CLINICAL INVESTIGATIONS ON PARASITIC ANAEMIA IN CATTLE

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled **"CLINICAL INVESTIGATIONS ON PARASITIC ANAEMIA IN CATTLE"** 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 **"CLINICAL INVESTIGATIONS ON PARASITIC ANAEMIA IN CATTLE"** is a record of research work done independently by **Dr. Rani Gopinath,V.(2002-13-13),** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

1. INTRODUCTION

Parasites particularly gastrointestinal helminths and protozoa are a major threat to animal productivity throughout the world. In many tropical countries protozoan diseases are of major importance. The tropical climate of Kerala favours the survival of lot of parasites. It has been frequently suggested that the nutritional status of the host can influence the pathogenesis of parasitic infection and it is generally accepted that well nourished animals withstand parasitism better than that are less adequately fed. Parasites are responsible for heavy economic losses to livestock industry. It causes death but even more important are the undesirable effects it produces in the host. One of the major effects of parasitism is anaemia

According to Katoch and Mandial (2003), anaemia may be caused due to helminthosis, haemoparasites, ectoparasitism or nutritional deficiencies and is one of the important clinical manifestations seen in animals under field condition. Anaemia adversely affects production, growth rate and reproductive efficiency.

Anaemia is a clinical entity most commonly seen in young animals. But now a days large numbers of clinical cases of anaemia with or without icterus are being encountered in adult cattle in Kerala. Though clinical signs are often suggestive of a blood parasite etiology, a precise diagnosis and initiation of suitable therapy is often found to be difficult.

India being a tropical country, diseases due to blood parasites in livestock are quite common in most of the states (Chandra *et al.*, 2000; Kumari *et al.*, 2000; Soodan *et al.*, 2000). Large numbers of clinical cases of blood parasite infection have been reported in the neighbouring states of Tamilnadu and Karnataka (Muraleedharan and Srinivas, 1985; Sunder *et al.*, 2001; Chitravel *et al.*, 1998) whereas in Kerala only isolated incidences of blood parasites have been reported. A systematic investigation on the prevalence of blood parasites infection causing anaemia has not been carried out so far in Kerala.

Hence the study entitled "Clinical investigations on parasitic anaemia in cattle" has been taken up with the following objectives;

- to identify the specific parasites causing anaemia in cattle.
- to study the haematobiochemical changes in parasitic anaemia.
- to formulate a specific therapy for clinical condition.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ANAEMIA IN CATTLE

Anaemia is a reduction below normal of the erythrocyte number and / or haemoglobin concentration per unit volume of blood. It generally reflects a secondary development and may develop when there is blood loss through haemorrhage or blood sucking parasites, accelerated erythrocyte destruction and reduced or defective erythropoiesis (Schalm *et al.,* 1975).

Anaemia is a sign of disease that results either from an increased rate of destruction or loss of erythrocytes or from a decreased rate of their production (Valli *et al.,* 1993).

Samanta *et al.* (1995) opined that anaemia often resulted in low productivity, depressed reproduction and suppressed resistance thus affecting the viability of livestock industry.

Srinivasan and Samuel (1999) recorded the haematological values in anaemic cattle. The values for packed cell volume (PCV), haemoglobin concentration (Hb), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were 22.66±0.67 per cent, 6.47±0.31 g/dl, 4.62±0.12 x 10^6/mm^3, 49.18±0.17 fl, 13.98±0.53 pg, and 28.52±1.01 per cent respectively.

The normal haematobiochemical levels were studied by Shrikande and Sarode (1999) in cows below five years of age. They recorded values for haemoglobin (g/dl), total protein (g/dl), albumin (g/dl) and globulin (g/dl) as 9.96±0.32, 7.04±0.10, 3.32±0.09 and 3.72±0.09 respectively.

The haematological values in a normal animal were postulated by Kramer *et al.* (2000) and were recorded as; PCV: 24-46 per cent, haemoglobin: 8-15 g/dl, TEC: 5-10 x10^6/µl, TLC: 4-12 x10^3/µl, MCV: 40-60 fl, MCH:11-17

pg, MCHC: 30-36 per cent, Thrombocytes: 100-800 $x10^3/\mu l$. The differential count (per cent) was recorded as N: 15-45, L: 45-75, M: 2-7, E: 4-6 and B: 0-2.

Tvedten and Weiss (2000) expressed the severity of anaemia depending on the packed cell volume in cattle as follows: Mild: 20-26 per cent; Moderate: 14 – 19 per cent; Severe: 10-13 percent and very severe < 10 per cent.

Benjamin (2001) postulated the reference range of serum biochemical parameters for normal cattle as total protein (g/dl) - 6.74-7.46; albumin (g/dl) - 3.03-3.55; globulin (g/dl) - 3-3.48; AG ratio - 0.84-0.94 ; total glucose (mg/dl) - 45-75 and total bilirubin (mg/dl) -.01-0.5.

2.2 PARASITIC ANAEMIA IN CATTLE

Schalm (1972) classified the different factors causing anaemia and suggested that parasitism was a major factor in causing anaemia in cattle.

Ramakrishna *et al.* (1992) concluded that the incidence of anemia in buffaloes was mostly of nutritional origin.

Samanta *et al.* (1995) observed that in anaemic cases of bovine, 80 percent were due to parasitism and 20 percent due to nutritional deficiency. They also noted that anaemia was highly prevalent in crossbred cattle which came to 67.5 per cent compared to 48.59 per cent in indigenous cattle.

Srinivasan and Samuel (1999) observed that haemoprotozoans were an important contributing factor for the development of bovine anaemia. They recommended oral haematinics as an effective and economical therapy for anaemia.

Metenawy (2000) recorded a higher prevalence of haemoparasites (77 per cent) causing anaemia in bovines. *Theileria annulata* was found to be most prevalent.

According to Carlson (2002), the parasitic infectious causes of haemolytic anaemia in cattle were anaplasmosis, babesiosis, haemobartonellosis, eperythrozoonosis, theileriosis and trypanosomosis.

2.2.1 Anaemia in Helminth Infections

Baker and Douglas (1957) studied the development of anaemia in strongyle infection in cattle.They observed that blood sucking activities of the parasites and abomasal haemorrhage contributed to the prevailing anaemia.

Mulligan *et al.* (1963) concluded that mild anaemia occurred in ostertagiasis due to a low plane of nutrition during the winter superimposed on abomasal dysfunctions characteristic of the disease.

Sinclair (1965) suggested that anaemia occurring in fascioliasis was secondary to a disorder of the reticuloendothelial system.

Jennings (1976) concluded that haemorrhage occurred in fascioliasis which predisposed to anaemia. Poor level of nutrition exacerbated the anaemia occuring in chronic fascioliasis

2.2.1.1 Age

A survey among young buffaloes revealed a higher percentage (73.8) of positive cases of helminths among 340 calves less than six months of age (Gupta and Chhabra, 1990). *Toxocara vitulorum* was the predominant infection (41.2 per cent) followed by *Strongyloides papillosus* (25.9 per cent), Strongyles (3.8 per cent) and *Moniezia* spp (2.6 per cent). Among calves over six months of age S. *papillosus* was found to be predominant (26.1 per cent) followed by *Moniezia* spp (15.2 per cent) and Strongyles (10.9 per cent).

In an epidemiological study conducted by Manna *et al.* (1994) a higher incidence rate of amphistomosis (56.51 per cent) was reported in adult cattle.

Borthakur and Das (1998) studied the incidence of gastro intestinal helminths in dairy animals. They observed that calves carried significantly higher

nematode burden than heifers and cows. Different genera of strongyles recorded were Haemonchus, Oesophagostomum, Trichostrongylus, Cooperia and Bunostomum. Toxocara, Strongyloides and Moniezia could be observed only in calves. Fasciola and Paramphistomes were present throughout the year in heifers and cows.

A study on the incidence of *Toxocara vitulorum* in buffalo calves was carried out by Rao *et al.* (2000). Faecal samples from 309 animals were screened and they could observe that the incidence was more in calves less than 30 days old and came to about 42.5 per cent.

A similar study was carried out in the Chattisgarh region by Pal *et al.* (2001).Their study included 659 animals and the results revealed a prevalence rate of 18.2 per cent amphistomes, 14.2 per cent strongyles, 6.6 per cent Trichuris, 2.4 per cent Toxocara and 1.2 per cent Moniezia. They also reported that the prevalence rate of the parasitic infections was higher in younger animals than adults.

2.2.1.2 Clinical Pathology

2.2.1.2.1 Haematological Changes

In an experimental study Ross *et al.* (1959) observed that the degree of haemonchosis was paralleled by a progressive anaemia as demonstrated by fall of PCV. The intensity of infection was determined by taking EPG counts. They reported a PCV as low as 22 per cent in heavily infected animals.

Ross and Armour (1960) considered packed cell volume percentage as an important index in determining the pathogenicity in helminthosis when considered in conjunction with a series of differential faecal egg count. They opined that a PCV lower than 32 per cent indicated pathogenic effect.

An investigation on the haematological and pathological aspects of naturally occurring bovine fascioliasis was made by Kumar *et al.* (1982). Affected animals revealed significant reduction in TEC, PCV and haemoglobin

content, increase in TLC, lymphocyte and eosinophil counts along with a decrease in neutrophil count.

