *et al.* (1956) and Tanwar (1987) reported sluggish/normal pupillary light reflex in CCN cases. The pupillary light reflexes depended on the functional integrity of the occulomotor nerve and optic nerve together with the mid-brain integrative centres and sympathetic pathways (Redding and Braund, 1978). A widely dilated pupil with normal vision suggested an occulomotor nerve lesion and this nerve could get damaged from diffused cerebral swelling in the forebrain, which exerted pressure on the brain stem (Mayhew, 1989). Sluggish pupillary reflex and dilated pupil as observed in certain cases could be due to the involvement of cerebellum and/or mid-brain or due to pressure on optic nerve. Cerebellum exerted both stimulatory and inhibitory influences on many cerebral functions including visual responses (McCormick *et al.*, 1984).

Assessment of pupillary reflex and occular movements gave an idea about the functional integrity of occulomotor nerve (Redding and Braund, 1978). Ventro-lateral and dorso-medial strabismus were not present in any of the cases. Dickie *et al.* (1979), Jackman (1985) and Tanwar and Malik (1995) also did not record ventro-lateral strabismus in PEM cases. This indicated that the nerve was not seriously affected in many cases of PEM. Dorsal strabismus due to stretching of trochlear nerve was reported by Radostits *et al.* (1994). Dorso-medial strabismus was not recorded in the observations of Lonkar and Prasad (1992a) and Maliekal (2000).

Medial strabismus, seen in affections of abducens nerve, was not recorded in any of the affected animals. Chahar *et al.* (1993) and Syamasundar and Malik (1993) also did not observe medial strabismus in PEM cases.

Palpebral and corneal reflexes were normal in all the affected animals and the goats produced an ipsilateral blink response when the margins of palpebral fissure or cornea were stimulated. All the affected animals resisted stimulation of nasal meatus by withdrawing its head. Animals resisted attempts to open the mouth irrespective of the severity of the condition. Tanwar *et al.* (1983) reported that manual opening of mouth was resisted and animals exhibited opisthotonus and extensor rigidity. These findings indicated that the function of trigeminal nerve was normal. The sensory part of the trigeminal nerve supplied the face and was examined by testing the palpebral reflex and the sensitivity of the face. The motor part of the nerve supplied the muscles of mastication and alterations were observed as abnormal jaw movements, asymmetry of muscle contraction and atrophy of muscles (Radostits *et al.*, 1994).

No abnormality could be detected on palpation of ears, lips, eyelids and muzzle. Normal tonicity was detected in all the cases. Radostits *et al.* (1994) opined that symmetry and posture of ears, eyelids and lips were the best criteria for assessing the function of facial nerve.

Bilateral vertical nystagmus or a combination of horizontal and vertical nystagmus in each eye were observed in the diseased animals. Nystagmus changed with variations in head position (positional nystagmus). Positional nystagmus was indicative of a central vestibular lesion. According to Mayhew (1989), with lesions involving the central components of vestibular system in medulla oblongata, spontaneous and positional nystagmus might be horizontal, vertical or rotatory and also might change direction with changes in head posture. Such lesions frequently affected adjacent structures such as the proprioceptive and motor pathways for voluntary limb movement, and the reticular formation resulting in ataxia, tetraparesis and depression. Nystagmus in PEM was a common feature and was reported by Dickie *et al.* (1979), Jackman (1985) and Tanwar (1995).

Gag reflex and laryngeal reflex were present in all the affected cases. There was no atrophy or paralysis of neck muscles – trapezius, sternocephalicus and brachocephalicus in any of the diseased animals. This finding suggested that glossopharyngeal, vagus and accessory spinal nerves were intact. The glossopharyngeal and vagus nerves innervates the pharynx and larynx. Dysfunctions were accompanied by paralysis of these organs with signs of dysphagia, regurgitation through the nostrils, abnormality of voice and interference with respiration. Loss of function of accessory spinal nerve lead to paralysis of neck muscles and lack of resistance to lift the head (Radostits *et al.*, 1994).

Hypoglossal nerve was intact since all the animals resisted pulling out of tongue from the mouth and the tongue was retracted as soon as the pressure on the tongue was relieved. Hypoglossal nerve, motor to the tongue, could be best examined by observing the motor activity of the tongue. There might be protrusion, fibrillation or deviation of the organ resulting in difficulty in prehension and drinking water (Mayhew, 1989).

