PREVALENCE AND TREATMENT OF BOVINE COCCIDIOSIS

By
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COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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2000

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

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TO MY PARENTS AND HUSBAND

IN
LOVE
AND
GRATITUDE

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INTRODUCTION

1. INTRODUCTION

"The protection of cattle is an article of faith. Apart from its sanctity, it is an ennobling creed. We should commence with ourselves and cover the land with useful propaganda leading to kindness in the treatment of cattle and scientific knowledge in the management of cattle farms and dairies"

-Mahatma Gandhi

Since time immemorial, cattle have been an integral part of Indian culture and way of life. Cattle domestication took place before 3000 B.C., as inferred from the fossils unravelled from the Siwalik hills of Northern India. The bovine population was truly a pivot around which civilizations and trades flourished. In our country, cattle are synonymous to wealth, indispensable to us, for they provide tractor power for agricultural operations, cheap but highly valuable organic manure for preserving soil fertility and then milk, the life sustaining fluid for the young and the old.

Today, India possesses the largest number of cattle and buffaloes in the world, ie. 201.5 million cattle and 20.8 million buffaloes comprising 15.9 per cent of the

world's cattle and 53.75 per cent of world's buffalo population (Banerjee, 1999). But, inspite of this enormous number, the alleles for productivity appear to have been lost under extremely poor managemental conditions.

Coccidiosis is an important protozoan disease of cattle causing profound economic loss. In India, outbreak of red dysentery in dairy cattle due to bovine coccidiosis has been reported from time to time. The disease generally occurs in calves less than a year old, but may also occur in older cattle. The cause for coccidiosis in cattle is attributed to the protozoan parasites of the genus *Eimeria*. Cattle become infected by oral ingestion of sporulated oocysts contained in faecal contaminated feed or water. Disease outbreaks are most likely to occur whenever there is an aggregation of young stock under conditions permitting the accumulation and sporulation of large number of oocysts.

Animals with subclinical infections irrespective of age may be a source of infection. Older cattle can also be severely stricken with the disease, usually when they

are stressed by factors such as crowding, changes of feed, severe weather, castration and dehorning.

The wet and humid climatic conditions prevailing in Kerala are very conducive in precipitating coccidial infections. Outbreaks of infection among young animals in small and large farms often lead to stunted growth rate, delayed maturity which inturn cause profound production losses and affects the national economy.

Taking into consideration the importance of the disease, this particular study was undertaken to

i) understand the prevalence of different coccidian species affecting bovines,
ii) assess the haematological alterations and
iii) evaluate the efficacy of sulphadimidine sodium, amprolium hydrochloride and salinomycin against bovine coccidiosis.

❁

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Prevalence

One of the earliest reports on the prevalence studies of *Eimeria zuernii* in Philippines was made by Tubangui (1931).

Boughton (1945) reported cases of bovine coccidiosis from the United States and isolated *E. ellipsoidalis, E. bovis* and *E. zuernii*. Hasche and Todd (1959) found *E. cylindrica, E. subspherica* and *E. brasiliensis* to cause coccidial infections in Wisconsin and *E. bovis* was identified as a prominent species in Utah by Fitzgerald (1962).

Patnaik (1965a) reported that the infection in the buffaloes of Agra were due to *E. bovis* (52 per cent), *E. zuernii*,(36 per cent), *E. subspherica* and *E. ellipsoidalis* (21 per cent each) , *E. cylindrica* (7 per cent) and *E. bukidnonensis* (3 per cent).

Bhatia *et al.*,(1968) conducted studies on coccidial infections prevalent in buffaloes from Mathura, India and they found species like *E. bovis, E. zuernii. E. subspherica, E. ellipsoidalis* and *E. bareillyi*.

Pande *et al.*, (1971) studied the morphology and endogenous life cycle of *E. bareillyi* from Mathura.

Ruiz (1973) recorded coccidial infections in beef cattle from three different states in Germany. A relatively higher prevalence of coccidia was recorded in summer, autumn and spring. Prevalence did not vary substantially with age. Mature cattle averaged fewer oocysts per gram (OPG) of faeces than immature animals. The nine species found in the order of decreasing prevalence were *E. bovis, E. alabamensis, E. ellipsoidalis, E. canadensis, E. subspherica, E. zuernii, E. cylindrica, E. auburnensis* and *E. bukidnonensis.*

A survey carried out by Majaro and Dipeolu (1981) in trade cattle in Nigeria, revealed coccidial oocysts in 56 per cent of the calves with *E. bovis* and *E. zuernii* as the predominant species.

Rodriguez and Blandino (1981) examined faecal samples from 1071 calves in six breeding centres in Havana, Cuba over five months and they could identify

species like *E. bovis, E. zuernii, E. ellipsoidalis, E. auburnensis, E. cylindrica, E. canadensis, E. alabamensis, E. subspherica, E. brasiliensis. E. bukidnonensis* and *E. wyomingensis.*

Yvore *et al.*,(1982) gave a preliminary report of a study of bovine coccidiosis in France, where in E. *bovis* and *E. zuernii* were the dominant species found.

Yvore *et al.*,(1983) found that *E. auburnensis* and *E. subspherica* were also common in France causing diarrhoea in calves.

It was observed by Ershaduzzaman *et al.* (1985) that coccidiosis in calves was significantly higher during rainy season than in dry season of the year and younger animals (less than six months old) showed a higher rate of infection with male calves being more prone to the infection than female calves.

Kasim and Al-Shawa (1985) made a survey of coccidial infections in cattle in five regions of Saudi Arabia to determine the relative incidence of *Eimeria sp.* in the country. The species recovered from 205

domestic cattle were *E. auburnensis, E. bovis, E. cylindrica, E. ellipsoidalis, E. subspherica, E. wyomingensis* and *E. zuernii* with a prevalence of 34.1 per cent. *Eimeria zuernii* and *E. bovis* occurred most frequently. Incidence was observed to be higher in areas with higher rainfall, humidity and intensive rearing methods than in dry arid regions.

Sanyal *et al.*, (1985) published a report on the incidence of bubaline coccidia at Hisar, Haryana. On examination of faecal samples of buffalo calves ranging from six months to 2½ years of age, the species prevalent were predominantly *E. bareillyi*, followed by *E. bovis, E. zuernii, E. canadensis* and *E. auburnensis*. It was observed that *E. bareillyi* was extensively common in young buffalo calves below six months of age. They opined that intensive cattle breeding programmes and excessive irrigation were the predisposing factors for coccidial infections in Haryana.

Ernst *et al.*, (1987) determined the prevalence of coccidian oocysts from cross bred beef calves raised on grass pastures in the coastal plains of Georgia. Although 86.3 per cent of the animals carried

subclinical infection, only one clinical case of coccidiosis due to *E. zuernii* was seen. The most prevalent species was found to be *E. bovis*, whereas on quantitative determination, *E. ellipsoidalis* had the greatest number of oocysts per gram (OPG).

Wee *et al.*, (1987) found 19.5 per cent out of 1424 cattle to be infected with coccidia in the Chonnam area, Korea. The eleven species identified were *E. bovis*, *E. zuernii*, *E. auburnensis*, *E. alabamensis*, *E. ellipsoidalis*, *E. subspherica*, *E. canadensis*, *E. bukidnonensis*, *E. cylindrica*, *E. brasiliensis* and *E. wyomingensis*.

Ali and Latif (1989) recorded 370 samples (31.5 per cent) as positive out of 1173, containing *Eimeria* oocysts of four different species, *E. bovis*, *E. zuernii*, *E. pellita* and *E. auburnensis* from dairy cattle farms in Baghdad province, Iraq. The highest rate of infection occurred in calves below one year. There was a significantly higher rate of infection in males (36.4 per cent) compared to females (28.1 per cent). No seasonal variation on the rate of infection was observed.

Munya and Ngotho (1990) determined the prevalence of coccidiosis in bovines in Kenya as 67.4 per cent. The species detected were *E. bovis, E. zuernii, E. ellipsoidalis, E. cylindrica, E. subspherica* and *E. wyomingensis.*

Oda and Nishida (1990) detected the prevalence as 59.0 per cent of 1015 faecal samples obtained from bovines in Japan. Prevalence was highest in the animals between 6 and 11 months old and decreased to 25 per cent in those more than 24 months old. *Eimeria bovis* and *E. ellipsoidalis* were the most prevalent species.

Faecal samples positive for coccidial oocysts amounted to 12.9 per cent in animals sent to pastures for grazing and 26.7 per cent in those housed loosely as per the study conducted by Hasbullah *et al.* (1990) at the university farm, Tohoku University, Japan. *Eimeria bovis* was the most predominant species followed by *E. auburnensis, E. canadensis, , E. alabamensis, E. bukidnonensis, E. ellipsoidalis* and *E. zuernii.*

Agnihotri (1993) found the occurrence of coccidia to be highest (43 to 45 per cent) in buffaloe calves of the age groups 2 to 4 months in a study conducted at Tarai region in Western Uttar Pradesh.

Senthilvel (1995) reported clinical coccidiosis in a bullock brought to a private veterinary clinic in Thrissur. This is one of the few reports on the prevalence of coccidiosis in aged cattle.

Gunning and Wessels (1996) identified *E. zuernii* as the causative agent of clinical coccidiosis in a herd of dairy cows at Langford, Bristol.

Grommes (1996) made an epidemiological study of subclinical *Eimeria* infections among 56 grazing calves, in Central West Germany. Species of coccidia identified were *E. bovis, E. ellipsoidalis, E. subspherica, E. canadensis, E. zuernii* and *E. pellita*.

Chibunda *et al.*, (1997) detected coccidial oocysts in 35 per cent of 445 cattle in Tanzania. Although the highest prevelance (56 per cent) was in animals aged between five and 18 months, the oocyst output was

highest in weaned calves. The species included *E. bovis* (68 per cent), *E. zuernii* (57 per cent) *E. ellipsoidalis* (25 per cent), *E. cylindrica* (23 per cent) *E. auburnensis* (22 per cent) and *E. alabamensis* (12 per cent).

2.2. Species identification

The methods of identification of species by studying the morphology of oocysts were elaborated by Jeffers and Shirley (1982). The characteristics of oocysts which were useful tools in describing various species were:

a) the dimensions, shape and colour of the oocyst,

b) the characteristics of the surface of the oocyst wall and appearance of polar cap or micropyle,

c) the characteristics of the sporocyst and

d) the presence or absence of granules or residual bodies in the oocyst or sporocyst.

2.2.1. *Eimeria zuernii*

Rivolta (1878) first observed coccidiosis in cattle and described a coccidium under the name *Cryptospermium zuernii* which was later renamed as *E. zuernii* by Martin (1909). Pellerdy (1965) compiled information on

morphology and sporulation time of oocysts in the light of earlier works and reports based on which various species of *Eimeria* were identified. The oocyst wall of *E. zuernii* was found to be smooth, uniformly thin, colourless without a visible micropyle. Sporulation took two or three days to complete at room temperature. There was no evidence of any oocystic residual body and the sizes were 14 to 21.5 μ by 10.5 to 17 μ (average 17.1 μ by 14.6 μ).

Mandal (1980) described the oocyst of *E. zuernii* as spherical, subspherical or bluntly ellipsoidal - ovoidal in shape, measuring 14 to 22 μ in length and 13 to 19 μ in width with a mean 17 μ by 16 μ. Oocyst wall was described to be smooth, double layered, light yellowish green in colour measuring 1.5 μ thick with micropyle, micropylar cap, oocystic residuum and polar granule absent. Sporocyst was observed to be elongate having a mean measurement of 9.9μ by 6.0μ with rounded ends and steida body present as faintly dark cap over the narrower anterior end. The sporozoites were stumpy 9μ by 3μ in size. The time for sporulation was three to four days.

2.2.2 *Eimeria bovis*

The oocysts of this species were first recognised by Zublin (1908) under the name *Coccidium bovis*. Fiebger (1913) renamed it as *E. bovis*.

Pellerdy (1965) described the oocyst as 28 to 34 µ in length and 19 to 28µ in width (average 27.7µ by 28µ). The shape varied from tapering ovoid, to elongate and pointed. A micropyle was observed at the narrower end. The wall was homogenous transparent gradually diminishing in thickness towards the micropyle and sporulation was completed in two to three days.

According to Mandal (1980) the oocysts were broadly ovoid, with a narrow micropylar end, 23 to 43µ by 15 to 26µ (mean 28µ by 21µ) in size. Oocyst wall was seen to be smooth, double layered, light yellowish brown in colour, 1.5 µ thick. Micropyle was present at the narrower end, as a dark knob. Sporocystic residuum was seen to be granular, arranged along the longitudinal axis of the sporozoite. Sporozoite was elongate, banana shaped and 13µ in size. Sporulation time was observed to be three to four days.

2.2.3. *Eimeria ellipsoidalis*

Becker and Frye (1929) were the first to report on the species *Eimeria ellipsoidalis.*

Pellerdy (1965) gave a description of the oocyst which measured from 20 to 26 μ in length and from 13 to 17 μ in width (average 23.4μ by 15.9μ). The oocysts were mostly ellipsoidal, but occasionally spherical or cylindrical forms were seen. No micropyle was present and the wall was homogenous, thin, transparent and colourless. Sporulation at room temperature required two to three days.