Pandey and Misra (1985) studied the haematological profile in anaemia associated with *Neoascaris vitulorum* infection in calves. The PCV, haemoglobin concentration and TEC of the infected calves ranged between 20-24 per cent, 6.8-7.4g/dl and 4.01-4.52 $x10^6/mm^3$ respectively.

Chakraborty (1999) evaluated the haematological changes in 40 non-descript calves infected with *Fasciola gigantica.* The values obtained for PCV, haemoglobin concentration,TEC and MCH were 28 per cent, 8g/dl, $3.4x10^6/mm^3$ and 20pg respectively. The corresponding values for healthy controls were 36 per cent, 11g/dl, $4.78x10^6/mm^3$ and 26.73 pg.

Haematological changes in crossbred calves experimentally infected with *H. contortus* were studied by Raman *et al.* (1999). A significant reduction in PCV and haemoglobin values with lymphopenia, neutrophilia and a mild eosinophilia were noticed.

Devi *et al.* (2000) reported that heavy infections of *T. vitulorum* in calves significantly lowered PCV, haemoglobin, TEC and MCV. A higher value was obtained for MCV indicating macrocytic to normocytic, hypochromic anaemia.

2.2.1.2.2 Serum Biochemical Changes.

Ross and Todd (1965) observed decreased serum albumin levels in calves infected with *Ostertagia ostertagi.* They reported that the serum albumin levels ranged between 1.3-1.96g/dl in heavily infected calves.

No significant reduction in serum protein fraction in buffaloes infected with *F. gigantica* was observed by Kumar *et al.* (1982).

Pandey and Misra (1985) reported a reduction in total protein level in Toxocara infected calves. The average total serum protein value for the affected calves was 4.99g/dl as against a value of 6.3g/dl of the healthy controls. The

blood glucose levels remained within the normal range. The affected calves showed marked improvement in their general condition with clinical disappearance of symptoms of anaemia after 10 days of treatment.

A drastic reduction in blood glucose and serum proteins were observed in calves infected with trichostrongyles. The lowest values noted for total protein, serum albumin and serum glucose were 5.23g/dl, 2.39g/dl and 40mg/dl respectively (Bandyopadhyay and Dasgupta, 2000).

Bharti and Prasad (2001) studied the biochemical profiles of cattle and buffaloes naturally infected with Paramphistomes and Fasciola. They recorded lower levels of total serum proteins, albumin, globulin and albumin globulin ratio and higher values of ALT and bilirubin. The values for these parameters were 5.93g/dl, 2.7g/dl, 3.23g/dl, 0.85, 29.26 IU/L and 0.23mg/dl respectively. Subsequent to treatment with oxyclosanide these values returned to normal.

2.2.1.3 Treatment

Comparative evaluation of three anthelmintic drugs febantel, piperazine adipate and tetramisole was done among 20 infected buffalo calves (Gupta and Chhabra, 1990). Febantel was used at the dose rate of 7.5mg/kg body weight, piperazine @ of 200mg/kg body weight and tetramisole @ 40mg/kg bodyweight. All drugs were administered orally. Febantel was found to be highly effective against *T. vitolorum* as well as *S. papillosus* and strongyles. Piperazine adipate showed good efficacy against *T. vitulorum* only. Tetramisole showed high efficacy against *S. papillosus* and strongyles but comparatively low efficacy against *T. vitulorum.* Both tetramisole and piperazine adipate required second dose of treatment.

Drug trials were conducted in 40 infected cattle to compare the efficacy of oxyclosanide, hexachlorophene and nitroxynil against natural infection of amphistomes (Manna *et al.,* 1994). Oxyclosanide proved to be more effective than the other two and it also increased the milk yield up to 25 per cent than that

of the untreated control. Oxyclosanide was used @ 3.4 per cent liquid 30ml/kg bodyweight orally

Sanyal (1998) conducted trials against natural and experimentally induced parasitic gastro enteritis due to *Haemonchus* spp. using in feed formulation of albendazole and evaluated its therapeutic and prophylactic efficiency in crossbred cattle and buffalo calves. In one trial albendazole was incorporated in feed pellets to deliver a minimum daily dose of 0.5mg/kg body weight for 10 days. In the second trial albendazole was administered at the therapeutic dosage of 7.5mg/kg body weight. Both trials proved to be beneficial.

A single dose of doramectin at the dose rate of 200µg/kg body weight has an outstanding efficiency against strongyle group of worms in cattle (Panda *et al.*, 2002).

Islam *et al.* (2003) indicated ivermectin to be cent percent effective against gastrointestinal nematodes and ectoparasites in calves. Ivermectin used @ 200µg/kg body weight subcutaneously showed a positive effect on body weight and hematological parameters.

2.2.2 Anaemia due to Ectoparasite Infestations

Anaemia, unthriftiness and lack of vigour were noted in bovines heavily infested with the short nosed sucking louse *Haematopinus eurysternus* on ranches of Alberta (Shemanchuk *et al.*, 1960).

Ectoparasites such as fleas, lice and ticks are considered mainly as disease vectors and not being directly responsible for the anaemia. However they can be responsible for the development of anaemia if the infections are severe enough (Jennings, 1976).

Pediculosis causes economic losses in dairy cattle due to anaemia, unthriftiness, poor feed utilisation resulting in decreased weight gain and lowered milk production (Joseph *et al.*, 1986).

Singh and Singh (1999) opined that *Boophilus microplus* was the major tick affecting the cattle population in India causing unthriftness and occasionally anaemia.

Otter *et al.* (2003) reported anaemia and mortality in young calves infested with long nosed sucking louse *Linognathus vituli* in the dairy farms of England.

2.2.2.1 Clinical Pathology

2.2.2.1.1 Haematological Changes

Mehrotra and Singh (1986) made obsevations on the changes in the blood constituents of the calves infested with *L. vituli.* The values obtained for PCV (per cent), haemoglobin concentration (g/dl), TEC ($x10^6/mm^3$), TLC ($x10^3/mm^3$), MCV (fl), MCH (pg) and MCHC (per cent) in healthy controls were 28.40±0.82, 13.88±0.71, 9.28±1.24, 32.03±1.58, 15.17±1.09 and 48.70±1.74 respectively while in infested animals these values were 23.83±2.94, 8.73±0.67, 10.46±1.35, 29.56±1.91, 9.77±0.81 and 32.85 ±0.71 respectively. The infested calves showed normocytic hypochromic anaemia.

Jonsson *et al.* (1998) suggested that infestation of cattle with *B. microplus* did not produce any significant change in the packed cell volume.

An observation on the blood parameters revealed PCV of 13 per cent, haemoglobin concentration of 4g/dl, TEC of $3.5x10^6/mm^3$ and platelet count of $533x10^5/mm^3$ in a calf severely infested with the sucking louse *L. vituli* (Otter *et al.*, 2003).

2.2.2.1.2 Serum Biochemical Changes

Mehrotra and Singh (1986) studied the serum biochemical changes in calves infested with *L. vituli*. They could not observe any significant difference in

the serum albumin and glucose concentration in infested calves from the control calves.

Burns and Titchener (1992) estimated the serum protein levels in calves infested with *L. vituli*. They reported a mean value of 2.85g/dl in infested calves which was not significantly lower from the control mean (2.89g/dl).

Jonsson *et al.* (1998) could not observe any significant decrease in serum protein fraction of the cattle infested with *B. microplus*.

In an experimental study Vatsya (2003) induced *B. microplus* infestation in 25 six to eight month old calves. Blood was collected prior to and seven days after tick infestation and analyzed immediately for biochemical changes. The total serum protein value reduced to 6.34g/dl from the initial value of 8.3g/dl while the serum glucose value reduced to 69.88mg/dl from the initial value of 83.39mg/dl.

2.2.2.2 Treatment

Singh and Chhabra (1991) experimented the acaricidal effect of fenvalerate 20 per cent EC against livestock ticks *in vitro* and in field trials. They used the drug at three different concentrations of 100ppm, 200ppm and 400 ppm. In *invitro* tests all the three concentration of the acaricide resulted in cent per cent mortality. All the engorged females were killed at 400 ppm concentration. The results of *in vivo* trials revealed that the two higher concentrations gave cent per cent good results while 100 ppm proved only partially effective.

Kagaruki (1996) studied the efficacy of amitraz in controlling cattle ticks. A comparative study of 25 per cent wettable powder and 12.5 per cent w/v EC was done and both were found to be equally effective.

Remington *et al.* (1997) evaluated the efficacy of moxidectin against *B. microplus*. The moxidectin was administered @ 0.2mg/kg body weight subcutaneously and was found to be 98.8 per cent effective.

Villeneuve and Daigneault (1997) suggested the efficacy of doramectin against sucking lice *L. vituli* in cattle. The drug was administered @ 200 µg/ kg body weight subcutaneously.

Tipacamu and Vivas (2003) investigated the effect of moxidectin against natural infestation of the cattle tick *B. microplus* in the Mexican tropics. Moxidectin was administered at the dose rate of 0.2mg/kg body weight subcutaneously. A comparative study with amitraz revealed that moxidectin possessed similar efficacy of 99 per cent.