Results of the cranial nerve examination suggested that there could be lot of individual variations in the manifestation of neurological signs in PEM cases and those nerves that were within/near to cerebral cortex and mid-brain showed more deficits.

5.2.4 Postural reaction

Animals with cerebral and/or mid-brain lesions might have a normal gait; however careful postural reaction testing will often reveal deficits (Braund, 1995).

Wheelbarrowing revealed deficits viz., asymmetric limb movements, stumbling or knuckling in diseased animals and this suggested a lesion in the brain stem or higher centres (Redding and Braund, 1978).

Proprioceptive positioning was absent in five of the seven affected animals. According to Mayhew (1989) proprioceptive deficits were caused by

lesions affecting general proprioceptive sensory pathways, which relayed information on limb and body position to the cerebellum and cerebral cortex.

5.2.5 Spinal cord reflexes

Although all reflexes are influenced by higher centres of control, reflex testing tests those segments of the CNS that are actually involved in the reflexes. The animals must be relaxed if the reflexes were to be elicited in the normal form (Braund, 1995).

Patellar reflexes were exaggerated in three of the affected animals, sluggish in two and others responded normally. Flexor reflexes were exaggerated in five cases and sluggish and normal in one each. Panniculus reflex testing evinced hyperesthesia in four cases and normal response in others. Perineal reflex displayed exaggerated contraction of anal sphincter in four cases, while the other three was normal.

Spinal cord reflexes along with the mental status of the animal suggested that the lesions were not in the spinal cord but in the higher centres like cerebrum and cerebellum. In a purely spinal cord lesion mental status of the animal would not be affected, but would be active and alert with normal appetite. Exaggerated response and hyperesthesia could be due to the lack of inhibitory action on the lower motor neurons (LMN) by the upper motor neurons (UMN). Radostits *et al.* (1994) suggested that exaggeration of nervous system activity occurred when lower neurons were released from the

inhibitory effects of higher centres. Sluggish responses in certain cases might be due to general weakness of the patients/inherent problems connected with reflex testing.

5.3 Cerebrospinal fluid

Collection of CSF from lumbo-sacral site was found to be easy and safe (Plate 1). Tanwar *et al.* (1994) and Scott (1995) also collected CSF from the lumbo-sacral site of ruminants. Local analgesia was not needed in any of the cases as all the animals co-operated well during the procedure. Presence of traces of blood (three cases) in the first few (discarded) drops was due to accidental puncture of small capillaries. Contamination of CSF by small quantities of blood usually does not interfere with CSF analysis or interpretation (Braund, 1986). No complication was noticed in any animals after the collection of CSF.

5.3.1 Physical and chemical characters

CSF collected from the diseased animals were clear, colourless and watery in consistency (Table 2) and was not apparently different from that of normal healthy animals. Similar findings were reported by Lonkar and Prasad (1992b), Tanwar *et al.* (1994) and Nair (1999).

Statistically significant increase ($P \leq 0.01$) was noted in the mean total protein content of CSF of diseased animals (35.17 ± 3.15 mg/dl) when

compared to healthy controls (14.50 ± 0.99 mg/dl) (Table 2, Figure 4). Scott (1992) reported a total protein concentration of 20 ± 0.15 mg/dl in polioencephalomalacia affected ovines. A range from 15 to 540 mg/dl with a mean value of 90 mg/dl was reported by Radostits *et al.* (2000). Sargison *et al.* (1994) observed normal values in PEM affected lambs.

Disorders in which neuronal necrosis was a primary feature, such as thiamine responsive PEM, typically had increased total protein (George, 1996). Brain oedema severe enough to result in ischemia, infarction or herniation resulted in dysfunctional blood brain barrier, vasogenic oedema and an elevated CSF protein. Even in the absence of vasogenic oedema, BBB leakage might occur, perhaps because of the biochemical effects of the disorder on the barrier cells (Bailey and Vernau, 1997). According to Radostits *et al.* (2000) ATP dependent sodium pump within the neurons failed resulting in intracellular oedema. This was followed by neuronal degeneration which was considered secondary.

5.3.2 Microscopical examination

5.3.2.1 Total leucocyte count

The increase in total leucocyte count (TLC) of PEM affected goats was not significant (Table 2, Figure 5). Pleocytosis was noticed by George (1996) in disorders with neuronal necrosis such as thiamine responsive PEM. Scott (1992) did not report any significant increase in TLC in PEM cases. A

significant increase in TLC was reported by Syamasundar *et al.* (1992) and Radostits *et al.* (2000). In brain oedema, if neuronal necrosis ensued, the TLC might also increase (Bailey and Vernau, 1997). Lack of significant increase in TLC suggested that majority of the animals were brought to the hospital in the initial stages of the disease before the occurrence of severe damage to the brain.