Mandal (1980) described the oocyst as ellipsoidal to slightly ovoid, 15 to 26 μ by 12 to 16 μ (mean 20 μ by 14 μ) in size. Oocyst wall was double layered, light yellowish green and 1.3 μ thick. Micropyle was present, with micropylar cap absent. Oocystic residuum and polar granule were absent. Sporocysts were elongate ovoid (mean 12.2 μ by 5.4 μ) in size. Steida body was seen as a dark knob. Sporocystic residuum was present as a mass of loose granules. Sporozoites were elongate, banana

shaped and 11 μ by 2.6 μ in size. Sporulation time was three days.

2.2.4. *Eimeria cylindrica*

Wilson (1931) described the species *E. cylindrica*. Reports were published by Pellerdy (1965) about the same species. The oocysts were cylindrical in shape measuring 16 to 27 μ by 12 to 15 μ (average 23.3 μ by 13.3 μ) ie., they were twice as long as broad. The wall was thin, colourless and smooth. No micropyle was visible, but a small area of cyst wall was lighter in colour than the rest. Sporulation was completed in two days.

Mandal (1980) found the oocyst to be cylindrical or somewhat sub-cylindrical measuring 20 to 34 μ by 12 to 17 μ (mean 26 μ by 14 μ) in size. Oocyst wall was double layered, colourless or somewhat straw coloured, 1.3 μ thick. Micropyle and micropylar cap were absent. Sporocysts were elongate and ovoid, 10.4 μ by 5.2μ in size. Steida body was present at the narrow end. Sporozoites were elongate measuring 7.6 μ by 2.1 μ. Sporocystic residuum was present as few scattered granules. Sporulation time was seen to be two to three days.

2.2.5 *Eimeria subspherica*

The species *E. subspherica* was identified by Christensen (1941).

Pellerdy (1965) reported that the oocyst of this species was the smallest among all other bovine species, measuring 9 to 13 μ length by 8 to 12 μ in width (average 11 μ by 10.4 μ). They were from thick set ellipsoid to subspherical in shape. No micropyle was present and the wall was uniformly thin, smooth and transparent. The sporocysts were spindle shaped. The species sporulated at four to five days at room temperature. The species was differentiated from *E. ellipsoidalis* by its longer sporulation time, thinner oocyst wall and lesser resistance of the cyst to indentation in a 40 per cent sugar solution.

Mandal (1980) found the oocysts to be colourless and subspherical in shape. The oocyst wall was smooth, sometimes pale yellowish in colour, double layered, 0.7 to 1.0 μ thick. The oocyst measured 9 to 14 μ in length and 8 to 12 μ in width with a mean of 11μ by 10.4 μ. Micropyle, oocystic residuum and polar granules were

absent. The sporocysts were elongate with a small steida body measuring an average of 8 μ by 3.5 μ containing no sporocystic residuum. The sporulation time was longer, four to five days.

2.2.6 *Eimeria bareillyi*

Gill *et al.*, (1963) discovered a new species of coccidium, *E. bareillyi* from buffaloes at Izatnagar. The description of the oocysts was given by Mandal (1980). They were pyriform, with the narrower anterior end, truncated and slightly flattened measuring 24 to 31 μ in length and 15 to 21 μ in width with a mean of 28μ by 19μ. Oocyst wall was double layered, yellowish brown, 1.3 μ thick. Micropyle was 3.5 to 6.0 μ wide. Micropylar cap, oocystic residuum and polar cap were absent. Sporocysts were elongate, measuring an average of 17 μ by 7.3 μ . Steida body was present as a protruberance. Sporocystic residuum was either centrally placed or scattered in the middle. Sporozoites were elongate (12 μ by 4 μ) with one end broader and the other end pointed. Sporulation time was noticed to be three to four days.

Sanyal *et al.* (1985) identified oocysts of *E. bareillyi* from faecal samples collected from progeny testing farm, Hisar. The oocysts were identified based on characteristics like sporulation time and morphology.

2.2.7 *Eimeria brasiliensis*

According to Supperer (1952) this species had a length of 33.7 to 49 μ and a width of 21.1 to 33.2 μ. The shape was described as oval, clear with a micropyle covered by a micropylar cap, 10 to 12 μ in width and 2 to 4 μ in height. The oocysts sporulated in 12 to 14 days only. The sporocysts measured an average of 16 by 8 μ. Sporocystic residual body was 5 μ in diameter, while the oocystic residual body was absent. Polar granule was present in both sporulated and unsporulated oocysts.

The oocysts of the same species were described as ellipsoidal or ovoidal by Mandal (1980), with a mean size of 39 μ by 27 μ. The oocyst wall was double layered, colourless to yellowish brown. Micropyle and micropylar cap were present and the sporocystic residuum was seen as a granular mass. The sporulation time was observed to be more than six days.

2.2.8 *Eimeria wyomingensis*

An almost perfectly egg shaped oocyst was reported by Huizinga and Winger (1942) and it was named as *E. wyomingensis*. The oocyst measured 37 to 44.9 μ in length and 26.4 to 30.8 μ in width. The oocyst wall was 3 μ thick and yellowish brown in colour. Sporulation took five days to be completed. In unsporulated oocyst, the sporont almost completely filled the oocyst. The sporocysts were described as fusiform, measuring 19 μ in length and 3 μ in width. Oocystic residual body was absent.

Mandal (1980) described the oocyst as egg shaped with an average size of 40 μ by 28 μ. Oocyst wall was brownish coloured and double layered with 2.0 μ thickness. Micropyle was present, oocystic residuum and polar granule were absent. Sporocysts were elongate with a mean size of 22 μ by 8.6 μ. Sporulation time was six to seven days.

2.3 Haematological alterations

Fitzgerald (1964) investigated on the effects of *E. bovis* infections on the serum proteins of calves. A detailed study was initiated to gain information on how coccidiosis caused by experimental infections with *E. bovis* affected blood serum proteins in new born Holstein- Friesian male calves. It was observed that serum albumin and total protein decreased three weeks after inoculation with sporulated oocysts. The alpha globulin increased slightly during this period while the beta and gamma fractions were unaffected. The degree of change in serum proteins was dependent upon the severity of clinical symptoms. The gamma globulins decreased during the period when the symptoms were most severe. The serum proteins did not return to preinoculation levels until six to eight weeks after the cessation of severe symptoms. The total serum protein averaged to 6.8 g/100 ml prior to inoculation with oocysts. The albumin globulin ratio changed from 1.48 / 1.00 prior to infection to 0.53/ 1.90, 24 days after inoculation, where symptoms were most severe. The total serum protein dropped to 3.15 g/ 100 ml three weeks after inoculation.

Total protein was determined by biuret method in a spectrophotometer. The experiments conducted showed that increase in alpha (α) globular fractions coincided with the decrease in albumin and total protein. The changes were attributed to generalised injury to the body, production of toxic materials, altered osmotic pressure, inefficiencies in lipid transport and discharge of blood, mucus, epithelium and the mechanical loss of fluids. The lack of detectable change in the gamma (γ) globulins of young animals was due to their reticulo-endothelial systems having a lower level of activity when compared to the situation in older animals. It was also suggested that the consistent changes in alpha (α) globulins, by contrast, showed that antibody response to bovine coccidiosis could be associated with this fraction. Gamma (γ) globulins were said to have lesser importance in immunological response associated with bovine coccidiosis.

Effects of bovine coccidiosis on certain blood components were studied by Fitzgerald and Mansfield (1972). Calves aged six weeks were inoculated with sporulated oocysts of *E. bovis, E. zuernii* and *E. ellipsoidalis*. Packed cell volume (PCV), haemoglobin

(Hb) content and total serum protein concentrations were determined for each calf once in a month. A marked decrease in the total serum protein concentration was noticed in association with the onset of clinical signs and discharge of oocysts. Haemoglobin and packed cell volume values altered in severely affected calves as a result of loss of intestinal tissue and bleeding into the intestinal lumen. In calves with low level infections, blood values were erratic and there were no significant differences statistically.

Stockdale *et al.*, (1981) studied on bovine coccidiosis caused by *E. zuernii* and observed on some patho-physiological changes associated with the infection. Calves aged two to four months were infected with sporocysts of *E. zuernii*. Blood parameters like differential leukocyte count, haemoglobin, packed cell volume and total serum proteins were assessed frequently. There were marked decreases in the packed cell volumes and haemoglobin levels. There appeared to be a mild reduction in the total plasma proteins. The pronounced decline in PCV and Hb values were coincidental with the damage to and removal of epithelium of large intestine. Interestingly, there was

a marked contrast to the disease caused by *E. bovis* in which an increase in Hb and PCV was reported.

Holst and Svensson (1995) studied the changes in the blood composition of calves during experimental and natural infection with *E. alabamensis*. The changes in blood parameters though significant were small. In acute infections, the white cell count, Hb and PCV levels declined along with total plasma protein concentration. The most severe changes coincided with the period when the second generation schizogony and gametogony caused the most severe damage to the intestinal epithelium.

2.4. Treatment

2.4.1. Sulphadimidine

In India, Biswal (1948) was one of the pioneer scientists who studied the efficacy of sulpha drugs as remedial measures adopted in the treatment of bovine coccidiosis. He employed sulphaguanidine at the rate of 0.05 to 0.1 g per kg body weight, administered in water, thrice daily for six to nine days till diarrhoeic symptoms subsided, to treat cases of clinical coccidiosis, in dairy farms. Sulphamethazine was also used at the rate of 12.5 g per 100 kg body weight. The

dosage was halved subsequently and used for four to six days. A cent per cent recovery was observed in the affected cases with absence of oocyst in the faecal samples, after treatment.

Trials on bovine coccidiostats with Sulphadimidine were carried out by Peardon *et al.* (1963) on two Guernsey heifers. One heifer was treated and the other heifer was an untreated control. On the first day, 1 g per 1b body weight, second day ¾ of the initial dose, third and fourth day, half of the initial dose was given. As the trial progressed, the number of oocysts shed decreased and other clinical signs became less apparent.

Observations on the effect of Sulphadimidine on *E. bovis* in naturally infected buffalo calves were made by Patnaik (1965b). Little information was then available, regarding the treatment of bubaline eimeriosis using Sulphadimidine. Eight subclinical cases of Murrah buffalo calves aged 4 to 13 week old which were raised in crowded calf pens and paddocks were selected and treated with Sulphadimidine with a daily dosage of 2.5 g for five days.

Arakawa and Todd (1968) studied the effect of treatment of calves with sulphonamides on the cellular response to first generation schizonts of *E. bovis*. Sulfamethazine was administered @ 0.2 g per kg body weight on days 12, 13 and 14 after exposure to oocysts. Degenerative changes occurring in the schizonts were demonstrated.

Todd and Thacher (1973) worked on the control of bovine coccidiosis using feed medicated with Sulphadimidine. Calves were experimentally infected with 30 to 5,00,000 oocysts from five Eimerian species affecting cattle. The infection failed to develop into a clinical condition in calves given feed containing Sulphadimidine equivalent to a daily dose of 350 mg. Administration of the medicated feed commenced five days before infection and continued for 30 days post infections.

McDougald (1982) reported Sulphadimidine as the drug of choice for treating *E. zuernii* and *E. bovis* infections at the rate of 5 g per calf orally for seven days.

Khahra *et al.*, (1983) administered Sulphadimidine @ 125 mg per kg body weight for three days orally in experimentally infected buffalo calves.

Celeda *et al.*, (1985) reported on the effect of intermittent treatment with Sulphadimidine on coccidiosis in pre-ruminant calves housed in groups. Repeated administration of Sulphadimidine for two weeks orally @ 50 mg per kg on the first day of treatment followed by a dosage rate of 37.5 mg per kg under the same housing condition kept the faeces free of oocyst, but it affected the immunoglobulin levels adversely. Sulphadimidine given repeatedly at a lower dose rate (30 mg per kg) for a week with medication free intervals of one week controlled the infection and no adverse effects were noted. Weight gains were greater in treated calves.

In the chemotherapeutic efficacy of Sulphadimidine in experimental *E. bareillyi* coccidiosis of buffaloes as studied by Sanyal *et al.*, (1985), Sulphadimidine was found to be highly effective, arresting the development of schizonts and early gamonts when administrated @ 30

mg per kg body weight for four consecutive days 10 to 13 days post inoculation.

Vottero and Suarez (1985) opined that parenteral or oral treatment with sulpha drugs at the time of maximum oocyst production produced no effect on the course of infection, but preventive and continous administration of sulpha drugs from the time of first exposure to infection limited the parasite multiplication and prevented the clinical disease.

Suresh *et al.*, (1990) reported the usefulness of Sulphadimidine @ 200 mg per kg body weight orally for five days in treating a cow that had clinical coccidiosis. Radostits *et al.* (1994) stated that Sulphadimidine was effective for calves against coccidiosis at the dosage rate of 140 mg per kg orally daily for three days.

The treatment of cattle coccidiosis using sulpha drugs was elaborated by Lindsay and Blagburn (1995). It was reported that Sulphadimidine could be administered orally to treat coccidiosis caused by *E. bovis* and *E. zuernii* with two five gram boluses per 45 kg for one

day followed by one five gram bolus per 45 kg upto four consecutive days.