2.2.3 Anaemia in Haemoparasite Infections.

Hemolytic anaemia is the primary manifestation of several rickettsial and protozoal diseases in domestic animals (Gaunt, 2000).

2.2.3.1 Anaplasmosis

Anaplasmosis is an infectious and transmissible disease of cattle characterized by progressive anaemia associated with the presence of intraerythrocytic inclusion bodies designated as anaplasma (Ristic, 1981). Of the three anaplasma species, *Anaplasma marginale* is the most pathogenic for the cattle. *Anaplasma centrale* causes a relatively mild form of bovine anaplasmosis.

2.2.3.1.1 Age

Comparative infectivity titrations on anaplasma infected blood were conducted by Roby *et al.* (1961) in splenectomised and nonsplenectomised calves and cows. They found the calves to be least susceptible to the disease.

Animals of all ages were susceptible but the severity of the resulting syndrome was directly related to age. In animals less than one year old it was usually subclinical; in yearlings and two years of age it was of moderate intensity; it was severe and often fatal in older cattle (Jones and Brock, 1966).

Cattle infected between six months and three years of age had increased risk of clinical illness and animals infected after three years of age was

commonly affected by a fatal per acute form of the disease (Radostits *et al.*, 2000).

2.2.3.1.2 Transplacental Transmission

Zaugg (1985) reported that transplacental transmission of bovine anaplasmosis could occur as early as second trimester of pregnancy.

Potgieter and Rensburg (1987) suggested that transplacental transmission must be considered as an important mode of transmission in anaplasmosis.

Ganesan *et al.* (1995) reported a case of transplacental transmission of anaplasmosis in a day-old calf. Both the dam and calf showed fever, anaemia, lymph node enlargement and anorexia. The peripheral smears from both animals were positive for intra erythrocytic organisms.

2.2.3.1.3 Clinical Signs and Gross Lesions

Baker *et al.* (1961) concluded that the major factor in the production of anaemia in anaplasmosis was acute extravascular haemolysis, and bonemarrow depression even if occurred had little significance.

Dimopoullos *et al.* (1962) studied the mechanism of anaemia resulting from infections of *A. marginale* in cattle. They demonstrated that in the acute stages of anaplasmosis a change in the ultrastructure of the erythrocytic membrane occurred as the result of a decrease in concentration of total phospholipid. The concentration of phospholipid was shown to be inversely proportional to the osmotic fragility of the erythrocyte.

The syndrome in anaplasmosis may be divided into a prepatent period, a period of increasing anaemia, maximal anaemia and convalescence. Maximal anaemia occurs one to three days after peak parasitemia (Jones and Brock, 1966).

Gautam *et al.* (1970) reported anaemia to be the chief syndrome of anaplasmosis as evident by the pale white conjunctiva, low hemoglobin values

and reduction in erythrocytes. The haemoglobin level was found to be less than 4g/dl in severe cases.

Jatkar and Krier (1970) demonstrated the presence of antierythrocytic and antiparasitic antibodies in the anaplasma infected cattle. The autoantibody coated erythrocytes were recognized and removed from the system which resulted in a reduction in the circulating erythrocytes. They stated that antierythrocytic antibodies appear after infection and persist during anaemia while antiparasitic antibodies persist even after recovery.

Chitravel *et al.* (1998) reported dullness, emaciation, dry muzzle, pyrexia, increased pulse and respiratory rate, pale and icteric mucous membranes and considerable weight loss in a Jersey bull infected with *A. marginale.*

Important clinical symptoms of anaplasmosis included pyrexia, lymphnode enlargement, weakness, anaemia and icterus (Soodan *et al.*, 2000).

The gross lesions in anaplasmosis were watery blood, icteric mucous membrane, enlarged liver and spleen, distended gall bladder and petechiael haemorrhages in heart (Basu, 2002).

Sivaseelan and Anna (2004) studied the gross pathological alterations of tissues and organs of crossbred dairy animals died due to anaplasmosis. On necropsy icteric discoloration and pallor of tissues and organs, splenomegaly and hepatomegaly with distended gall bladder, petechiae under epicardium and catarrhal gastroenteritis were observed. Organ impression smears were found to be better than the peripheral blood smears for the detection of *A. marginale.*

2.2.3.1.4 Clinical Pathology

2.2.3.1.4.1 Haematological Changes.

Anaplasmosis in a group of crossbred heifers was reported by Ravindranath *et al.* (1982). Hematological studies revealed a mean haemoglobin

concentration of 5.2±0.2 g/dl which increased to 9 g/dl after treatment with oxytetracycline.

Sharma (1986) reported mean PCV and haemoglobin levels of 15.7 per cent and 4.8 g/dl in crossbred cow calves experimentally infected with *A. marginale.*

Banerjee and Guha (1989) determined the changes in the haematological parameters in 17 crossbred cattle naturally infected with *A. marginale.* These parameters were determined one day before and seven days after treatment. Mean PCV, haemoglobin level and TEC were increased from 20.06±0.48 per cent, 6.66±0.16g/dl and 3.34±0.08 $x10^6/mm^3$ to 24.76±0.76 per cent, 8.24±0.25 g/dl and 4.24±0.16 $x10^6/mm^3$ respectively.

Palmer and Lincoln (2002) opined that a packed cell volume less than ten per cent in anaplasmosis resulted in the death of the animal.

Soodan *et al.* (2000) reported erythrocytopenia, leucocytosis and reduction in haemoglobin in a clinical case of anaplasmosis. The values recorded for haemoglobin concentration, TEC and TLC count were 7.5g/dl, $3.46x10^6/mm^3$ and $9.06x10^3/mm^3$ respectively. DLC revealed 47 per cent neutrophil, 40 per cent lymphocyte, three per cent eosinophil, two per cent basophil and eight per cent monocyte.

2.2.3.1.4.2 Serum Biochemical Changes.

Dimopoullos *et al.* (1960) investigated the serum protein changes in anaplasmosis. They recorded a plasma protein concentration of 6.93 g/dl in the infected animals, which was within the normal range. The AG ratio also was not significantly altered but serum globulin was increased slightly.

The alterations in the serum bilirubin concentration induced by *A. marginale* infection were determined in 16 adult cows experimentally infected with virulent isolates (Allen *et al.,* 1981a). A high serum bilirubin value of 2.4mg/dl was recorded.

In another study regarding the total protein changes Allen *et al.* (1981b) opined only slight decrease in serum protein level. The albumin level was recorded as 3.07±0.36g/dl.

Soodan *et al.* (2000) reported no significant changes in serum protein concentration in anaplasmosis infection in a crossbred cow.

2.2.3.1.5 Diagnosis

James *et al.* (1990) evaluated the dot enzyme linked immunosorbent assay for serodiagnosis of anaplasmosis in cattle. The test allowed the processing of multiple sera in less than three hours and was found to be sensitive as the indirect fluorescent antibody test. The sensitivity was 93 percent while the specificity was 96 percent.

Goff *et al.* (1990) compared the sensitivity of DNA probe, complement fixation test and indirect immunofluorescence test for diagnosing *A. marginale* in an enzootic area. The study revealed that DNA probe and indirect immunofluorescence were more sensitive in detecting carrier infections than CFT.

A highly sensitive and specific polymerase chain reaction (PCR) based assay for the detection of the minute levels of *A. marginale* present in the blood of long-term carrier cattle was developed by Gale *et al.* (1996). The sensitivity limit of the PCR-ELISA was 0.00015 per cent parasitaemia (24 infected erythrocytes per microlitre of blood). No cross-reactivity of the assay was observed when *A. marginale* negative blood infected with *Babesia bovis* or *Theileria orientalis* was tested. The PCR-ELISA was shown to be 92 per cent efficient in the detection of long-term *A. marginale* carrier cattle. No false-positive results were obtained.

Chitravel *et al.* (1998) detected *A. marginale* bodies in Giemsa stained peripheral blood smears from two bulls suspected of the disease.

In properly stained thin blood smear *A. marginale* bodies appeared as spherical granules measuring 0.2-0.5 microns near periphery of the cell. The bodies had slight haloes surrounding them (Basu, 2002).

Sivaseelan and Anna (2004) examined both blood smears and impression smears from a dead crossbred cow which was highly icteric and anaemic. Blood smears had one to two inclusions in erythrocyte and parasitemia was around 30 per cent. Impression smears of lung, spleen and liver revealed the typical dark staining *A. marginale* organisms when compared to blood smears.

2.2.3.1.6 Treatment

Magonigle *et al.* (1975) observed that the intravenous administration of oxytetracycline hydrochloride at the dose rate of 22mg/kg bodyweight intravenously daily for five days was effective in treating 11 adult cattle that were naturally infected *A. marginale* carriers.

Magonigle and Newby (1982) studied the efficacy of oxytetracycline in eliminating naturally acquired chronic *A. marginale* infection. Four injections of a long acting oxytetracycline at the dose rate of 20mg/kg body weight were administered at three days intervals to 14 naturally infected anaplasma carrier cattle.

Lincoln *et al.* (1982) suggested that treatment of cattle in the prepatent period with long acting oxytetracycline formulation was effective in reducing the occurrence frequency of clinical disease.