5.3.2.2 Differential leucocyte count

No difference in differential leucocyte count was noticed between the diseased and normal animals (Figure 6). Lymphocytes were predominant in the CSF of normal animals and this finding agreed with the observation of Scott (1992).

5.4 Electroencephalography

Recording of EEG using disc electrodes, with the animal in lateral recumbent position, under proper restraint was a simple and safe procedure (Plates 2 and 3). Disc electrodes glued to the skull with the help of kaolin paste and a small piece of cotton was sufficient to withstand minor movements of head. These findings were similar to the reports of Redding and Knecht (1984). According to them clip electrodes were unsuitable to equine and bovine scalp since their skin were tightly adhered to the cranium. Needle electrodes could not penetrate the thick, tough scalp of these animals. Silver or gold or disc electrodes could be secured to the scalp of equine and

bovine patients using thick electrode paste, each electrode being covered with a gauze pad for additional adhesion. They also opined that recordings obtained from physically restrained animals in the alert, relaxed and auditory stimulated states were most useful for clinical diagnosis.

5.4.1 EEG recordings

EEG patterns in normal goats were characterized by low amplitude (voltage) fast waves or high amplitude slow waves or a mixture of low amplitude fast waves and high amplitude slow waves (Plates 9 and 10). This was in accordance with the findings of Sugawara (1971).

Abnormal patterns in PEM included slow waves and asymmetry (Plate 11). Most of the animals showed slow waves of high or low amplitude which were intermittent with normal waves. Continuous slow waves of low or high amplitude were observed by Itabisashi *et al.* (1990) in PEM affected sheep. Similar observations were also recorded by Syamasundar *et al.* (1996). Another abnormality recorded was slow activity. Similar finding was also reported by Suzuki *et al.* (1990). They also opined that high amplitude slow activity (HASA) was the characteristic EEG pattern of a CCN patient. Long lasting spindles as reported by Dunlop *et al.* (1981) and Itabisashi *et al.* (1990) was not a finding in the present study.

Slow waves of high or low amplitude was suggestive of a degenerative change in the cerebrocortical areas. Klemm and Hall (1974) reported that

local EEG abnormalities indicated a cortical rather than a subcortical lesion. High amplitude slow activity (Plate 12) and low amplitude slow activity were seen prominently in two cases each. In other cases the pattern was a combination of these two. Redding (1978b) described that high voltage slow activity was produced by various degenerative diseases such as trauma, vascular disorders and hydrocephalus. It was noticed that the abnormal wave pattern was not confined to any one part of the brain and abnormalities were noticed in all the montages. This indicated the diffused nature of involvement of cerebral cortex in polioencephalomalacia. Subcortical lesions also caused EEG abnormalities to appear in several areas of the cortex because the subcortical brain tissue send projecting fibre tracts to scattered areas on the cerebral cortex, but clinical signs and results of nervous system examination were suggestive of predominant cerebrocortical involvement.

5.5 Radiographic examination

No abnormalities could be detected in the radiographs of skull of diseased goats (Plate 13). Brain damage from direct or indirect trauma could be diagnosed based on the history of trauma, radiographic examination to detect the presence of fractures, and presence of neurological signs (Whittick, 1974).

5.6 Response to treatment

All the animals except one severe case responded to the treatment. Improvement was recorded in all the animals the very next day itself. Two animals became completely normal by fourth day, three on fifth day and one severe case on eighth day (Plates 4-7). Treatment with 50 mg/kg thiamine hydrochloride intravenously twice daily along with 15 g bismuth carbonate orally for four days was very effective. The conventional treatment is administration of thiamine hydrochloride at the rate of 10 mg/kg body weight every three hours intravenously for a total of five treatments (Radostits *et al.*, 1994). Bismuth carbonate given orally would change the rumen pH favourably and suppress the growth of thiaminase producing bacteria. Oral administration of bismuth carbonate had beneficial effects in PEM due to sulfur toxicosis (Bulgin *et al.*, 1996). The dissociation rate of sulfide might be reduced by adminstering bismuth carbonate, which would increase rumen pH, thus, limiting the absorption of sulfur from the rumen (Kandylis, 1984).