Svensson and Olofsson (1996) used Sulphadimidine boluses to treat *E. alabamensis* coccidiosis in grazing calves. The animals were dosed at the rate of one bolus (14.4 g Sulphadimidine for 200 kg body weight).

Another study was carried out by Svensson (1998) to investigate the efficacy of a baquiloprim Sulphadimidine bolus in preventing *E. albamensis* coccidiosis when administered to first season grazing calves two days after turnout onto a contaminated pasture. The study revealed that the treatment with the drug @ one bolus (1.6 g baquiloprim and 14.4 g Sulphadimidine) can be used as a valuable complement to the pasture hygienic measures in farms where coccidia free pastures may not be readily available.

2.4.2 Amprolium

Hammond *et al.,* (1966) used Amprolium for the control of experimental coccidiosis in cattle at a dosage rate of 145 mg per kg for 21 days.

McDougald (1982) stated that Amprolium was one of the safest anticoccidial drugs to be used extensively and it could be fed several times the recommended dose with no ill effects. The beneficial effects of Amprolium in clinically ill calves were especially interesting because they were almost immediately visible. The drug was said to have miraculous cures of extremely ill animals, so as to become the product of choice for the treatment of clinically ill cattle. Dosage rate of 10 to 25 mg per kg body weight for five days was seen to be effective. Treatment with Amprolium at 145 mg per kg for 21 days was found to be very effective against experimental infections of *E. bovis.*

Amprolium was used @ 20 mg per kg body weight for 5 to 7 days orally in the therapy of experimental coccidiosis in buffaloes by Khahra *et al.,* (1983).

Sanyal *et al.,* (1985) studied the chemotherapeutic efficacy of Amprolium in comparison with other anticoccidial drugs in buffaloes experimentally infected with *E. bareillyi* oocysts. Amprolium (Amprolsol 20 per cent soluble powder) given orally at the rate of 10 mg per kg body weight for 10 consecutive days post

infection revealed only mild lesions and few oocysts in the treated calves and they gained weight. The histologic findings proved that the drug was effective against schizonts and early gamonts.

Radostits *et al.*, (1994) used Amprolium against coccidiosis in calves @ 10 mg per kg body weight twice daily for five days.

Lindsay and Blagburn (1995) administered Amprolium acting on the first generation schizonts, sexual stages and the sporulating oocysts in the feed/ drinking water @ 5 mg per kg body weight for 21 days to prevent coccidiosis or @ 10 mg per kg body weight for 21 days to treat coccidiosis caused by *E. bovis* or *E. zuernii*.

2.4.3 Salinomycin

Salinomycin, a polyether antibiotic was first used in the treatment of experimental coccidiosis by *E. bovis* in calves by Benz and Ernst (1979) by conducting three experimental trials. The first experiment consisted of dosing 18 animals with 0.33, 0.66 and 1 mg per kg body weight orally, starting 2 to 3 days prior to *E. bovis* inoculation and continuing for

21 days post inoculation. In the second experiment, 15 calves were dosed @ 0.5, 1.0 and 2.0 mg per kg body weight. In the third experiment, seven calves were given Salinomycin at the rate of 2.0 mg per kg. Best results with reduction in the prevalence of severe diarrhoea, absence of blood in faeces and reduced oocyst count could be obtained with the daily drug administration @ 2 mg per kg. Salinomycin was seen to be active apparently against the first asexual generation of *E. bovis* which develops 8 to 12 days post inoculation. It was interpreted that the drug was coccidiocidal rather than coccidiostatic.

Based on the above findings, McDoughald (1982) also reported the therapeutic use of Salinomycin @ 2 mg per kg body weight for 21 days.

Salinomycin was also found to be effective in controlling coccidiosis in a farm, Nanjing Agricultural University, China, by Xui-Kui-Wu *et al.* (1995).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Prevalence

Data on the prevalence of coccidial infections in cattle belonging to different age groups were collected from suspected cases brought to the University Veterinary Hospitals (Mannuthy and Kokkalai), including ambulatory units at Cherumkuzhy and Valakkavu and also from localities in and around Thrissur. Faecal samples were screened at University Livestock Farm and Buffalo breeding station, Mannuthy and also University farm, Pattambi. Studies related to the effect of season, area, sex and age on the prevalence of coccidiosis were made round the year, from June 1999 to May 2000.

3.2. Diagnosis

3.2.1. Clinical signs and history

The animals were suspected for being infected with *Eimeria* species based on the manifestation of characteristic signs like persistent diarrhoea with traces of blood and mucus, accompanied by listlessness, weakness, rough hair coat, soiled hind quarters etc. (Ernst and Benz, 1981). History of the clinical illness of the animals was also taken which helped to understand the chronic nature of the disease and to

interpret if the managemental condition in a particular household or farm were conducive for the development of the infection.

3.2.2 Microscopical examination of the faecal samples

The faecal samples were screened for the presence of oocysts by concentration methods viz., sedimentation technique and floatation technique using Sheather's sucrose solution as the floatation fluid (Hammond and Long, 1973). Sheather's sucrose solution was prepared by adding 500 g of sucrose to 320 ml of water. This mixture was boiled gently, with frequent stirring, until the solution was clear. The solution was allowed to cool and 6.5 g of melted phenol was added as a preservative. Sheather's sucrose solution had the advantage that the solution evaporated rather slowly and did not distort the oocyst as some salt solutions did (Ray, 1953).

3.3. Identification of species

The identification of *Eimeria sp.* was done on the basis of sporulation time and oocyst morphology.

3.3.1. Sporulation

Culture of coccidian oocysts for sporulation to find out the sporulation time was carried out. A concentrated suspension of oocysts was mixed with 2.5 per cent potassium dichromate solution in petridishes at room temperature in shallow layers. Drying was avoided by constantly adding potassium dichromate solution and the mixture was aerated at regular intervals using glass pipettes to aid sporulation. It was daily examined microscopically to judge the progress of sporulation. The time required for the sporulation of oocysts was noted.

3.3.2 Morphology and micrometry

The morphological details of the sporulated and unsporulated oocysts were studied under high power as well as oil immersion objectives. The features useful in the identification of unsporulated oocysts included the size, shape, colour and texture of oocyst wall and the presence or absence of a micropyle. The sporulated oocysts were examined for the sporocyst and sporozoite shapes, presence or absence of steida body, oocystic residuum, sporocystic residuum and polar body (Pellerdy, 1965 and Mandal, 1980).

The size of the oocysts and structure like sporocysts and sporozoites were measured by micrometry. Measurement of 20 to 50 oocysts were taken and the average was determined (Bowman, 1995).

3.4 Analysis of blood parameters

The haematological values including blood haemoglobin, packed cell volume, total leukocyte count, differential count and serum protein fractions were studied in 10 clinically infected animals. The haematological and serum values of 10 healthy animals were also taken as control.

3.4.1 Haemoglobin

Haemoglobin was estimated by acid haematin method, using Sahli's haemoglobinometer (Benjamin, 1998).

3.4.2. Packed cell volume

Packed cell volume was determined by the Wintrobe method, using a special Wintrobe pipette (Wintrobe, 1974).

3.4.3 Total leukocyte count

The total leukocyte count was determined by the method described by Benjamin (1998).

3.4.4. Differential leukocyte count

The differential leukocyte count was assessed as per the method described by Benjamin (1998).

3.4.5 Serum proteins

3.4.5.1 Total protein

The total protein concentration was determined colorimetrically by Biuret method. The reagent and standard were provided in a kit supplied by Agappe diagnostics. This colorimetric test was carried out using Photometer 5010 (Gomall, 1949).

3.4.5.2 Albumin

Albumin concentration of the samples were determined by Bromo cresol- green method (Doumasa, 1971) using the kit from Agappe diagnostics. The change in colour was read and values recorded using Photometer 5010.

3.4.5.3 Globulin

The globulin concentrations were derived from the known total protein and albumin values.

3.5. Evaluation of anticoccidial drugs

The efficacy of three drugs viz., Sulphadimidine sodium, Amprolium hydrochloride and Salinomycin were evaluated in the treatment of clinical cases of bovine coccidiosis. For the study, 18 infected animals showing apparent clinical signs were divided into three groups, each group consisting of six animals. The efficacy of drugs was evaluated based on the reduction in the number of oocysts on the seventh day after treatment in the case of Sulphadimidine and Amprolium and on the 21st day after treatment in the case of Salinomycin.

3.5.1. Sulphadimidine sodium

The first group of animals was treated with Sulphadimidine sodium boluses (Sarabhai) @ 125 mg per kg body weight. The drug was administered orally for three consecutive days.

3.5.2 Amprolium hydrochloride

The second group was administered Amprolium hydrochloride (Amprolsol, Glaxo) @ 20 mg per kg body weight orally for five days.

3.5.3. Salinomycin

The third group was given Salinomycin sodium (Sacox, Hoechst Roussel Vet) at the rate of 2 mg per kg orally for 21 days.

3.6. Determination of oocysts per gram (OPG)

The number of oocyst per gram of faeces was recorded as per the method described by Bowman (1995) before treatment and on the seventh day after treatment, in case of Sulphadimidine and Amprolium and on the 21st day after treatment in the case of Salinomycin.

RESULTS

4. RESULTS

4.1. Prevalence

Out of the total number of 1821 cattle screened during the period from June 1999 to May 2000, from hospitals at Kokkalai, Mannuthy, Cherumkuzhy and Valakkavu, Pattambi cattle farm, University livestock farm and buffalo breeding station, Mannuthy, 40 animals were found to be infected with coccidiosis. The prevalence rate thus found was 2.20 per cent. Month wise, the maximum prevalence rate was observed in August 1999 (5.84 per cent). Lowest prevalence rates were encountered during June 1999 and December 1999 (0.74 per cent). The month wise prevalence is presented in Table 1 and depicted in Fig.s 1 and 2. The figures revealed a steep peak for the month of August 1999 and short peaks for June and December 1999.

The season wise prevalence of coccidiosis is depicted in Table 2 and Fig. 3. The infection rate was found to be higher during the cold-wet, South-West Monsoon season during which an average rainfall of 527.8 cm and temperature of 26.1°C was recorded. Prevalence of

Table 1. Month wise prevalence of coccidiosis

Month	Number examined	Number positive	Per cent positive
June '99	135	1	0.74
July '99	104	5	4.81
August '99	154	9	5.84
September '99	135	4	2.96
October '99	121	3	2.48
November '99	137	2	1.46
December '99	271	2	0.74
January 2000	138	5	3.62
February '00	175	2	1.14
March '00	157	2	1.27
April '00	147	2	1.36
May '00	147	3	1.36
Total	1821	40	2.2

Table 2 Season wise prevalence of coccidiosis

Season	Month	Number of samples examined	Number positive	Per cent positive
Cold wet South-West Monsoon (heavy rainfall)	June - August	393	15	3.82
Warm wet North-East Monsoon (low rainfall)	September - November	393	9	2.29
Dry	December - May	1035	16	1.54

infection was seen to be reduced during the summer season when an average rainfall of 31.61 cm and temperature of 28.46°C was recorded (Department of Meteorology, Kerala Agricultural University, Vellanikkara, Thrissur).

Out of 40 positive cases, female animals (72.5 per cent) were found to be affected more than the males (27.5 per cent). The prevalence was higher in animals less than one year and that was 65 per cent (Table 3 and Fig. 4). No male animals above one year could be examined since samples were not obtained.