Swift *et al.* (1983) also experimented the elimination of carrier state with injectable long acting oxytetracycline. Three to four doses of long acting oxytetracycline was administered intramuscularly at the dose rate of 20mg/kg bodyweight at three days intervals to each of 16 naturally infected *A. marginale* carrier cows.

Banerjee and Guha (1989) evaluated the effect of five daily intravenous injections of oxytetracycline hydrochloride on clinical cases of anaplasmosis in

crossbred cattle. Animals were also given supportive therapy with B complex, liver extract, paracetamol and haematinics for five consecutive days.

Pipano *et al.* (1992) stated that animals vaccinated with *A. centrale* that receive tetracyclines for other infections might be unintentionally disinfected of *A. centrale* infection and may lose their immunity against anaplasmosis

2.2.3.2 Theilerioses

Theilerioses are tick borne protozoan diseases caused by *Theileria* spp. in ungulates (Radostits *et al.*, 2000). The diseases are characterized by fever and lymphoproliferative disorders, which may be associated with leukopenia and anaemia.

2.2.3.2.1 Age

Koch *et al.* (1990) reported that mortality in calves owing to *T. parva bovis* infection was significantly lower in animals younger than seven months than in older cattle.

All age group of animals were affected with theileriosis but mortality was high in young animals (Al-Atya *et al.*, 1991)

Tropical theileriosis caused by *T. annulata* was an important disease of exotic cattle and their crossbred progeny leading to morbidity and mortality especially in calves causing considerable economic loss (Chandra *et al.*, 2000).

2.2.3.2.2 Clinical Signs

Sharma and Gautam (1970) reported anaemia and leukopenia as the pertinent and constant heamatological findings in calves infected with *T. annulata.*

In 1979, Deore *et al.* described a case of cutaneous theileriosis in a ten-month-old Holstein Friesian heifer. He observed numerous erythematous more or less hard nodular convex lesions on the skin over the neck extending over the

back upto the posterior border of scapula. Lesions were also seen on the vulval lips and udder. Smears were taken from the lesions and on microscopical examination revealed Koch blue bodies.

Another case of cutaneous theileriosis was reported by Shastri *et al.* (1982) in two 36-day-old calves. Urticarial eruptions were noted over the neck, withers and back.

Shastri (1991) opined that in mild cases of theileriosis clinical symptoms consisted of anorexia, pyrexia and lymph node enlargement. In severe cases manifestations were pyrexia, lymph node enlargement, anorexia and pale conjunctival mucous membranes showing petechiae.

Clinical signs in *T. parva* infections in Zebu cattle in Tanzania included dullness, diarrhoea, froth in nostrils, severe dyspnoea, diffuse subcutaneous oedema, enlargement of lymph nodes, pyrexia, petechial haemorrhges under the tongue, inappetance, salivation, corneal opacity, lacrimation, serous nasal discharges and weakness (Mbassa *et al.*, 1994).

Patel *et al.* (2001) described the clinical signs in calves experimentally induced tropical theileriosis as dullness, anorexia, profuse lacrimation and pyrexia. During the febrile stage the calves were constipated and later on developed diarrhoea resulting in dehydration, emaciation and weakness. Icterus was indicated by yellowish discolouration of mucous membranes. Respiratory symptoms were manifested as increased respiratory rate and watery cough, which was suggestive of pulmonary oedema.

2.2.3.2.3 Clinical Pathology

2.2.3.2.3.1 Haematological Changes.

A comparative study of the diseases in cattle caused by *T. parva* and *T. lawrenci* infection was done by Maxie *et al.* (1981). The PCV decreased to 23 per cent in *T. parva* infection while there was no significant change in *T. lawrencei* infected group. Significant leukopenia occurred in all infected groups with a

decrease to $5x10^3/mm^3$ in *T. lawrenci* and $2x10^3/mm^3$ in *T. parva*. Platelet count reduced to $2x10^5/mm^3$ from an initial count of $5x10^5/mm^3$.

Mbassa *et al.* (1994) reported normocytic normochromic anaemia in natural cases of *T. parva* infections in cattle. The erythrocyte and leukocyte count, haematocrit and haemoglobin concentrations were greatly decreased when compared to the healthy cattle. The mean values were recorded as 2.85±1.10 $x10^6/mm^3$, 2.78±1.70 $x10^3/mm^3$, 19±0.06 per cent and 4.07±1.62 g/dl respectively.

Sandhu *et al.* (1998) reported significant progressive decrease in haemoglobin concentration, PCV and TEC whereas the TLC showed and initial nonsignificant leukocytosis followed by significant leukopenia.

Patel *et al.* (2001) experimentally induced bovine tropical theileriosis in six cross bred male calves and observed the changes in the haematological and biochemical parameters. The recorded values for PCV (per cent), haemoglobin concentration (g/dl), TEC ($10^6/mm^3$), TLC ($10^3/mm^3$), MCV (fl), MCH (pg), MCHC (per cent) were 20.23±0.55, 6.21±0.28, 5.46±0.19, 5.28±0.40, 46.91±0.67, 12.48±0.60 and 29.43±0.57 respectively.

Singh *et al.* (2001) studied the changes in blood parameters in crossbred calves with experimental *T. annulata* infections. They observed progressive decrease in haemoglobin and PCV to 3.7±0.3 g/dl and 14.6±0.9 per cent respectively. Coagulopathies included thrombocytopenia (2.27 x $10^5/mm^3$) and an increased prothrombin time along with a nonsignificant increase in bleeding time.

2.2.3.2.3.2 Serum Biochemistry

Dhar and Gautam (1977) reported an increase in the serum bilirubin level in *T. annulata* infections. They experimentally induced acute and chronic theileriosis in 16 crossbred male calves of four months of age. Total serum bilirubin level increased from 0.236 and 0.225mg/dl of serum to 1.117 and

0.62 mg/dl in acute and chronic infections respectively. They pointed out the increased destruction of erythrocytes and hepatic cell insufficiency as the causative factors.

Total serum protein and protein fractions were estimated quantitatively and qualitatively in experimentally induced acute and chronic *T. annulata* infection of cattle by Dhar and Gautam (1979). The serum proteins reduced to a level of 5.63g/dl from an initial value of 7.47g/dl in acute infections. The albumin globulin ratio decreased to 0.71 from 1.01. In chronic phase these fractions became almost normal but gamma globulins increased significantly.

Maxie *et al.* (1981) studied the biochemical profile in Theileria infected cattle. The plasma protein concentration decreased significantly from an initial value of 8 to 6g/dl. The total bilirubin concentration increased significantly to 2.3mg/dl.

Yadav and Sharma (1986) conducted a study on the changes in blood chemical components during experimentally induced *T. annulata* infections in cattle. They noticed a rapid fall in blood glucose and serum protein levels to 48.2±5.7mg/dl and 6.2±0.5g/dl during period of peak parasitemia. They attributed this to the utilization of these components by the parasites. They also reported an increase in the serum bilirubin levels to 0.61 ±0.13mg/dl by tenth day of infection.

Patel *et al.* (2001) studied the biochemical changes in crossbred calves experimentally infected with *T. annulata*. The biochemical changes included reduction in serum protein (g/dl) to 5.33±0.27, and serum albumin (g/dl) to 2.63±0.09. The serum bilirubin increased to 2.06±0.20mg/dl.

Singh *et al.* (2001) investigated the changes in the serum protein profile in experimental *T. annulata* infection. The serum protein and albumin concentrations decreased to 4.6±0.2g/dl and 2.9±0.2g/dl by 20th day post infection from pre infection concentration of 6±0.2 g/dl and 4.5±0.2g/dl

respectively. A concurrent nonsignificant increase in serum globulin value was also recorded.

2.2.3.2.4 Diagnosis

Shastri *et al.* (1982) reported the presence of small sized oval and round piroplasms inside the RBCs of a peripheral blood smear obtained from a case of cutaneous theileriosis. Smears taken from the urticarial lesions revealed large numbers of macro and micro schizonts.

Blood smears were taken from both acute and mild infections of *T. annulata* in crossbred calves (Shastri, 1991). In mild cases intraerythrocytic parasitemia ranged from 0.5 to 5 per cent and either stray or around 5 per cent schizont affected lymphoblasts were observed in the lymph node smears. In acute cases erythrocytic parasitemia was 40-90 per cent with multiple numbers of piroplasms and numerous schizonts detected in the lymph node smears.

Christine *et al.* (1995) demonstrated that PCR detected *T. annulata* parasites at low parasitemias in carrier cattle. A value of 75 per cent sensitivity was recorded in the experiment.

Soundarajan *et al.* (2000) detected *T. annulata* infection in cattle and buffaloes by ELISA. 66.4 per cent of cattle and 41.9 per cent of buffaloes showed antibodies to *T. annulata* by ELISA

Sunder *et al.* (2001) reported 35 per cent parasitemia in a ten-day-old crossbred HF calf. The peripheral blood smear and lymph node biopsy smears revealed the presence of piroplasms of *T. annulata* and Koch blue bodies respectively.

2.2.3.2.5 Treatment

Narasimhamurty *et al.* (1970) reported the efficacy of camoquin, the human antimalarial drug in treating *T. annulata* infections in cattle.

Singh *et al.* (1980) conducted clinical trials to study the chemotherapeutic efficiency of oxytetracycline in *T. annulata* infection. He revealed that oxytetracycline at a higher dose of 10-15mg/kg bwt for four to six days is appropriate in treating the cases.