The treatment regimen adopted in this study was much easier and effective and hence recommended for adoption in field conditions.

5.7 Gross and histopathology

Brain was grossly soft, swollen and spongy in appearance with yellowish discolouration of the cerebral hemispheres and haemorrhages on

meninges. This was consistent with the findings of Dickie *et al.* (1979), Jackman (1985), Tanwar *et al.* (1993) and Fakhruddin *et al.* (1987a) and might have occurred due to the increase in intracranial pressure.

Histological changes observed in the cerebrum were necrosis of neurons with neuronophagia (Plate 14), focal accumulation of neutrophils in cortico-medullary area (Plate 15), perivascular cuffing of lymphocytes (Plate 16), neovascularisation (Plate 17), perivascular oedema and satellitosis (Plate 18). Thornber *et al.* (1979), McAllister *et al.* (1992), Chahar *et al.* (1993), Tanwar (1987) and Nair (1999) observed similar findings in PEM cases. These changes were indications of degeneration of brain along with secondary inflammatory process (Nair, 1999).

Cerebellum also revealed changes like perivascular oedema (Plate 20), perivascular cuffing (Plate 19), neovascularisation and congestion of vessels.

No histopathological lesions could be detected in pons, medulla and hippocampus.

Glial cell transketolase (thiamine dependent enzyme) played an important role in the metabolic activities of brain (Hamlen *et al.*, 1993). The first change that occurred in PEM was an oedema of the intracellular compartment, principally involving the astrocytes and plasma cells. It was suggested that the oedema might be due to a reduction in ATP production following a defect in carbohydrate metabolism in the astrocytes (Radostits

et al., 2000). They also opined that diets high in sulphur resulted in hydrogen sulphide production in the rumen, which could be inhaled during eructation, and often systemic absorption inhibited cellular respiration leading to hypoxia which might be sufficient to create neuronal necrosis in PEM.

Laminar necrosis of cerebral cortex as reported by Tanwar (1987) and McAllister *et al.* (1992) was not detected in this study.

Clinical signs exhibited by the animal like dullness, hyperesthesia, head pressing, opisthotonus, postural reaction deficits, visual impairment and spasticity of limbs were suggestive of cerebral involvement. Neurological and EEG examination had also suggested that the lesion was in the cerebral cortex. Absence of histopathological changes in the pons and medulla suggested that brain stem was not primarily involved in PEM and some of the clinical signs suggestive of brain stem involvement could only be functional disturbances.

Summary

6. SUMMARY

In the present study polioencephalomalacia affected goats were examined in detail for their clinical and neurological manifestations, CSF changes, EEG, cranial radiography and histopathological changes in brain. Therapeutic efficacy of thiamine hydrochloride intravenously at the rate of 50 mg/kg body weight twice daily along with bismuth carbonate at the rate of 15 g/animal orally as a drench for four days was assessed.

Seven clinical cases of polioencephalomalacia were utilized for the study. Six apparently healthy goats maintained under identical conditions served as the control. Detailed clinical and neurological examination were carried out and recorded. Cerebrospinal fluid samples were collected and analysed for its physical (colour, transparency and consistency), chemical (total protein) and microscopical characters (total leucocyte count and differential leucocyte count). Electroencephalogram of all the patients were recorded and radiographs of skull were taken. The brain of the animal that did not respond to treatment was subjected to histopathological studies.

History of the affected animals showed that majority of the animals were fed with rice gruel (a carbohydrate rich diet) which could have created a favourable environment for the growth of thiaminase producing bacteria in the rumen.

The characteristic clinical signs were head tilt, lateral deviation of head, nystagmus, circling, staggering gait, broad-based stance, head pressing and lying only on one side. Other symptoms noticed were hyperesthesia, low carriage of head, extension of head and neck, aimless wandering, ataxia, vertical or horizontal nystagmus, tonic-clonic convulsions and opisthotonus. All the symptoms mentioned above were not present in all the animals.

The rate of respiration and pulse were higher at the time of presentation, but the values decreased steadily after the initiation of the treatment. The body temperature remained normal throughout the course of the disease.

Neurological examination was carried out to assess the behaviour, posture, gait, integrity of cranial nerves, postural reactions like wheel-barrowing and proprioceptive positioning and spinal cord reflexes like patellar reflex, flexor reflex, panniculus reflex and perineal reflex. The examination revealed depressed behaviour and deficits in posture and gait which suggested cerebrocortical involvement. Most of the cranial nerves did not show serious deficits. Postural reaction deficits and abnormal spinal cord reflexes indicated upper motor neuron (UMN) involvement.