Area wise, the maximum prevalence rate was observed at Valakkavu Ambulatory Unit (13.63 per cent) and the minimum (zero per cent) at the University livestock farm, Mannuthy (Table 4 and Fig. 5)

4.2. Diagnosis of infection

4.2.1. General health status and clinical signs of infected animals

Infection with a few oocysts produced mild or no apparent signs. In mild cases, the animals revealed signs like diarrhoea, weakness and anorexia. Streaks of blood

Table 3. Sex wise prevalence of coccidiosis in cattle from birth to one year and above one year

Age	Number of males	Per cent	Number of females	Per cent	Total number	Per cent
Up to one year	11	42.3	15	57.6	26	65
Above one year	-	-	14	100.0	14	35
Total	11	27.5	29	72.5	40	100

Table 4. Prevalence of bovine coccidiosis at different places

	Number screened	Number positive	Prevalence per cent
Mannuthy Hospital	765	8	1.04
Kokkalai Hospital	769	21	2.73
Cherumkuzhy	70	6	8.57
Valakkavu	22	3	13.63
Pattambi Cattle Farm	21	1	4.76
Buffalo Breeding Station, Mannuthy	10	1	10.00
University Livestock Farm, Mannuthy	164	0	0
Total	1821	40	2.2

Figure 1 **Monthwise prevalence of coccidiosis**

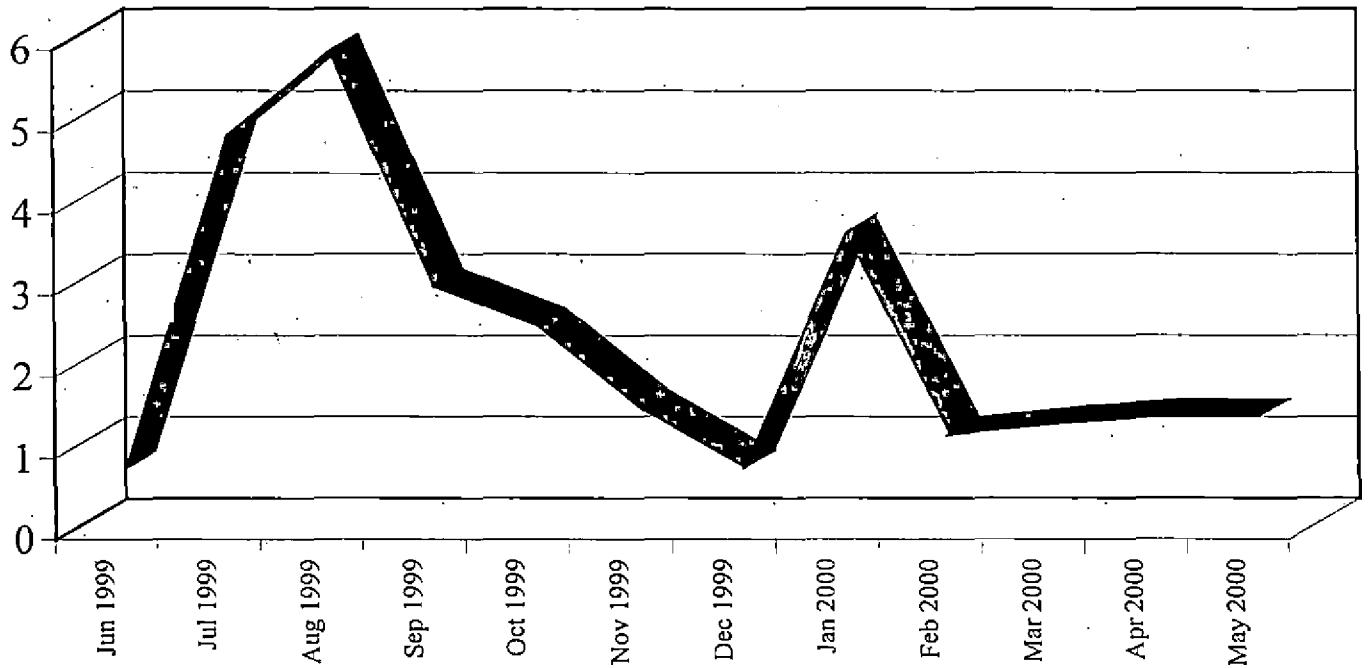


Figure 2 **Monthwise prevalence of coccidiosis**

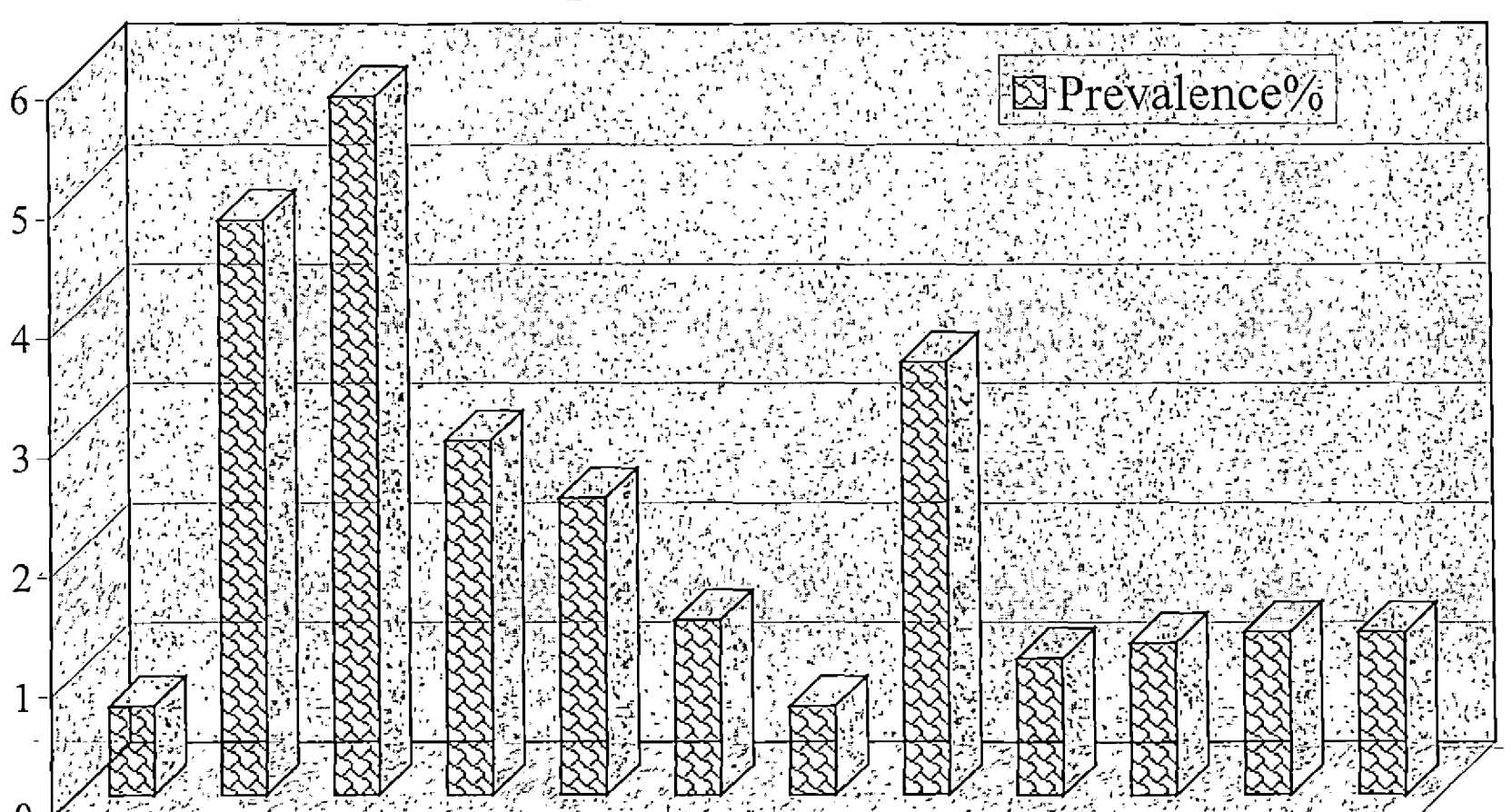


Figure 3 **Seasonwise prevalence of coccidiosis**

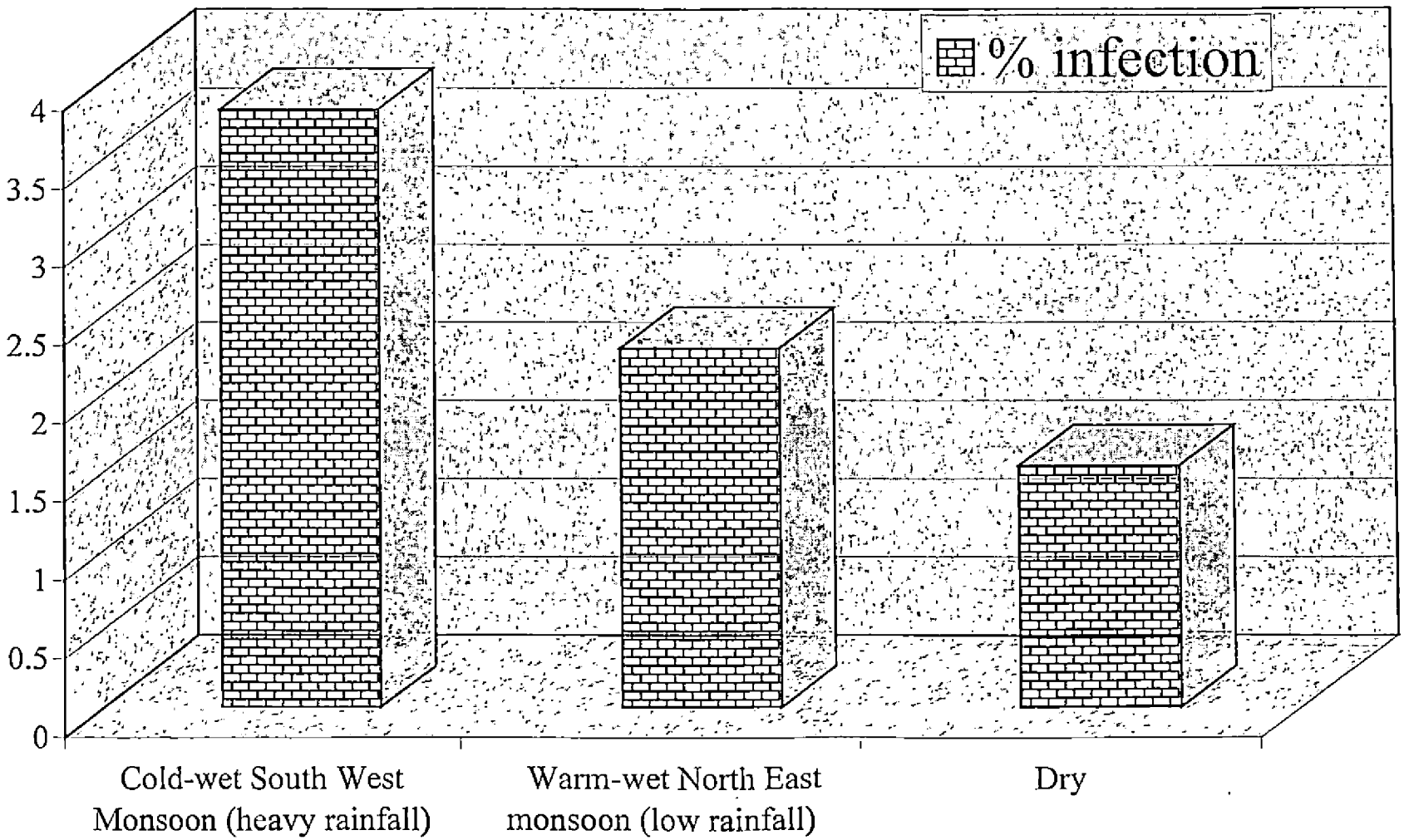


Figure 4. **Sexwise prevalence of coccidiosis in cattle from birth to 1 year and above 1 year**