Vos and Roos (1983) reported that of halofuginone at the dose rate of 1mg/kg body weight orally for two days was effective in treating *T. parva lawrenci* infections in cattle.

Pradhan *et al.* (1993) emphasized the role of oral haematinics in the treatment of anaemia due to tropical theileriosis.

Kumari *et al.* (2000) successfully treated a mixed infection of theileriosis and tyrpanosomosis in a crossbred cow with chloroquin phosphate and berenil.

Sunder *et al.* (2001) reported the rare occurrence of theileriosis in a 10 day old crossbred Holstein Friesian female calf and successful treatment with a single injection of buparvaquone @ 2.5mg/kg body weight.

2.2.3.3 Trypanosomosis

Trypanosomosis of cattle is a disease caused by several species of extracellular haemoprotozoan parasites, which are cyclically transmitted by tse tse flies (Morrison *et al.*, 1981). More commonly lower levels of parasitemia, which may persist for many months and is associated with the development of anaemia, which is the major contributory factor to the disease, characterize the disease.

Amole *et al.* (1982) reported that the anaemia manifested in the chronic or prolonged stages of *Trypanosoma evansi* was due to the failure of haemopoiesis and marrow dysfunction. They explained that the anaemia in the acute phase of trypanosomosis is caused by the immune response of the host.

Dead dying or living trypanosomes released potentially pathogenic biological or chemical substances that were capable of lysing RBCs and caused

the development of anaemia (Suliman and Feldman, 1989). Mechanical injury to erythrocyte due to lashing action of parasite added to the pathogenic effect.

2.2.3.3.1. Clinical Signs

The major feature of the disease in cattle was anaemia clinically manifested as pallor of the mucous membranes (Morrison *et al.*, 1981).

Singh and Misra (1986) reported that infection in cattle and buffalo was generally subclinical but acute outbreaks of the disease occurred when animals become debilitated due to some intercurrent disease or stressfactors like lactation and pregnancy.

Reduction in PCV and TEC were the most characteristic features in trypanosomosis (Suliman and Feldman, 1989).

Characteristic signs of surra in buffaloes were intermittent fever (upto105^{0}F), salivation, lacrimation, nasal discharge, loss of milk production and congestion of conjunctivae. Anaemia, emaciation, staggering gait and falling on the ground were noticed in chronic cases (Singh and Joshi, 1991).

Tuntasuvan *et al.* (1997) reported cases of cerebral trypanosomosis due to *T. evansi* in cattle. The affected cattle were showing nervous symptoms including circling, excitation, jumping, aggressive behaviour, lateral recumbency, convulsion and finally death. The impression smears and smears prepared from the blood and CSF revealed the presence of organism.

Trypanosome affected animals were dull anorectic, apathetic, had an ocular discharge and lost condition. Superficial lymph nodes became visibly swollen, mucous membranes were pale, diaarhoea occasionally occurred and some animals had oedema of the throat (Radostits *et al.*, 2000).

Gupta *et al.* (2003) reported high fever, muscle twitching, anorexia, dyspnoea, increased salivation, dehydration and acute abdominal pain in a herd of cattle and buffaloes in Punjab.

Clinical signs like fever, lacrimal discharge, progressive emaciation and weakness of hind quarters were observed in crossbred calves experimentally inoculated with *T. evansi* (Singh and Chaudhri, 2003).

2.2.3.3.2 Clinical Pathology

2.2.3.3.2.1 Haematological Changes.

Singh and Misra (1986) revealed significant decrease in PCV (20±0.80 per cent) and haemoglobin (5.9±0.34 g/dl) concentration in *T. evansi* infection in calves. Other haematological parameters like TEC, MCV, MCH and MCHC were also significantly reduced revealing microcytic hypochromic anaemia.

Rajguru *et al.* (2000) determined the haematological changes in trypanosomosis in neonatal calves. The PCV and haemoglobin concentration were recorded as 22 per cent and 7.2 g/dl respectively. The differential count revealed neutrophilia.

Kaur and Juyal (2003) induced experimental surra infection in 12 crossbred cow calves and observed the haematobiochemical changes. The mean values obtained for haemoglobin, PCV, TEC and TLC count on 14th day post infection were 11.06g/dl, 29 per cent, 6.76x10^6/mm^3 and 10.47 x 10^3/mm^3 while in the noninfected control groups these values were obtained as 13.07 g/dl, 32 per cent, 7x106mm^3 and 9.55x10^3/mm^3.

Singh and Chaudhri (2003) evaluated the haematological changes in the crossbred calves experimentally infected with *T. evansi*. They noticed a considerable reduction in these parameters and the values were recorded as PCV (per cent) - 23.67±1.20, haemoglobin (g/dl) -7.30 ±0.35, and TEC (x10^6/mm^3) – 4.76±0.15.

2.2.3.3.2.2 Serum Biochemistry

Significant decrease in blood glucose levels (43.13±1.9mg/dl) in 33 surra affected buffaloes were recorded (Singh and Joshi, 1991). This was attributed to

rapid consumption of blood glucose by trypanosomes. Significant decrease in total serum protein (7.32±0.18g/dl) and nonsignificant changes in albumin and albumin globulin ratio were also recorded.

A decreased serum albumin concentration of 1.80g/dl was recorded in buffalo calves experimentally infected with *T. evansi* (Rajora *et al.*, 1986).

Kulkarni *et al.* (1994) studied the blood glucose profile in trypanosomosis in buffaloes. They recorded a mean value of 27.21mg/dl in the infected animals.

Singla *et al.* (2000) investigated the serum protein changes of crossbred calves experimentally infected with *T. evansi*. In infected calves the total protein decreased significantly (6.49±0.31 g/dl) in comparison to the preinfection level (8.49±0.778). The albumin concentration also decreased while the serum globulin showed increased levels.

The serum biochemical changes in experimental surra were determined by Kaur and Juyal (2003). They observed a reduction in albumin fraction to 2.71g/dl by 14th day postinfection while in control group the value was 3.7g/dl. AG ratio was 0.71 in infected and 1.25 in uninfected control group.

2.2.3.3.3 Diagnosis

Bossche *et al.* (2000) suggested that examination of the buffy coat and uppermost layer of red blood cells was ideal for detection of trypanosomes, as these organisms tend to aggregate in these portions of a centrifuged sample.

Krishnappa *et al.* (2002) considered passive haemagglutination test as a suitable, economical and reliable test for the studies on seroprevalence of *T. evansi* infection in bovines.

Masake *et al.* (2002) described the application of PCR-ELISA to the detection of the *T. brucei* and *T. vivax* infections in the livestock.

The diagnostic sensitivity and specificity of direct card agglutination, indirect card agglutination and ELISA was calculated on the basis of

parasitological results obtained by mouse inoculation and compared for all assays (Verloo *et al.,* 2000). Diagnostic specificity was highest in direct card agglutination (98 per cent) followed by ELISA (95 per cent) and indirect agglutination (82 per cent).

Davila *et al.* (2003) used the polymerase chain reaction test to detect the prevalence of trypanosomosis in Brazil. They reported the high sensitivity of PCR when compared to other techniques.

2.2.3.3.4 Treatment

Comparative evaluation of the drugs against surra was done by Singh and Joshi (1991) in buffaloes. Single doses of quinapyramine (4.4mg/kg body weight) and isometamidium (0.5mg/kg bodyweight) proved beneficial in the study.

Kulkarni *et al.* (1994) evaluated the different drug combinations in trypanosomosis in buffaloes. A combination of berenil and intravenous 20 per cent dextrose proved to be beneficial.

Kaur and Juyal (2003) investigated the therapeutic value of diminazene aceturate (3.5mg/kg body weight) along with antipyrine (4.16mg/kg bodyweight) and procaine (0.2mg/kg bodyweight) in experimental surra in cow calves. Following treatment the altered haematological parameters returned to normal.

Singh and Chaudhri (2003) evaluated the efficacy of diminazene di aceturate against *T. evansi* in crossbred calves. It was used at the dose rate of 3.5mg/kg bodyweight intramuscularly in crossbred calves experimentally infected with *T. evansi*. The parasitaemia did not reappear throughout the post treatment period.

2.2.3.4 Babesiosis

Babesiosis is a tick borne disease of cattle caused by the protozoan parasites *B. bovis, B. bigemina, B. divergens* and others. The principal pathogenic

effect of babesiosis in cattle was haemolytic anaemia due to intravascular haemolysis (Pandey and Misra, 1987).

2.2.3.4.1 Age

Roychoudhury *et al.* (1976) observed clinical cases of babesiosis in calves below 30 days of age.

Latif *et al.* (1979) opined that the age of cattle was an important factor in prevalence of *B. bigemina* infection. They succeeded in inducing the infection in six month and one year old animals experimentally and observed typical signs of disease in the latter case.

Mallick *et al.* (1980) reported rare occurrence of babesiosis in a ten day old calf. He considered such incidences to be rare since the young calves are considered to be resistant to natural infection of babesiosis.

Bhikane *et al.* (2001) stated that most cases of bovine babesiosis occurred in cattle of six months to five years.