Cerebrospinal fluid was collected from the lumbo-sacral site and the physical characters were similar to that of healthy animals. A significant

increase in the mean total protein content of CSF was noticed in the diseased animals. This could be due to altered blood-brain-barrier. TLC and DLC of CSF remained within the normal physiological limits.

The abnormal patterns detected in the EEG included continuous slow waves and asymmetric waves. Slow waves were of low or high amplitude. These abnormalities also suggested a cerebrocortical involvement.

Radiography of the skull did not reveal any pathological change.

Histopathology of the brain of a succumbed goat revealed necrosis of neurons of the pyramidal and fusiform cell layers of the cerebral cortex with neuronophagia, satellitosis, perivascular oedema, perivascular cuffing and neovascularisation of grey matter in certain gyri.

All the animals, except one severe case, responded to the treatment with thiamine hydrochloride intravenously and bismuth carbonate orally for four days.

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NEUROLOGICAL INVESTIGATION AND TREATMENT OF POLIOENCEPHALOMALACIA AFFECTED GOATS

By

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ABSTRACT OF THE THESIS
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ABSTRACT

Seven goats diagnosed to be suffering from polioencephalomalacia were used for the present study. It included detailed clinical and neurological examination, CSF collection and analysis, electroencephalography, cranial radiography, histopathology of brain and assessment of the therapeutic efficacy of thiamine hydrochloride intravenously and bismuth carbonate orally in PEM cases.

History of the affected animals suggested that feeding of rice gruel (a carbohydrate rich diet) could be one of the predisposing causes for the development of PEM of goats in Kerala. The characteristic clinical signs were head tilt, lateral deviation of head, nystagmus, circling, staggering gait, broad based stance and lying only on one side.

Nervous system examination revealed depressed behaviour and deficits in posture and gait. Majority of the cranial nerves did not show deficits. Defects in postural reaction and abnormalities in spinal cord reflexes were detected.

CSF examination revealed a significant increase in the mean total protein concentration and a non-significant increase in the total leucocyte

count. Electroencephalograms revealed continuous slow waves and asymmetric waves. Another abnormality was high amplitude slow activity. Histopathology of brain revealed necrosis of neurons of the pyramidal and fusiform cell layers of the cerebral cortex with neuronophagia, satellitosis, perivascular oedema, perivascular cuffing and neovascularisation of grey matter. Out of the seven cases, six animals responded to the treatment with thiamine hydrochloride at the rate of 50 mg/kg body weight twice daily intravenously and bismuth carbonate, 15g per animal, orally as a drench for four days.

Appendix

APPENDIX

PROFORMA FOR CLINICAL EXAMINATION

Case No: Date:

I. Patient data

Animal	Species	Breed
Age	Sex	Color

II. History

III. Physical examination

Head, neck & forelimbs-
Trunk & hindlimbs-
Tail & anus-

IV. Neurological examination

1. Mental status-Dullness/somnolence/stupor/coma/active & alert/mania/frenzy/aimless wandering/head pressing/narcolepsy.

2. Posture & gait-Paresis
-Ataxia
-Spasticity
-Total deficit
-Others

3. Cranial nerve examination

(a) *Olfactory*-Normal/hyposmia/anosmia		
(b) *Optic*- Menace	R	L
Pupillary	R	L
(c) *Occulomotor*-Pupillary		
Ventrolateral strabismus	R	L
(d) *Trochlear*-Dorsomedial strabismus	R	L
(e) *Abducens*-Medial strabismus	R	L
(f) *Trigeminal*-Palpebral	R	L
Corneal	R	L

Avoiding stimulation of nasal meatus
Resistance in opening the jaw

(g) *Facial*-Palpate ears, lips, eyelids & muzzle
Palpebral, corneal, menace & avoiding stimulation of nasal meatus

(h) *Vestibular*-Physiological nystagmus

(i) *Glossopharyngeal, Vagus & Accessory spinal*
Gag reflex
Laryngeal reflex
Muscles of neck

(j) *Hypoglossal*
Tongue stretch

4. Postural reaction

a. Wheel barrowing
b. Proprioseptive positioning

5.Spinal cord reflexes

a. Patellar reflex
b. Flexor reflex
c. Panniculus reflex
d. Perineal reflex

V. Ancillary diagnostic procedures

VI. Clinical assessment