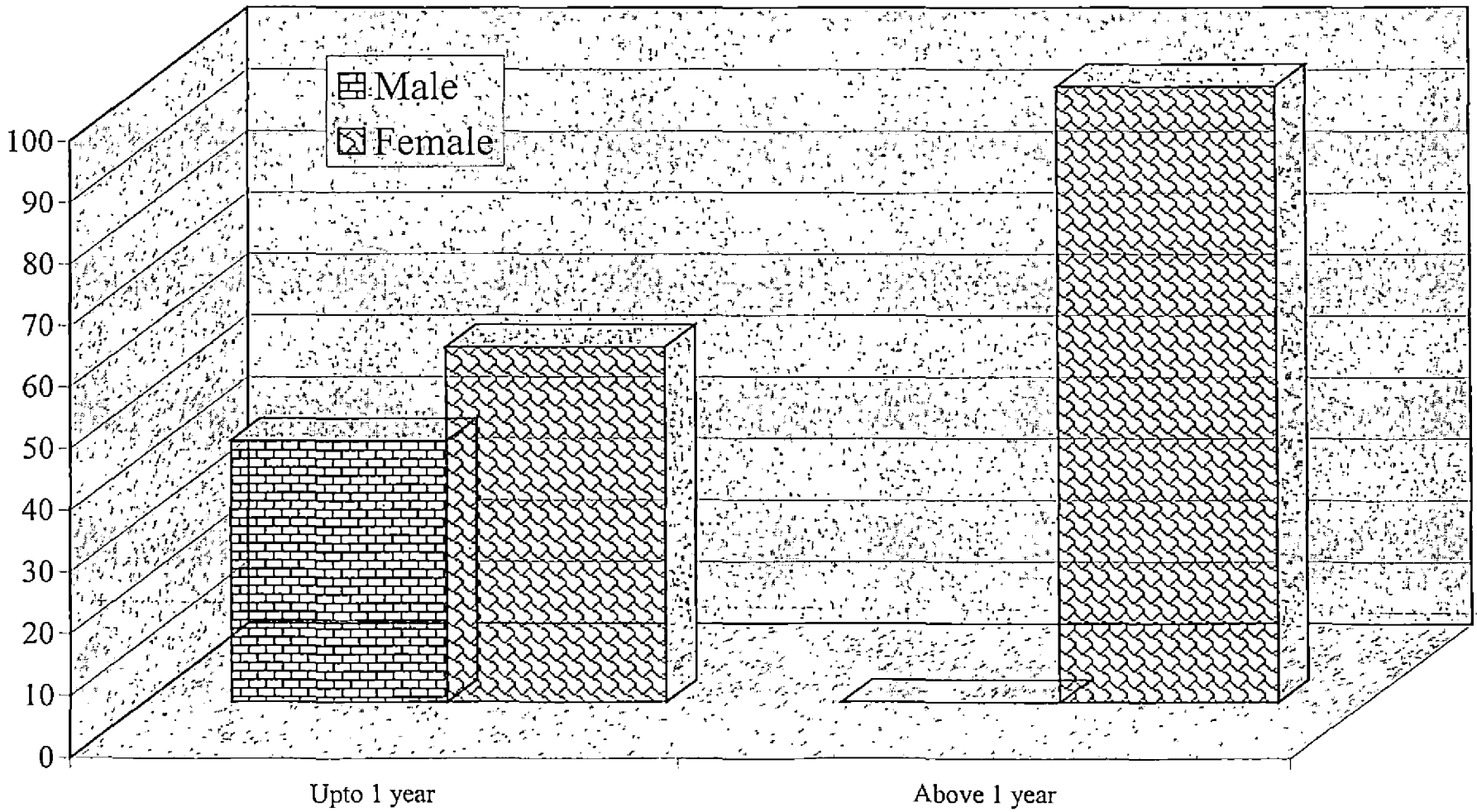
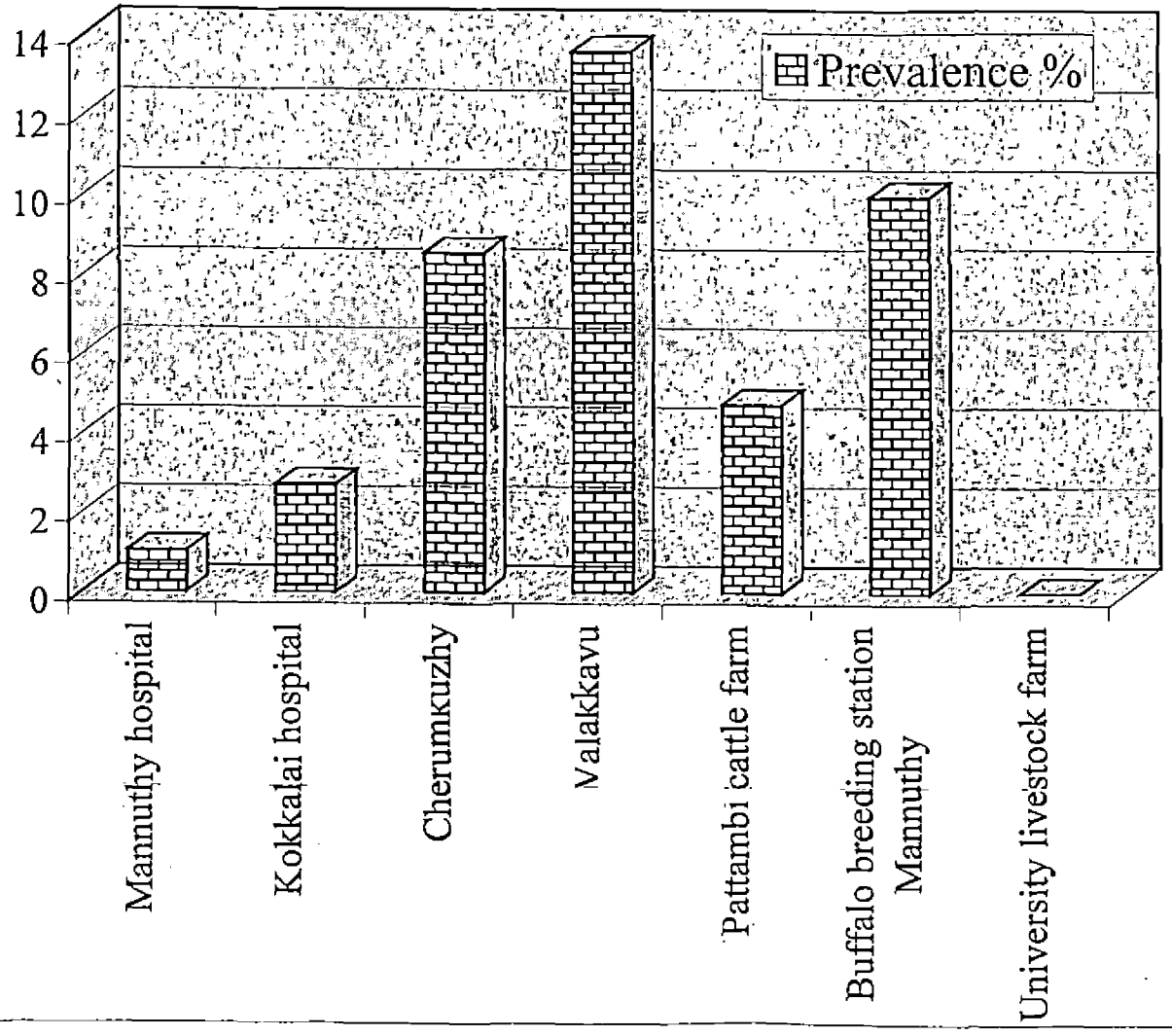


Figure 5 **Prevalence of bovine coccidiosis at different places**



and mucus were occasionally found in the faeces. In such cases, the number of oocysts per gram (OPG) ranged from 1000 to 8000. In acute infections, very large numbers of oocysts were passed in the dung and OPG was found to be as high as 7,00,000. The animals showed signs of severe diarrhoea, foul smelling and blood tinged. They were also found to have rough hair coat, dehydration, emaciation, tenesmus and soiled hind quarters.

4.2.2 Microscopic examination

The diagnosis was carried out by microscopic examination of the dung samples, which revealed oocysts of different *Eimeria* species. Concentration methods like floatation and sedimentation techniques were employed to concentrate oocysts in faeces. Floatation method using Sheather's sucrose solution had the advantage that the solution evaporated slowly and did not distort the oocysts. Direct method of faecal examination was helpful especially in heavily infected cases. The intensity of infection was determined using oocyst counts.

4.3. Identification of various species of *Eimeria*

The oocysts obtained from the faecal samples of infected cattle were identified to species, based on the morphologcal characters and sporulation time. It was possible to identify the species on the basis of unsporulated oocyst itself, owing to their distinct shapes. But the sporulated oocysts were more useful in differential diagnosis because they had definite morphological characters compared to the unsporulated forms. The per cent distribution of *Eimeria* species observed to infect the animals (Fig. 6) was as follows - *E. zuernii* (55 per cent), *E. bovis* (35 per cent), *E. ellipsoidalis* (60 per cent), *E. subspherica* and *E. wyomingensis* (10 per cent each), *E. cylindrica*, *E. bareillyi* and *E. brasiliensis* (5 per cent each). The different species of *Eimeria* with distinct characteristics identified are furnished in Table 5.

4.3.1. ***Eimeria bovis*** (Plate I, Fig.s 1 and 2)

The oocysts belonging to this species were broadly ovoid with a narrower micropylar end. Oocyst wall was smooth, double layered and light yellowish brown in colour. Micropyle was present and micropylar cap absent.

Oocystic residuum and polar cap were also absent. Sporozoites were elongate and banana shaped. The oocyst measured 24.71µ long and 17.6µ wide. The oocystic wall was 1.5µ thick and the sporocyst measured 14.12µ by 7.06µ. Sporulation time was found to be three to four days.

4.3.2. *Eimeria zuernii* (Plate I, Fig.s 3 and 4)

Oocysts were spherical to ovoidal in shape. Oocyst wall was smooth and double layered. Structures like micropyle, micropylar cap, oocystic residuum and polar granule were absent. Sporocysts were slightly elongate and oval in shape. Sporozoites were stumpy in appearance. Average dimensions observed were oocyst 14.12 µ by 14.12 µ, oocyst wall 1.5µ thick and sporocyst 10.6µ by 3.53µ. Sporulation time was three to four days.

4.3.3. *Eimeria subspherica* (Plate I, Fig. 5)

The oocysts were colourless and smaller compared to *E. zuernii*, subspherical in shape. The oocyst wall was smooth and double layered. Micropyle, oocystic residuum and polar granules were absent. The sporocysts were elongate - ovoid. The oocyst measured an average 10.5µ by

8 μ. The oocyst wall was 1.0μ thick. Oocysts failed to sporulate and hence sporulation time could not be noted.

4.3.4. *Eimeria ellipsoidalis* (Plate I, Fig.s 6 and 7)

The oocysts had an ellipsoidal shape. Oocyst wall was double layered. Micropyle was present as a slight thin area at one pole. Micropylar cap, oocystic residuum and polar granule were absent. Sporocysts were elongate and sporozoites banana shaped. Mean dimensions of oocysts were 17.65 μ by 14.12 μ, oocyst wall 1.3μ thick, sporocyst 10.59μ to 3.5μ and sporulation took three to four days.

4.3.5. *Eimeria cylindrica* (Plate I, Fig. 8)

The oocysts were cylindrical, with a two layered oocyst wall. Micropyle and micropylar cap were absent along with oocystic residuum and polar granule. Sporocysts and sporozoites were elongate. Average size- oocyst- 18.5 μ by 10.59 μ, oocyst wall- 1μ thick and sporocyst 16 μ by 4μ. Sporulation time was three days.

4.3.6. *Eimeria bareillyi* (Plate I, Fig. 9)

Oocysts had a typical pyriform shape with a narrow anterior end. A small granular body was seen lying below the micropyle. The oocyst wall was double layered and yellowish brown. Oocystic residuum and polar granules were absent. Sporocysts were elongate and wide. Mean dimensions: Oocyst- 34.29µ by 22.18µ, oocyst wall- 1.3µ thick. The oocyst of this species failed to sporulate.

4.3.7. *Eimeria brasiliensis* (Plate I, Fig. 10)

The oocysts were ovoidal with a double contoured oocyst wall. Micropyle and micropylar cap were present. This was a distinct feature for identification. Oocysts had a mean size of 39µ by 27µ and the oocyst wall was 1µ thick. Sporulation time could not be detected.

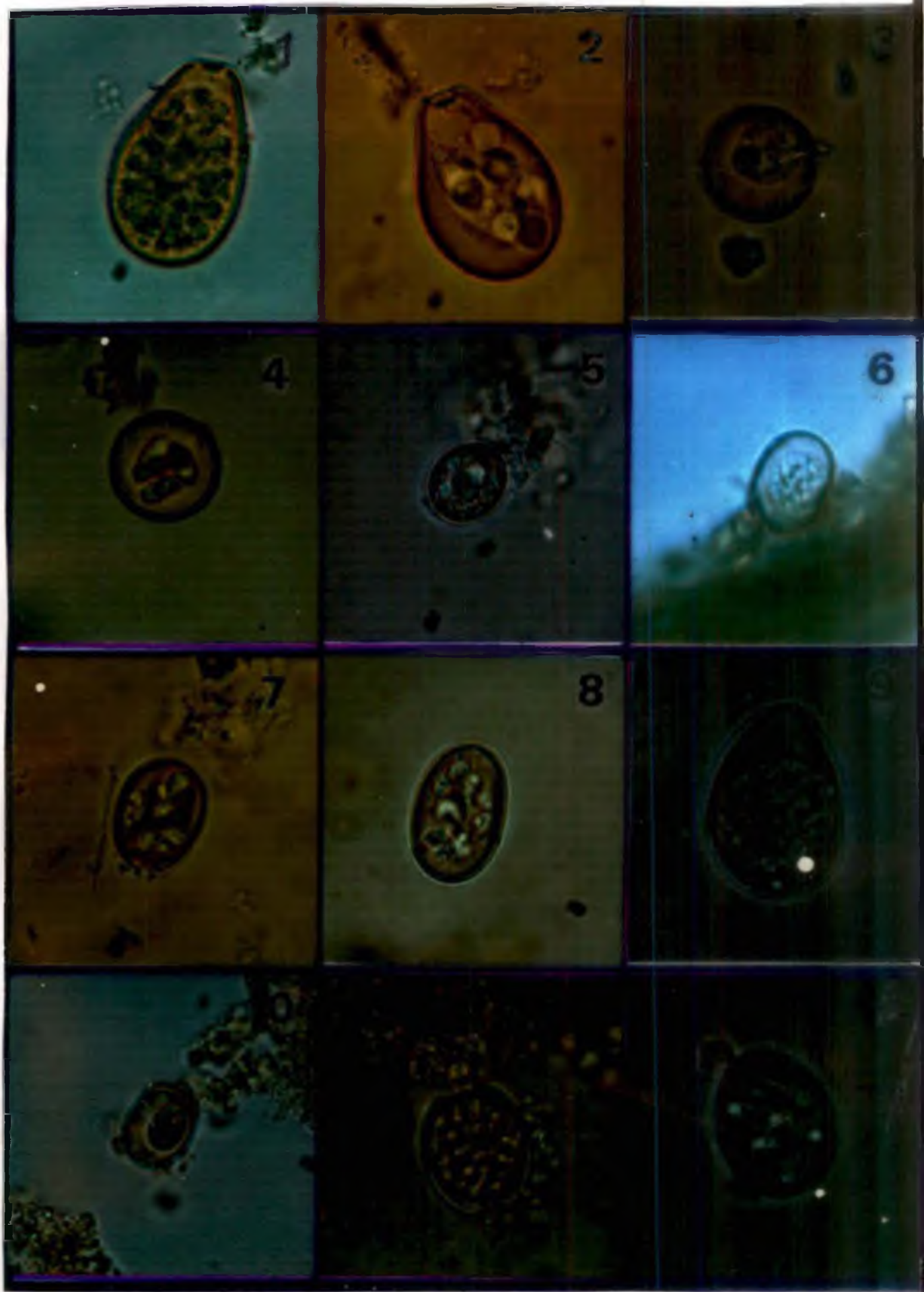
4.3.8. *Eimeria wyomingensis* (Plate I, Fig.s 11 and 12)

The oocysts were distinctly egg shaped with a double layered wall. Micropyle was present, but oocystic residuum and polar granule were absent. In the unsporulated oocyst, the sporont was seen to completely fill the cyst. The sporozoites were elongate with

Plate I

Figures

1. Unsporulated oocyst of *E. bovis* (x 1000)
2. Sporulated oocyst of *E. bovis* (x 1000)
3. Unsporulated oocyst of *E. zuernii* (x 1000)
4. Sporulated oocyst of *E.zuernii* (x 1000)
5. Unsporulated oocyst of *E. subspherica* (x 1000)
6. Unsporulated oocyst of *E.ellipsoidalis* (x 1000)
7. Sporulated oocyst of *E. ellipsoidalis* (x 1000)
8. Sporulated oocyst of *E. cylindrica* (x 1000)
9. Unsporulated oocyst of *E. bareillyi* (x 1000)
10. Unsporulated oocyst of *E. brasiliensis* (x 400)
11. Unsporulated oocyst of *E. wyomingensis* (x 1000)
12. Sporulated oocyst of *E. wyomingensis* (x 1000)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refractile globules. Average dimensions- Oocyst- 35.5µ by 23.8µ oocyst wall- 2.4µ thick, sporocyst- 20 µ by 8.6µ and sporulation time- five days.