2.2.3.4.2 Clinical Signs

High rise of temperature and haemoglobinuria were recorded as the chief clinical symptoms in bovine babesiosis by Dwivedi *et al.* (1976).

Vos *et al.* (1976) reported a case of cerebral babesiosis in a day old calf, which became infected with *B. bovis* inutero. The calf refused to suckle and showed ataxia, haemoglobinuria and yellowish mucous membranes.

Mallick *et al.* (1980) described the clinical signs in a newborn indigenous calf as pyrexia, dullness, depression, anorexia, haemoglobinuria and pale mucous membranes.

Post mortem examination of bovine died due to babesiosis was done by Shastri *et al.* (1991). The important lesions observed were enlarged liver, spleen and kidney, pale myocardium, haemorrhages in the epicardium, blood-tinged

urine in the bladder and dark yellowish exudates in the abdominal, pericardial and thoracic cavities. They opined that the mortality rate was high in the age group of 5-18 months.

Bhikane *et al.* (2001) reported abortion in one pregnant cow due to babesiosis.

2.2.3.4.3 Clinical Pathology.

2.2.3.4.3.1 Haematological Changes.

Mallick *et al.* (1980) reported PCV of 19 per cent, haemoglobin concentration of 7g/dl, TEC of 2.4 $x10^6/mm^3$ and TLC of $8.7x10^3/mm^3$ in a newborn indigenous calf affected with babesiosis.

PCV, haemoglobin concentration, TEC, TLC, MCV, MCH and MCHC were determined in cattle infected with clinical babesiosis (Pandey and Misra, 1987). The values observed for these parameters were 17.80±0.68 per cent, 6.48±0.34 g/dl, 3.44±0.23 $x10^6/mm^3$, 12.85±439.12 $x10^3/mm^3$, 52.59±2.19 fl, 19.04±0.46 pg and 36.24±0.83 per cent respectively. The blood picture revealed normocytic normochromic anaemia.

Shastri *et al.* (1991) recorded a haemoglobin level of 3g/dl in a three year old crossbred cow affected with babesiosis.

An evaluation of the haematological parameters in babesiosis infected cattle was done by Bhikane *et al.* (2001). The values recorded for PCV, haemoglobin, TEC and TLC were 18.4±1.81 per cent, 6.46±0.60 g/dl, 3.4±0.32 $x10^6/mm^3$ and 11.52±0.52 $x10^3/mm^3$ respectively. The corresponding values in healthy controls were 32.60±1.92 per cent, 10.52±0.75g/dl, 6.20±0.33 $x10^6/mm^3$ and 8.28±0.68 $x10^3/mm^3$. DLC revealed neutrophilia and lymphopenia, which could be due to the stress during the course of the disease.

Zaugg (2002) stated that in babesiosis, PCV values dropped rapidly from 35 per cent to below 10 per cent in a week after the onset of clinical signs. They

opined that acute cases with PCV values above 12 per cent usually responded well to treatment and the prognosis decreased for cases with PCV values below 10 per cent.

2.2.3.4.3.2 Serum Biochemical Changes

Pandey and Misra (1987) studied the biochemical response to haemolytic anemia due to babesiosis in cattle. The serum protein levels did not show any significant decrease while the blood glucose level (46.64±1.41 mg/dl) showed drastic decrease when compared with the healthy control (62.07±20.38 mg/dl).

Radostits *et al.* (2000) reported a decrease in serum protein profile of cattle affected with babesiosis.

Bhikane *et al.* (2001) investigated the serum biochemical response in cattle affected with babesia. They observed significant increase in the serum glucose (73.9±4.05 mg/dl), protein (8±0.15 g/dl) and bilirubin(2.38±0.170 mg/dl) values. These changes were attributed to intravascular haemolysis.

2.2.3.4.4 Diagnosis

Vos *et al.* (1976) conducted detailed microscopic examination of blood smears and brain impression smears taken from a day old calf which died due to cerebral babesiosis. The blood smear revealed typical single or paired forms of *B. bovis* and the parasitised cells were found to form aggregations. Massive accumulation of parasitised red blood cells in the capillaries with marked distension was noted in the brain smears. The percentage infection of the red blood cells was found to be 90 per cent with many of the cells harbouring up to four parasites.

Bose *et al.* (1995) claimed that the method of choice to detect babesial parasites in acute condition was the examination of Giemsa stained thin blood films since excellent demonstration of morphological details of the parasites and species were possible. They opined that thick films were necessary for detections of some strains of *B. bovis*.

Soulsby (1982) described *B. bigemina* as a large piroplasm 4-5µm in length pear shaped lying in pairs forming an acute angle in the red blood corpuscles. Round oval or irregular shaped forms occured depending on the stage of the development of the parasite in the red cell. *B. bovis* was seen as a small piroplasm in pairs or single.

Machado *et al.* (1997) described ELISA as a reliable serological test for detection of bovine babesia infection.

Thammasirirak *et al.* (2003) evaluated the PCR-ELISA for the detection of *B. bovis* in cattle in Thailand. The detection of around $24x10^{-8}$ percent parasitemia was achieved by PCR amplification followed by ELISA. The PCR-ELISA also showed high specificity to *B. bovis* with no cross reaction to other endemic parasites except for *A. marginale.*

Rejitha (2003) compared the immunoflourescence test, examination of Giemsa stained blood smears, and slide ELISA techniques for determination of babesiosis in cattle. Examination of Giemsa stained blood smear could detect only 12.68 per cent of infections while IFAT and slide ELISA detected 52.11 and 54.93 per cent infections respectively. These two serological tests were found to be equally efficient with slide ELISA proving more suitable for use as a field diagnostic test.

2.2.3.4.5 Treatment

Dwivedi *et al.* (1976) treated clinical cases of babesisois with berenil at the dose rate of 6mg/kg bodyweight. Belamyl was given as supportive treatment in the therapy.

Pandey and Misra (1987) successfully treated cases of clinical babesiosis with berenil and haematinics.

Mallick *et al.* (1980) treated babesiosis in an indigenous calf with berenil and liver extracts. It was administered at the rate of 6mg/kg bodyweight through intramuscular route.

Bhikane *et al.* (2001) opined that berenil along with haematinics was effective in treating haemolytic anaemia due to babesia. He also reported a mortality rate of nine per cent among the treated animals.

2.2.3.5 Ehrlichiosis

Ehrlichiosis was a tick borne febrile debilitating disease of bovines caused by *Ehrlichia bovis*, a rickettsial parasite found in circulating mononuclear cells of bovines (Kolte *et al.*, 2003).

2.2.3.5.1 Clinical Signs

Karunamoorthy *et al.* (1992) observed fever, tremors, in coordination of movements, convulsions, anorexia, pale visible mucous membranes, enlargement of prescapular lymphnode, salivation, lacrimation, grinding of teeth and frequent micturition in a bullock infected with *E. bovis*. In another case of ehrlichiosis in aged nondescript she buffalo they noticed blood tinged urine and diarrhoea.

Devada *et al.* (1996) reported emaciation, anaemia, pyrexia, enlargement of lymph nodes in cow infected with *E. bovis*.

Kolte *et al.* (2003) observed pyrexia, stiffness of neck, sudden drop in milk production, inappetance, nervous symptoms and arthritis in buffaloes from Vidharba.

2.2.3.5.2 Clinical Pathology

2.2.3.5.2.1 Haematological Changes.

Devada *et al.* (1996) studied the haematological parameters in a crossbred cow with *E. bovis* infection. They reported 25 per cent PCV, 7.2g/dl hemoglobin concentration, $3.8x10^6/mm^3$ TEC and $3.5x10^3/mm^3$ TLC. The DLC was neutrophil-16 per cent, lymphocyte-72 per cent, monocyte-8 per cent and eosinophil-4 per cent.

Kolte *et al.* (2003) observed leucocytopenia and reduced number of neutrophils with relative increase in monocyte count. Haemoglobin concentration was found to be reduced.

2.2.3.5.3 Diagnosis

Sreekumar *et al.* (1995) made attempts to inoculate laboratory mice and rabbit with *E. bovis*. Mice showed clear inclusions in both blood monocytes and peritoneal macrophages while symptoms of disease were not observable in rabbits. They suggested mice as better laboratory models for *E. bovis* infection and infection in mice could be enhanced by immunosuppression.

Devada *et al.* (1996) reported the presence of small roughly spherical inclusions in the cytoplasm of monocytes and lymphocytes of a cow infected with *E. bovis*. The inclusions included mostly small bodies measuring up to 1μm and a few medium sized ones measuring up to 3-5μm.The inclusions were reddish and purplish in shade.

A PCR-based assay was developed for detecting DNA of granulocytic ehrlichiae in blood samples from dogs, horses, and cattle (Engwall *et al.,* 1996). They concluded that PCR was the most reliable method, useful in the clinical laboratory for specific and early diagnosis of granulocytic ehrlichiosis in animals.

Kolte *et al.* (2003) described the inclusion bodies in ehrlichiosis as intracytoplasmic, coccoid and elongated bodies in mononuclear leucocytes. The organism appeared red to dark purple in colour.

2.2.3.5.4 Treatment

Karunamoorthy *et al.* (1992) successfully treated two cases of ehrlichiosis in buffaloes with tetracyclines and berenil. Berenil was given as a single injection of 4g intramuscularly.