4.4. Clinical pathology

4.4.1 Haematology

Haematological parameters were recorded in 10 infected cases and also in a control group of another 10 healthy non-infected animals. Alterations were significant in severely infected animals and less so in the mildly infected ones. The haematological values in the infected cases are furnished in Table 6.

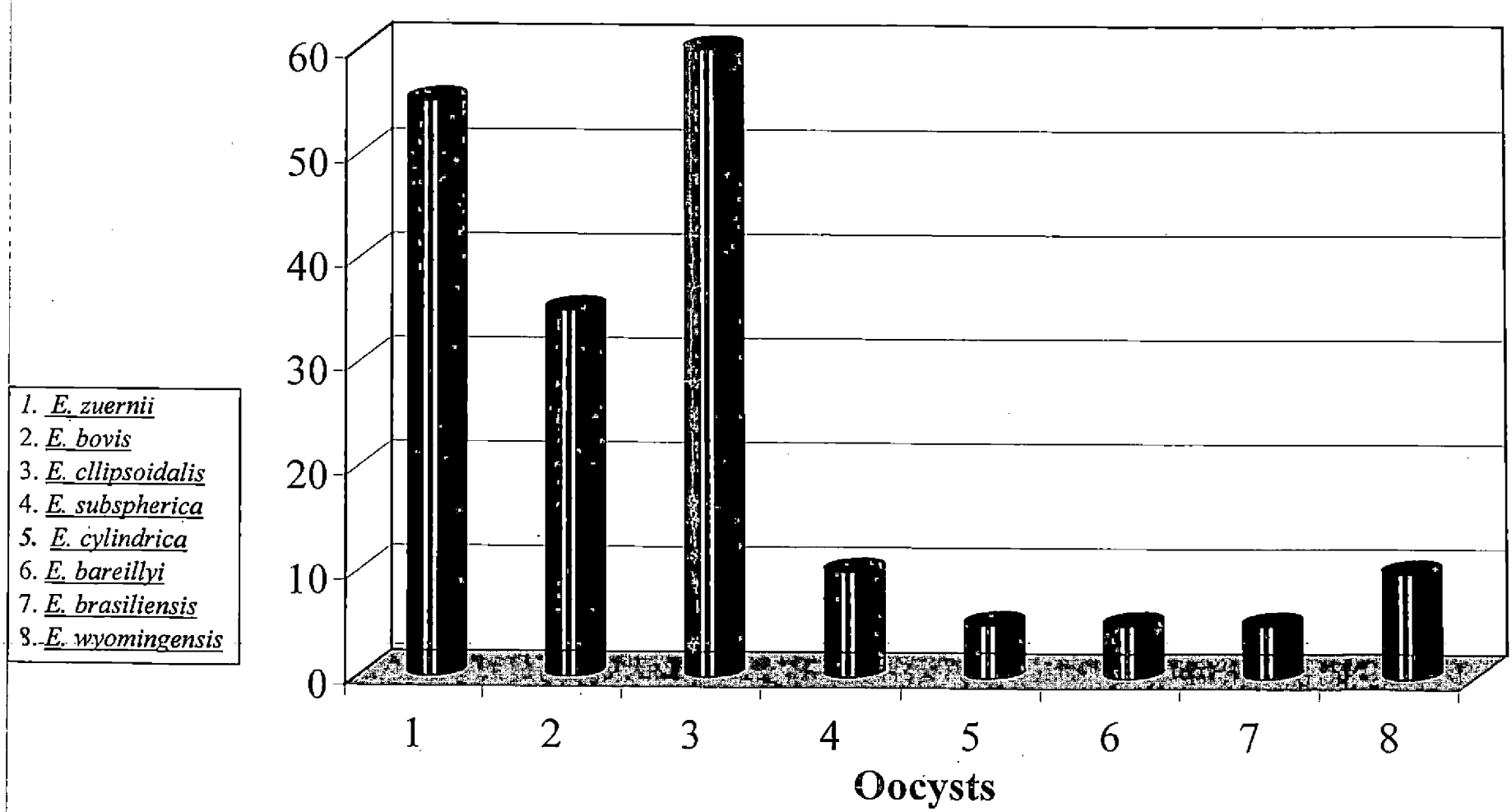
4.4.1.1 Haemoglobin

A reduction in the percentage of haemoglobin was noticed in the infected group, which was significant ($p \leq 0.05$) in the heavily infected group. The mean values were 12.49 ± 2.34, 11.44 ± 1.67 and 8.50 ± 1.66 in the non-infected, mildly infected and severely infected groups respectively. The change in haemoglobin value was nonsignificant in the mildly infected group.

Table 5 Morphology, micrometry and per cent distribution of oocysts of different species of *Eimeria*

Species	Shape and mean size of oocyst (µm)	Micro pyle	Micro-pylar cap	Oocystic residuum	Sporocyst mean size (µm)	Shape of sporo zoites	Oocyst wall mean thick ness (µm)	Sporulat ion time (days)	Oocysts per cent distrib ution
E.bovis	Broadly ovoid,narrow micropylar end, 24.71 by 17.6	Present	Absent	Absent	14.12 by 7.06	Elongate, Banana shaped	1.5	3 - 4	35 %
E.zuernii	Spherical and ovoidal, 14.12 by 14.12	Absent	Absent	Absent	10.6 by 3.53	Stumpy	1.5	3 - 4	55 %
E.subspherica	Subspherical 10.5 by 8µ	Absent	Absent	Absent	-	-	1.0	Not recorded	10 %
E.ellipsoidalis	Ellpsoidal, 17.65 by 14.12	Present	Absent	Absent	10.59 by 3.5	Banana shaped	1.3	3 -4	60 %
E.cylindrica	Cylindrical, 18.5 by 10.59	Absent	Absent	Absent	16 by 4	Elongate	1.0	3	5 %
E.bareillyi	Pyriform, 34.29 by 22.18	Present	Absent	Absent	-	-	1.3	Not recorded	5 %
E brasiliensis	Ovoidal, 39 by 27	Present	Present	Absent	-	-	1.0	Not recorded	5 %
E.wyomingensis	Egg shaped 35.5 by 23.8	Present	Absent	Absent	20 by 8.6	Elongate with refractile globules	2.4	5	10 %

Figure 6 Per cent distribution of oocysts of different species of bovine coccidia



4.4.1.2 Packed cell volume

The packed cell volume per cent was low in the infected animals compared to the non-infected ones, the mean values being 32.55 ± 4.91, 25.40 ± 6.02 and 28.64 ± 10.68 in the infected, mildly infected and severely infected groups. The difference was found to be significant ($p \leq 0.05$) in the mildly infected group.

4.4.1.3. Total leukocyte count

A nonsignificant reduction in the total leukocyte count was observed in the mild and severe group in comparison with the non-infected group. The mean values were 22615.00 ± 3875.33, 10980.00 ± 2430.17 and 12510.00 ± 6964.40 in the non-infected, mild and severe groups respectively.

4.4.1.4. Differential leukocyte count

Neutrophil

Mild neutrophilia was observed in the infected group though nonsignificant in the mild group. The non-infected, mildly infected and severely infected groups

had the mean values 25.0 ± 6.92, 41.20 ± 13.88 and 36.60 ± 8.73 respectively.

Lymphocyte

A significant reduction ($p \leq 0.05$) in the percentage of lymphocytes was noticed in both the mild and severe groups when compared to the infected group. Mean values were 66.40 ± 9.54, 46.00 ± 13.13 and 49.60 ± 13.90 in non-infected, mild and severe groups respectively.

Eosinophil

The respective mean values of eosinophils were 5.80 ± 3.97, 1.80 ± 2.17 and 4.80 ± 8.53 for non-infected, mildly infected and severely infected cases. The change was found to be of no significance.

Monocyte

A highly significant monocytosis ($p \leq 0.01$) was observed in the mildly infected group, compared to the severely infected group. The monocyte values were 1.20 ± 1.47, 13.00 ± 2.55 and 9.00 ± 8.43 respectively for uninfected, mild and severe groups.

Table 6. Haematology of healthy and infected cattle

Category	Healthy	Infected			
		Mild (OPG- 1000-8000)		Severe (OPG 8000 to 7,00,000)	
Number of animals examined	10	5		5	
Parameters	Mean ± SE	Mean ± SE	't' value	Mean ± SE	't' value
Haemoglobin (g/ 100 ml)	12.49 ± 2.34	11.44 ± 1.67	0.89^{NS}	8.50 ± 1.66	3.38*
Packed cell volume (%)	32.55 ± 4.91	25.40 ± 6.02	2.47*	28.64 ± 10.68	0.78^{NS}
Total leukocyte count (millions/ cu.mm)	22615.00 ± 3875.33	10980.00 ± 2430.17	0.95^{NS}	12510.00 ± 6964.40	0.80^{NS}
Neutrophil %	25.60 ± 6.92	41.20 ± 13.88	2.37^{NS}	36.60 ± 8.73	2.67*
Lymphocyte %	66.40 ± 9.54	46.00 ± 13.13	3.46*	49.60 ± 13.90	2.77*
Eosinophil %	5.80 ± 3.97	1.80 ± 2.17	2.08^{NS}	4.80 ± 8.53	0.25^{NS}
Monocyte %	1.20 ± 1.47	13.00 ± 2.55	11.50**	9.00 ± 8.43	2.05^{NS}
Basophil %	-	-	-	-	-

NS- Non significant
*- Significant at five per cent ($p< 0.05$)
**- Significant at one per cent ($p\leq 0.01$)

Basophil count

Basophil values were not observed in any of the groups.

4.4.2. Serum protein fraction assays

The serum total protein values were significantly lower ($p \leq 0.05$) in the infected animals (5.97 ± 1.77) than that of the non-infected animals (8.11 ± 2.16). The albumin values were observed to be significantly greater ($p \leq 0.05$) in the infected cattle (3.37 ± 1.59) compared to the non-infected ones (3.26 ± 0.71). It was found that the globulin values of the non-infected group (4.85 ± 2.13) were slightly greater than the infected group (2.96 ± 2.22), but not significant (Table 7 and Fig.s 7,8). Albumin / globulin ratio was found to be 0.77 ± 0.08 in the case of non-infected animals and 1.85 ± 1.44 in the case of infected animals. The change was found to be significant.

Table 7 Serum protein fractions of non infected and infected cattle

Fraction	Non-infected		Infected		't' value
	No of animals	Mean ± SE	No of animals	Mean ± SE	
Total protein (g/dl)	10	8.11 ± 2.16	10	5.97 ± 1.77	2.42*
Albumin (g/dl)	10	3.26 ± 0.71	10	3.37 ± 1.59	0.19*
Globulin (g/dl)	10	4.85 ± 2.13	10	2.96 ± 2.22	1.94^{NS}
Albumin/ globulin ratio	10	0.77 ± 0.08	10	1.85 ± 1.44	2.34*

NS- Non significant,

*- Significant at five per cent ($p \leq 0.05$)

4.5. Treatment with anticoccidial drugs (Table 8)

4.5.1 Sulphadimidine sodium

Sulphadimidine sodium at a dose rate of 125 mg per kg body weight was given orally to animals that showed apparent clinical signs of coccidiosis for three

Figure 7 **Total protein, albumin and globulin in non-infected and infected animals**

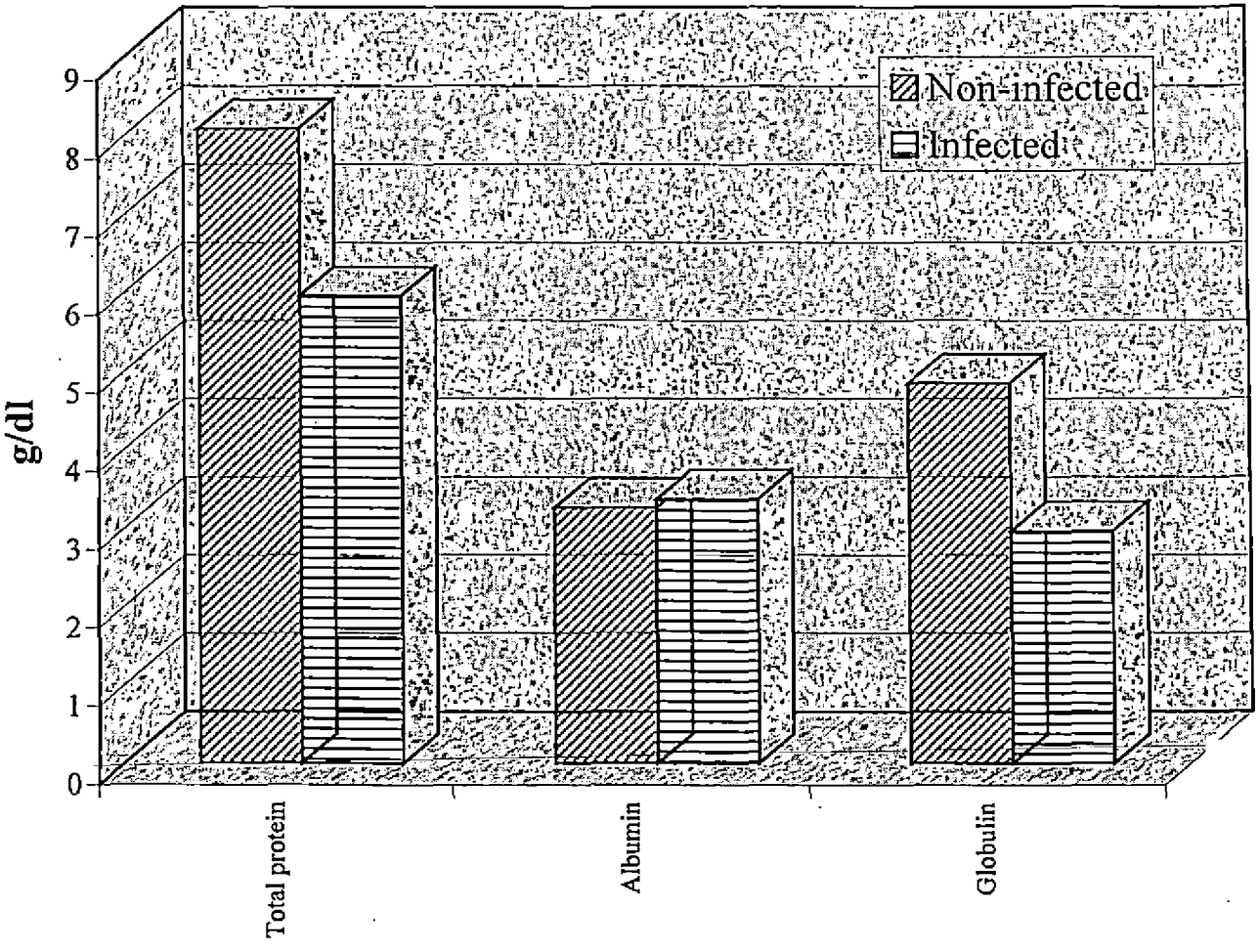
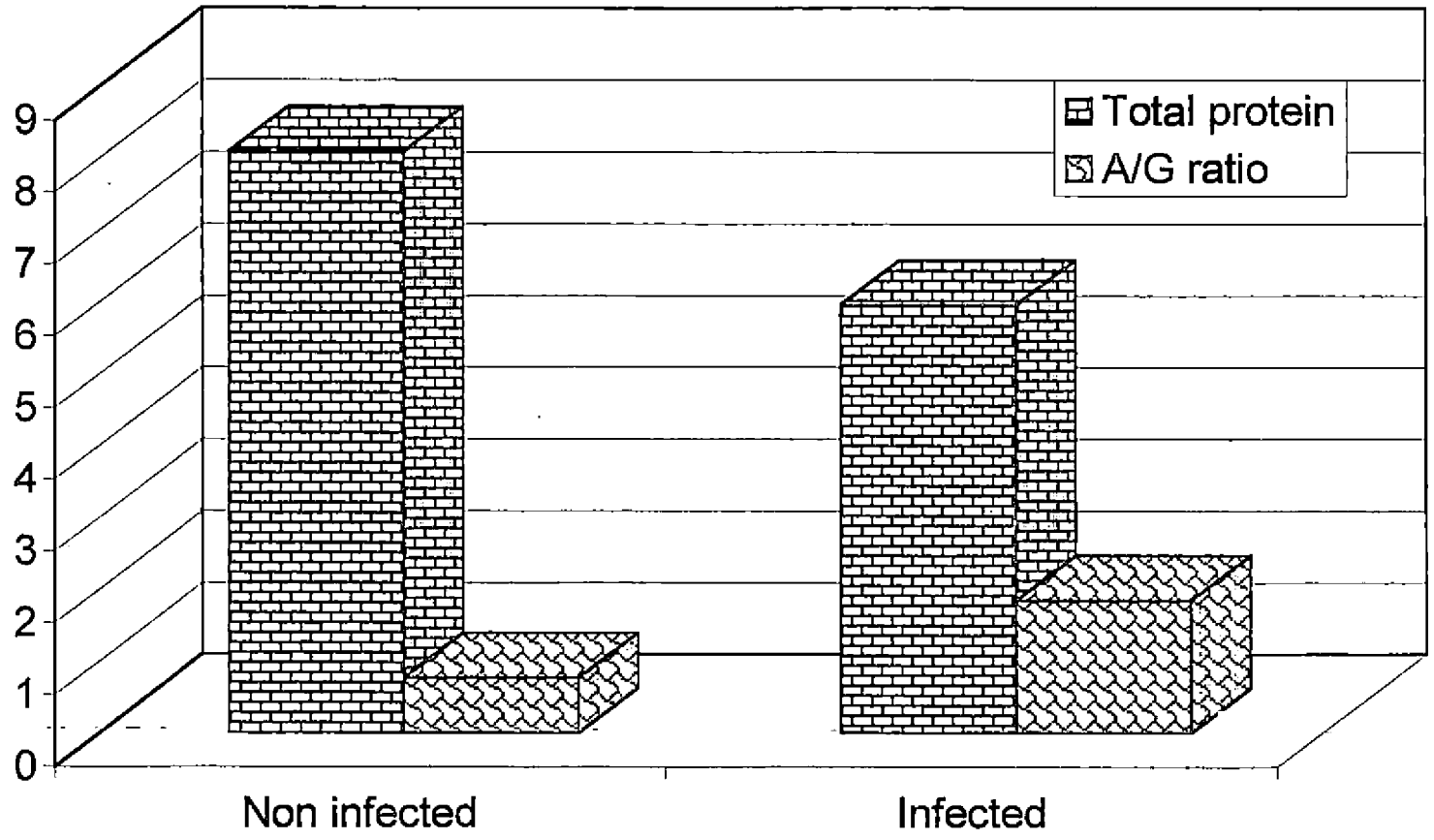


Figure 8 Total protein and albumin / globulin ratio (A / G ratio) in non-infected and infected cattle.



consecutive days. Out of the six animals treated with this drug, two animals had only mild infection. The oocyst count reduced immensely seven days after treatment when compared to the first day. The efficacy was evaluated as 99.9 per cent in the mildly infected animals and 98.4 per cent in the severely infected ones. The clinical signs like diarrhoea, blood and mucus streaks in the faeces etc., reduced markedly with a single dose and completely disappeared after treatment.

4.5.2. Amprolium hydrochloride

The treatment with Amprolium hydrochloride (Amprolsol) was carried out at a dose of 20 mg per kg body weight for five days and was found to be very effective in eliminating the infection. Four severely and two mildly infected animals were treated with amprolium. The drug showed an efficacy of 100 per cent in mild infections and 99.9 per cent in heavy infections. In most of the cases, the oocysts in faecal samples disappeared completely after a week. The clinical signs gradually reduced and the animals regained their appetite and health.

4.5.3. Salinomycin

The treatment carried out with salinomycin consisted of dosing the animals at the rate of 2 mg per kg body weight for a period of 21 days, orally. This drug was tried on three mildly infected and another three severely infected animals. The oocysts were completely obliterated in four infected cases. Thus recovery was satisfactory. The clearance rates were 99.96 per cent and 99.98 per cent respectively in the mild and severe groups.

4.5.4. Comparison between Sulphadimidine sodium, Amprolium hydrochloride and Salinomycin administered against bovine coccidiosis (Table 8)

The mean efficacies (clearance rates) of Sulphadimidine, Amprolium and Salinomycin were observed to be 99.15 ± 0.78, 99.58 ± 0.41 and 99.98 ± 0.99 respectively. 'ANOVA' showed no significant difference between the clearance rates of these three drugs. Thus a comparison between them also proved to be insignificant.

Table 8. Efficacy of anticoccidials against coccidiosis in cattle.

Drug	Dose	Intensity	Number of animals treated	'zero' day Mean oocyst count	7th day Mean oocyst count	21st day Mean oocyst count	Mean clearance efficacy %	Clearance efficacy Mean ± SE
Sulphadimidine sodium	125 mg/ kg body weight orally for 3 days	Mild	2	6000	6	-	99.90	99.15 ± 0.78
		Severe	4	16025	255.5		98.40	
Amprolium hydrochloride	20 mg / kg body weight orally for five days	Mild	2	1750	0	-	100	99.58 ± 0.41
		Severe	4	231250	132.5		99.9	
Salinomycin	2 mg per kg body weight orally for 21 days	Mild	3	4695		1.67	99.96	99.98 ± 0.99
		Severe	3	15333.3		17.50	99.98	

DISCUSSION

5. DISCUSSION

5.1. Prevalence

Coccidia were found to be prevalent in the faecal samples of 40 animals (2.2 per cent) out of 1821 cattle screened at University hospitals Kokkalai, Mannuthy, Cherumkuzhy and Valakkavu, Pattambi cattle farm, University livestock farm and Buffalo breeding station, Mannuthy, round the year. The prevalence of infection was observed to be highest in August 1999 (5.84 per cent). The cool and wet months of the year had a higher prevalence rate. This brings to light the fact that heavy rainfall and the subsequent humidity were extremely conducive for the precipitation of the infection, which was in accordance with the observations of Kasim *et al.*,(1985) who reported higher incidence in areas with high rainfall, humidity and intensive rearing methods.

The cold wet South-West Monsoon season also gave way for increased occurrence of the infection (3.82 per cent) which corroborated with the findings of Ershaduzzaman *et al.*,(1985).

Coccidiosis was also found to occur during the dry months of the year, though at a lower rate (1.54 per cent). This could be attributed to the poor hygiene and managemental conditions in the households and farms which aided the sporulation of oocysts. A similar observation was made by Ruiz (1973) who found coccidiosis to occur in summer, autumn and spring.

In the present study, female animals were more affected (72.5 per cent) than the males (27.5 per cent) which contradicted the findings made by Ali and Latif (1989) who reported significantly higher rate of infection in male animals. The most probable reason for the less number of male animals to be infected could be that the number of male animals maintained were less generally. Most of the male animals were disposed for slaughter at an early age due to economic constraints faced by the farmers. Besides, the number of male animals brought to the hospitals for routine check up were comparatively few.

The age wise study revealed that the age group of less than one year (65 per cent) was more affected. This is in agreement with the findings of

Sanyal *et al.*, (1985a), Chibunda *et al.*, (1997), Ali and Latif (1989), Oda and Nishida (1990) and Agnihotri (1993). The probable reason for the young animals to be more affected with the disease could be due to low immunotolerance exhibited by the young ones and also the fact that older animals became gradually resistant due to repeated exposure to coccidian infection (Ershaduzzaman, 1985). Coincidentally, it should be noted that young calves after a month of age start nibbling and picking up oocysts by their licking habits resulting in severe infection which may be aggravated by stress factors like weaning, poor feeding etc.

The disease was not uncommon in older cattle and this concurs with the reports of Oda and Nishida (1990) and Senthilvel (1995). These older animals would not have been exposed to a previous infection. The possible reason could be that, the previously unexposed older animals were more susceptible to coccidiosis.

Kasim *et al.*, (1985) and Sanyal *et al.*,(1985a) observed that areas with intensive rearing methods, excessive irrigation and humidity had a higher prevalence rate than dry-arid regions. The higher

incidence rates at Valakkavu (13.63 per cent), a village area, could be due to dampness and crowding in animal houses. Also, the flooring of cattle sheds were not always made of impervious cement, which aided the survival of oocysts. The reason for the absence of coccidiosis at the University livestock farm, Mannuthy may possibly be due to the hygienic management and preventive measures adopted.

5.2. Diagnosis of infection

In the present study, diagnosis was carried out based on the clinical signs followed by the microscopic examination of dung samples of suspected animals. Mild infection revealed an OPG of 1000 to 8000 in faecal samples and clinical signs like diarrhoea, anorexia and weakness. Blood in faeces was only occasionally found. In acute infections, the typical signs like blood tinged foul smelling diarrhoea, dehydration, emaciation, soiling of hind quarters and an OPG of 8000 to 7,00,000 were noticed.