Treatment with oxytetracycline at dose level of 10mg/kg bodyweight intravenously for five days was reported to be effective by Kolte *et al.* (2003).

Materials and Methods

3. MATERIALS AND METHODS

The study was conducted in the Department of Clinical Medicine, College of Veterinary and Animal Sciences, Mannuthy over a period of three semesters from May 2003 to July 2004. Cattle presented at the various Government Veterinary Hospitals of Thrissur, University Veterinary Hospitals and those belonging to University livestock farm, Mannuthy with clinical signs suggestive of anaemia were selected and utilized for the study.

3.1 DESIGN OF THE STUDY

The study consisted of apparently healthy animals as control and clinical cases.

3.2 SELECTION OF ANIMALS

Fifty cattle, which had clinical signs suggestive of anaemia, were chosen for the study. Six apparently healthy cattle that were brought to the AI centre for the purpose of insemination were selected as control for studying the normal haematological parameters.

3.3 OUTLINE OF STUDY

3.3.1 Clinical Examination

Detailed clinical examination of the patients was conducted as described by Boddie (1962).

3.3.2 Examination of Clinical Materials

Relevant clinical materials like peripheral wet film, blood smear, lymph node aspiration smear and dung sample were collected and detailed examination was done for detection of the parasites.

3.3.2.1 Screening of Blood Smears

Thin smears were prepared by collecting one drop of the peripheral blood from the ear tip of the animal on clean grease free glass slide and stained using the Giemsa stain. Smears were examined under the oil immersion objective of a light microscope to detect the presence of the blood parasites.

3.3.2.2 Screening of Lymph Node Aspiration Smears

The area over the prescapular lymph node was prepared aseptically. Using a sterile 18-gauge needle and syringe, lymph node aspirates was taken and smeared onto grease free clean glass slides to make thin and thick smears. These were stained using Giemsa stain and observed under the oil immersion objective of a light microscope to detect the presence of parasitic stages.

3.3.2.3 Screening of Faecal Samples

Faecal samples were thoroughly mixed with water using a mortar and pestle then sieved and the filtrate was collected and centrifuged at 3000 rpm for five minutes. The sediment was examined under the low power objective of a light microscope for the presence of ova of parasites.

3.3.2.4 Animal Inoculation Tests

Animal inoculation tests were done in cases suspected for trypanosomosis as described by Verloo *et al.* (2000). A volume of 0.25 ml of blood from the suspected animals was collected and inoculated intraperitoneally into the mice. Development of parasitemia in the inoculated mice was checked in the subsequent days by microscopical examination of a small drop of blood taken from the tail tip.

3.4 CLINICAL PATHOLOGY

3.4.1 Collection of Clinical Materials

Blood was collected in clean and dry vials by puncturing the jugular vein. Sodium citrate at the rate of 1mg/ml of blood was used as the anticoagulant. Blood smear for differential leukocyte count was prepared on a clean and dry

glass slide with a drop of blood collected from the ear tip. Blood was collected without anticoagulant in a clean and dry test tube and nonhaemolysed serum was separated for determination of serum biochemistry. The clinical materials were collected both on first day and tenth day for evaluating the anaemia and assessing the clinical response to therapy.

3.4.2 Examination of Clinical Materials

3.4.2.1 Evaluation of Haematological Parameters

Parameters viz; packed cell volume (PCV), haemoglobin (Hb), total erythrocyte count (TEC), total leucocyte count (TLC), platelet count, differential leucocyte count (DLC), mean corpuscular cell volume (MCV),mean corpusclar haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were estimated as described by Schalm *et al.* (1975)

3.4.2.2 Serum Biochemistry

Total serum protein, albumin, globulin, AG ratio, serum glucose and serum total bilirubin were estimated by spectrophotometry in Photometer 5110 (Boehringer Manheim)

Serum total protein[1] was estimated by modified Biuret method described by Weichselbaum (1940) while albumin[2] was estimated by bromocresol green dye binding method as described by Doumas *et al.* (1971). Total bilirubin[3] was estimated using the method of Jendrassik and Grof (1938) and serum glucose[4] was estimated by the method of Burtis *et al.* (1996)

3.5 TREATMENT OF ANAEMIC CASES

After detailed investigation, necessary steps were adopted in the therapeutic regimen. Suitable therapy was adopted depending on the

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[1] Merck Ecoline Total Protein [2] Merck Ecoline Albumin

[3] Merckotest Bilirubin [4] Merckotest Glucose

etiological factors. The drugs indicated in the present study were;

3.5.1 Group I - Haemoparasites

1. Oxytetracycline- Intravenous administration of oxytetracyclines @ 10mg /kg bodyweight was done in cases of anaplasmosis, theileriosis and ehrlichiosis. The therapy was continued for a period of five days.

2. Diminazene aceturate- Intramuscular injection of diminazene aceturate @ 8mg/kg bodyweight was indicated in cases of babesiosis. In most cases clinical cure was observed after a single injection except for a case wherein the drug was repeated after 48 hours.

3.5.2 Group II – Intestinal Helminths

1. Ivermectin- Two Subcutaneous administrations @ 200 µg/kg bodyweight one week apart were given in the treatment of strongyloides.

2. Oxyclosanide- Cases of amphistomiasis were treated with single oral administration of oxyclosanide @ 10mg/kg body weight.

3. Albendazole- In case of strongyle and trichuris infections oral administration of albendazole @ 5mg/kg body weight orally was given.

Apart from the specific therapy supportive therapy was adopted in appropriate cases which included intravenous fluids, haematinics and vitamin supplements. Five per cent dextrose was the fluid of choice in the present study. Haematinics included both parenteral and oral pharmacy preparations.

Pharmacy preparation of tonic mixture consisted of:

Ferrous sulphate exicatus - 60g

Copper sulphate - 10g

Cobalt chloride - 200mg

Calcium gluconas - 250g

Tr Calumba - 150 ml

Liq Arsenicalis -100ml

Aqua ad 500ml

The anaemic cattle were given this preparation at the dose rate of 50ml daily orally for a period of ten days. Intramuscular administration of iron dextran (imferon) at the dose rate of 6 ml /animal was attempted in ten cases (Pradhan *et al.*, 1993).

Response to treatment was assessed by noting the clinical improvement of the animal in the subsequent days and by assessing the improvement in the haematobiochemical profile by tenth day of treatment.

3.6 STATISTICAL ANALYSIS

Data obtained were analysed statistically as per Snedecor and Cochran (1980). The means of all the groups were compared with that of the control using analysis of variance (ANOVA).

Results

4. RESULTS

In the present study 50 cattle showing clinical signs of anaemia were subjected to detailed clinical examination and all parameters under study such as signalment, history, physical examination, screening of clinical materials for parasites, haematology, serum biochemistry and treatment response were carried out. The data obtained were analyzed statistically.

The clinical cases positive for parasitic etiology were grouped into three.

Group I (n= 32) - Animals positive for haemoparasites.

Group II (n=8) - Animals positive for intestinal helminths.

Group III (n=1) - Animals positive for ectoparasites.

Remaining nine cases were excluded from the study as no parasitic etiology could be detected.

4.1 INCIDENCE OF PARASITIC ANAEMIA

Out of the 50 anaemic animals screened 41 turned to be positive for parasites. The positive cases comprised of haemoparasites, helminths and ectoparasites. Thus a significantly higher value of 82 per cent was recorded for occurrence of parasitic anaemia in the present study (Fig.1).

4.1.1 Group I - Haemoparasites

Peripheral blood smears and lymph node aspiration smears from suspected cases were screened for the presence of parasites. The various haemoparasites observed in the study were Anaplasma (Fig.2), Babesia (Fig.3), Theileria (Plate 1), and Ehrlichia (Fig.4). A total of 32 cases were recorded which included 15 cases of babesiosis, 12 cases of anaplasmosis, four cases of theileriosis and one case of ehrlichiosis infection. Thus babesiosis was found to be the predominant infection with an incidence of 47 per cent followed by anaplasmosis (37.5 per cent), theileriosis (12.5 per cent) and three per cent