Concentration method ie., floatation technique using Sheather's sucrose solution was useful in maintaining the structural integrity of oocysts, which

helped in diagnosis and also identification of *Eimeria* species. It was found that direct examination of faecal smear was a simple and fast method for diagnosing heavy infections as stated by Ernst and Benz (1981).

5.3. Identification of species

The species of coccidia of cattle encountered during the study were identified based on the morphology of the oocyst and sporulation time. The shape and size of the oocysts, details like presence or absence of micropyle, micropylar cap, characteristics and size of sporocysts and sporozoites were taken into consideration for speciation, as elaborated by Jeffers and Shirley (1982). It was observed that oocysts of some species failed to sporulate. This could be due to unfavourable temperature and climatic conditions or due to the storage of samples in narrow mouthed containers. Delay in setting up cultures also predisposed to the failure of sporulation.

Slight variation was observed in the micrometry of a few species obtained in this study, compared to those of earlier workers. The formation of different shapes

in the same species upon a wet mount could be the reason for variation in dimension.

The morphological characteristics of *Eimeria bovis* were more or less similar to the reports made by Mandal (1980). Similarly, *E. zuernii* identified in the study concurred with the morphological descriptions made by Mandal (1980).

The oocysts of *E. subspherica* were the smallest ones identified in the present study. The morphology of the oocyst was agreeable with the details compiled by Pellerdy (1965) and Mandal (1980). Characteristics of sporocysts and sporozoites could not be recorded.

Ellipsoidal shaped oocysts of *E. ellipsoidalis* were obtained from most of the clinical cases of coccidiosis encountered. The morphological details observed, accorded with those by Mandal (1980).

Cylindrical shape and other morphological characters of *E. cylindrica* obtained in the present study were similarly stated by Mandal (1980).

A pyriform shape was typical of oocysts belonging to the species *E. bareillyi*. In this study, the oocyst had an average measurement of 34.29µ by 22.18µ, which varied from the observation made by Mandal (1980) who recorded the mean size as 28µ by 19µ. Sporulation time was not recorded.

Oocysts of *E. brasiliensis* were ovoidal and had both the micropyle and micropylar cap. This species was encountered rarely, in very few faecal samples. The average size of the oocysts was 39µ by 27µ which is in agreement with the reports of Mandal (1980). The oocyst of this species failed to sporulate.

In this study, it was noted that oocysts of the species *E. wyomingensis* had almost the perfect shape of an egg. Oocyst dimension and sporulation time agreed with the findings of Huizinga and Winger (1942).

5.4. Clinical pathology

5.4.1. Haematology

The results of the haematological studies in the present study, indicated significant alterations in the severely infected animals. Haemoglobin and packed cell

volume (PCV) values were found to decrease significantly in coccidial infections with a slight decrease in total leukocyte count. The serum total protein values were also found to reduce.

Coccidial infection may result in damage to epithelial cells, loss of intestinal tissue, bleeding into the intestinal lumen along with the discharge of raw blood in severe cases (Stockdale *et al.*, 1981). This could be the reason ascribable to the significant reduction in the haemoglobin per cent in the heavily infected group and this finding is in agreement with the observations made by Fitzgerald and Mansfield (1972), Stockdale *et al.*, (1981) and Holst and Svensson (1995).

The packed cell volume was also found to reduce in the heavily infected group though not very significant. This could be correlated with haemorrhagic lesions in the intestine (Holst and Svensson, 1995).

A mild reduction in the total leukocyte count was observed in this study which was similar to the findings of Holst and Svensson (1995). This could be

attributed to a significant reduction in the percentage of lymphocytes in both mild and severe cases.

In the study on differential count, mild neutrophilia was noticed in the infected group. This change can be substantiated by the statements of Benjamin (1998) that neutrophilia is encountered in inflammatory disorders due to protozoan infections.

An obvious reduction in the percentage of lymphocytes was noticed in both mild and severe groups and this is in accordance with the haematological interpretation of Benjamin (1998). Lymphopenia could be the aftermath of coccidial infection and systemic stress.

Eosinophil and basophil counts were of no significance in this study.

A significant increase was noticed in the monocyte count of infected animals in the present study. Benjamin (1998) also stated that monocytosis was found in granulomatous inflammatory conditions following protozoan infections.

5.4.2. Serum protein fraction assays

In the present study, total protein values were significantly lower in the infected animals This is similar to the findings of Fitzgerald (1964), Fitzgerald *et al.*, (1972), Stockdale *et al.*, (1981) and Holst and Svensson (1995). The pronounced decline in serum protein values could be coincidental with generalised injuries, production of toxic materials, altered osmotic pressure, inefficiencies in lipid transport and loss of blood. Benjamin (1998) interpreted this change as dilutions of plasma protein by extravascular fluid movement in response to a reduction in blood volume.

An increase in the albumin fraction and decrease in globulin fraction was noticed in infected cattle which was contrary to the findings of Fitzgerald (1964) who observed that serum albumin decreased and alpha globulin fraction increased slightly in experimental coccidiosis. He also stated that gamma globulins decreased during the manifestation of severe symptoms.

The albumin globulin ratio was greater in the infected animals, again contrary to the findings of Fitzgerald (1964).

The increase in the albumin fractions could probably be relative to the decrease in globulin fraction observed in the study. The reduction in globulin values could be due to a lack of sufficient immune response, not letting the rapid rise of alpha globulin fractions which play a crucial role in the antibody response to bovine coccidiosis.

5.5. Treatment with anticoccidial drugs

5.5.1. Sulphadimidine sodium

Treatment of coccidiosis with Sulphadimidine was carried out at the rate of 125 mg per kg body weight orally for three consecutive days in six infected animals. A marked reduction in the oocyst count and clinical signs like diarrhoea was found. Complete cure was noticed after treatment. This is in accordance with the findings of Biswal (1948), Peardon *et al.*, (1963), Celeda *et al.*, (1985), Sanyal *et al.*, (1985b) and Vottero and Suarez (1985).

5.5.2. Amprolium hydrochloride

Amprolium hydrochloride at the rate of 20 mg per kg body weight orally for five days was also found to be very effective in eliminating coccidiosis and a 100 per cent efficacy was observed. This finding is in agreement with the findings of McDougald (1982) who reported on the safety and curative properties of the drug.

5.5.3. Salinomycin

Salinomycin, at the dose rate of 2 mg per kg body weight was administered for 21 days orally. This drug considerably reduced the oocyst count and diarrhoeic symptoms. Similarly, Benz and Ernst (1979) had also reported best results with reduction in severe diarrhoea, absence of blood in faeces and reduced oocyst count with salinomycin at the rate of 2 mg per kg body weight for 21 days. Some animals were seen to develop constipation, which was corrected by the administration of liquid paraffin.

5.5.4. Comparison between Sulphadimidine sodium, Amprolium hydrochloride and Salinomycin administered against bovine coccidiosis

Treatment trials with Sulphadimidine, Amprolium and Salinomycin proved beyond doubt that all the three drugs were equally effective against bovine coccidiosis. The efficacies were 99.15 ± 0.78, 99.58 ± 0.41 and 99.98 ± 0.99 respectively. The oocyst count and the predominant clinical signs like diarrhoea reduced markedly following the treatment.

During this study, it was noticed that Sulphadimidine and Amprolium were easily available drugs and convenient for administration, to the animals. The drug Salinomycin had short falls like lack of easy availability and the need for a longer course of treatment. The development of constipation in some animals was another practical difficulty encountered during the administration of Salinomycin.

SUMMARY

6. SUMMARY

A detailed study on the prevalence, clinical pathology and treatment of coccidial infections in cattle belonging to all age groups was undertaken. Faecal samples for diagnosis were collected from suspected cases brought to the University veterinary hospitals at Mannuthy and Kokkalai, Ambulatory units at Cherumkuzhy and Valakkavu, University Livestock farms, Buffalo breeding station, Mannuthy, Cattle farm, Pattambi and from localities in and around Thrissur, during the period from June 1999 to May 2000. The salient findings of the study were as follows.

1. Out of 1821 samples collected, 40 animals were positive for coccidial oocysts showing an overall prevalence rate of 2.2 per cent. The prevalence of coccidiosis in cattle was found to be high during the months with heavy rainfall and high humidity conducive for the persistence of oocysts. The highest prevalence rate (5.84 per cent) was recorded in the month of August. Crowding and unhygienic management practices predisposed to greater incidence in rural areas. Female animals (72.5 per cent) were found to be more affected than males (27.5 per cent). The

maintenance of less number of male animals could be the probable reason for this. Coccidiosis was found to affect animals below one year (65 per cent) more severely than the other age groups, which could be attributed to low immunotolerance in young animals.

2. For the diagnosis of coccidiosis, direct microscopical examination of the faecal samples and also concentration methods like floatation technique using Sheather's sucrose solution were useful apart from the clinical signs like blood tinged foul smelling diarrhoea, dehydration, emaciation, soiled hind quarters etc. characteristic of the infection. Sheather's sucrose solution had the advantage that it did not distort the shape of the oocysts.

3. Eight species of *Eimeria* affecting cattle were identified in the present study, on the basis of morphological details and sporulation time. They were *Eimeria bovis* (35 per cent), *E. zuernii* (55 per cent), *E. subspherica* (10 per cent), *E. ellipsoidalis* (60 per cent), *E. cylindrica* (five per cent), *E. bareillyi* (five per cent), *E. brasiliensis* (five per cent) and *E. wyomingensis* (10 per cent). The

commonly encountered species were *E. ellipsoidalis*, *E. zuernii* and *E. bovis*. The sporulation time of species like *E. cylindrica*, *E. brasiliensis* and *E. bareillyi* could not be recorded. There was no difference in the species affecting the calves and the adult animals. Delay in setting up of cultures, unfavourable temperature and climatic conditions could be the predisposing factors leading to failure of sporulation in some species.

4. Haematological studies were carried out in a group of 10 infected animals and a control group of another 10 healthy animals. A marked reduction in the haemoglobin (8.50 ± 1.66) and packed cell volume (28.64 ± 10.68) values was observed in the severely infected groups. This change could be attributed to haemorrhagic lesions in the intestine and subsequent blood loss. A decrease in the total leukocyte count (10780.00 ± 1362.00) could be ascribable to a significant decrease in the proportion of lymphocytes. The number of neutrophils (36.60 ± 8.73) and monocytes (13.00 ± 2.55) increased as encountered in inflammatory disorders due to protozoan infections.

5. Serum protein fraction assays revealed significant reduction in the level of total protein (5.97 ± 1.77) in the serum of infected animals, which could be coincidental with generalised injuries and blood loss. An increase in the albumin fraction (3.37 ± 1.59) and decrease in the globulin fraction (2.96 ± 2.22) was observed with a greater albumin-globulin ratio (1.85 ± 1.44) in the infected group. The reduction in globulin values could be due to a lack of sufficient immune response.

6. The anticoccidial drugs administered orally in 18 clinically infected animals were Sulphadimidine sodium (125 mg per kg body weight for three days), Amprolium hydrochloride (20 mg per kg body weight for five days) and Salinomycin (2 mg per kg body weight for 21 days). Each drug was administered orally to a group consisting of six animals. Efficacies of the three drugs were 99.15 ± 0.78, 99.58 ± 0.41 and 99.88 ± 0.99 respectively. It could be concluded that all the three drugs were equally effective against bovine coccidiosis; except that the treatment with Salinomycin had the difficulty of having a longer course and constipation as a side effect.

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PREVALENCE AND TREATMENT OF BOVINE COCCIDIOSIS

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ABSTRACT OF A THESIS

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ABSTRACT

A detailed study on the prevalence, clinical pathology and treatment of coccidial infections in cattle belonging to all age groups was undertaken at the Veterinary Hospitals, University Livestock Farm, Buffalo Breeding Station, Cattle Farm, Pattambi, Kerala Agricultural University and other localities in Thrissur during the period from June 1999 to May 2000. The prevalence of the infection was found to be 2.2 per cent. On the whole, the incidence of coccidiosis was found to be high in animals below one year, and female animals, during the rainy and humid months. Clinical signs; predominantly blood tinged or foul smelling diarrhoea and the microscopical examination of the faecal samples from suspected cases were made use of for diagnosis.

Eight Eimerian species causing coccidial infections in cattle were identified and they were *Eimeria bovis* (35 per cent), *E. zuernii* (55 per cent), *E. subspherica* (10 per cent), *E. ellipsoidalis* (60 per cent), *E. cylindrica* (five per cent), *E. bareillyi* (five per cent), *E. brasiliensis* (five per cent) and *E. wyomingensis* (10 per cent). The most commonly

encountered species were *E. ellipsoidalis, E. bovis,* and *E. zuernii.*

Haematological studies of clinically infected animals revealed reduction in the values of haemoglobin, packed cell volume, total leukocyte and lymphocyte counts. A reduction in serum total protein and globulin fractions was also observed during the assay of serum protein fractions in these animals.

Treatment trials against bovine coccidiosis using drugs, Sulphadimidine sodium (125 mg per kg body weight for three days), Amprolium hydrochloride (20 mg per kg body weight for five days) and Salinomycin (2 mg per kg body weight for 21 days) orally resulted in nearly cent per cent efficacy in the clinically affected animals. All the three drugs were found to be equally effective.