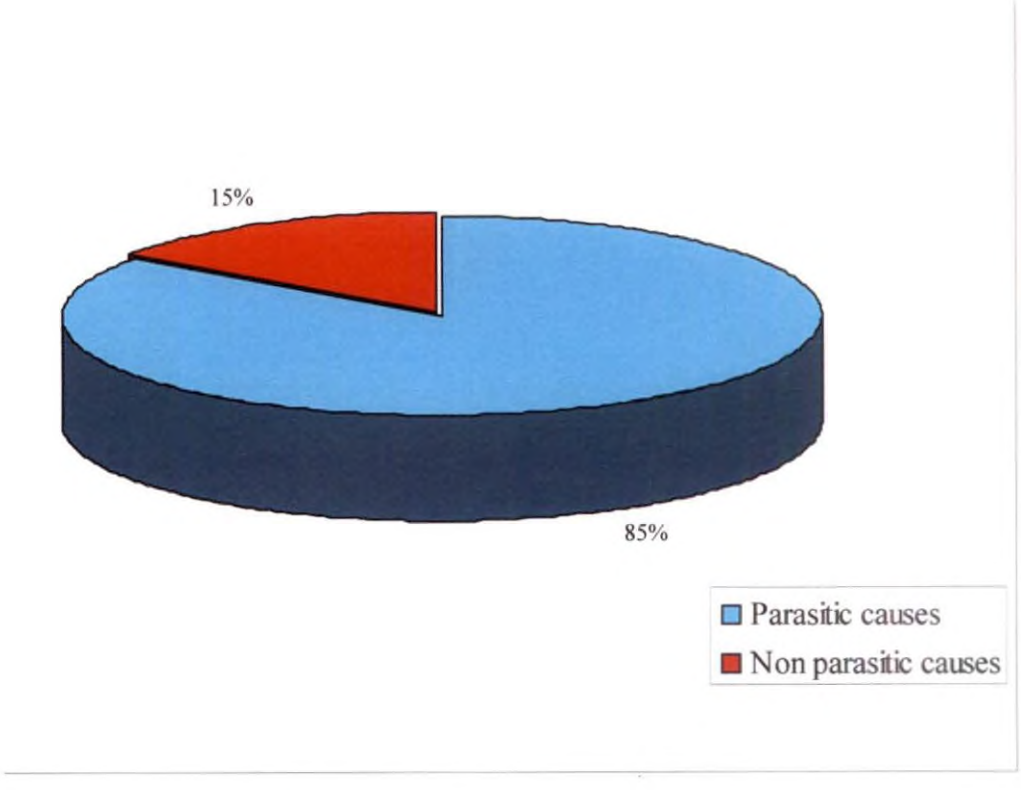


Fig. 1: Incidence of parasitic anaemia

ehrlichiosis (Fig.5). Altogether haemoparasites constituted 78 per cent of parasitic anaemia in the present study (Fig.6).

4.1.2 Group II - Helminths.

Only eight cases of anaemia due to helminth infections were recorded which included four cases of Trichuris, two cases of Strongyloides and one case each of Paramphistome and Strongyles. This comprised about 19.5 percent of the parasitic anaemia cases under study (Fig.6).

4.1.3 Group III - Ectoparasites

A single case of tick infestation leading to anaemia was recorded (Fig.6).

The percentage of occurrence of different parasites are summarized in the table 1 below.

Table 1. Status of parasitic anaemia encountered

ETIOLOGY		NUMBER OF CASES	PER CENT	TOTAL (PER CENT)
HAEMOPARASITES	Anaplasma	12	29.27	78.06
	Babesia	15	36.59	
	Theileria	4	9.76	
	Ehrlichia	1	2.44	
HELMINTHS	Strongyloides	2	4.88	19.5
	Strongyles	1	2.44	
	Trichuris	4	9.76	
	Amphistomes	1	2.44	
ECTOPARASITES	Ticks	1	2.44	2.44

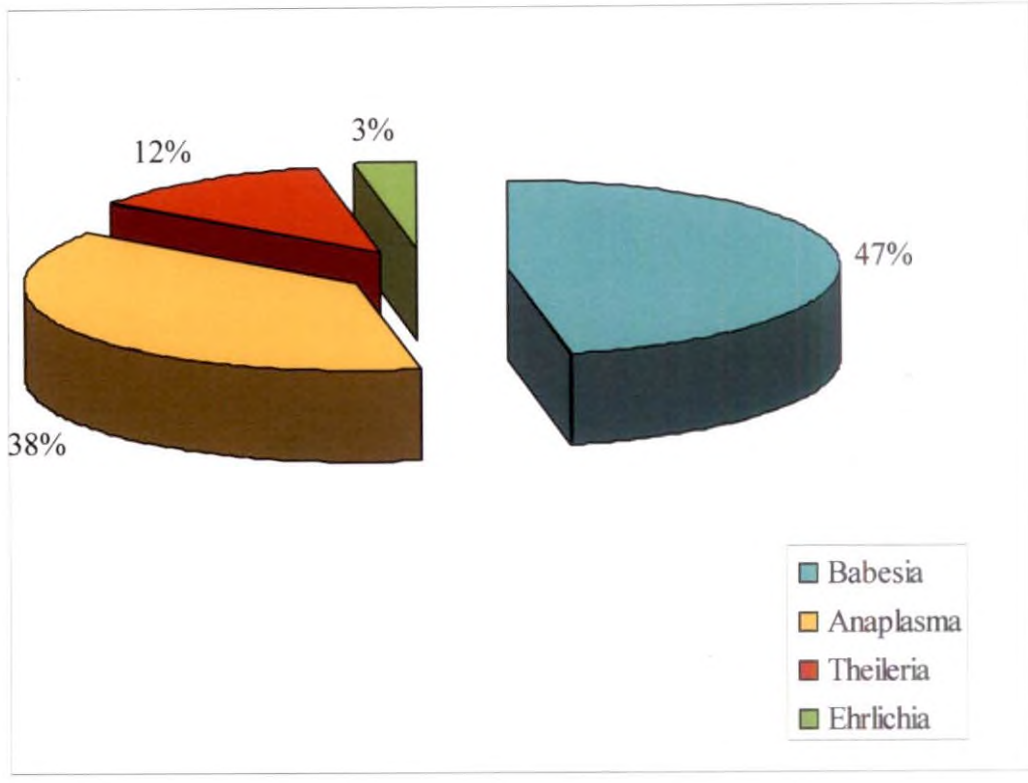


Fig. 5: Distribution of Haemoparasites

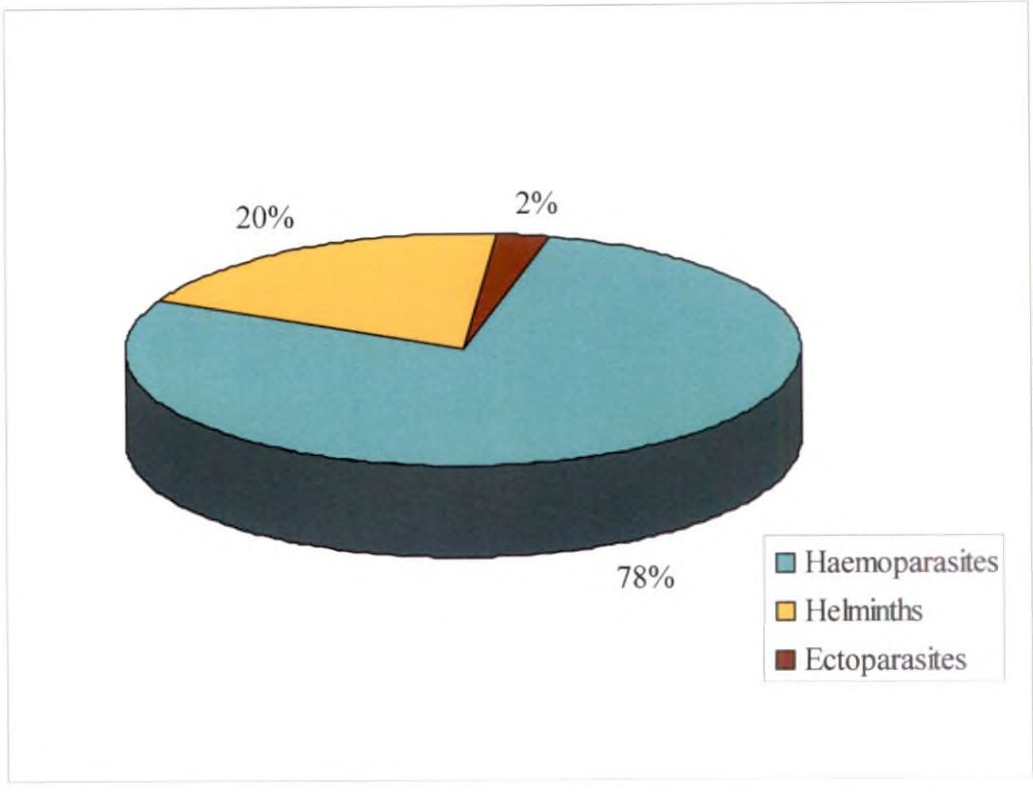


Fig. 6 Distribution of parasitic anaemia

4.2 EFFECT OF AGE

Age was found to be an important deciding factor in determining susceptibility to different infections (Fig.7). Anaemia due to intestinal helminthosis was encountered mostly in young animals except for a case of amphistomosis in a cow. Out of the eight cases recorded seven were observed in calves below six months of age. Regarding haemoparasites, cases were recorded in all age groups though the major percent was comprised of animals above one year of age. One case each of Babesia and Anaplasma was reported in calves below 15 days of age. This could be due to transplacental transmission. However the dams were not available for screening for parasites.Anaemia due to tick infestation was encountered in a ten day old calf. No case of tick infestation leading to anaemia was recorded in adult cattle.

The age wise distribution of animals with parasites is given in the table 2 below;

Table 2. Agewise distribution of animals positive for parasites

PARASITES	AGE		
	< Six months	Six months-one year	>one year
HAEMOPARASITES Anaplasma	2	-	10
Babesia	1	1	13
Theileria	-	-	4
Ehrlichia	-	-	1
HELMINTHS Strongyles	1	-	-
Trichuris	4	-	-
Strongyloides	2	-	-
Amphistomes	-	-	1
ECTOPARASITES Ticks	1	-	-

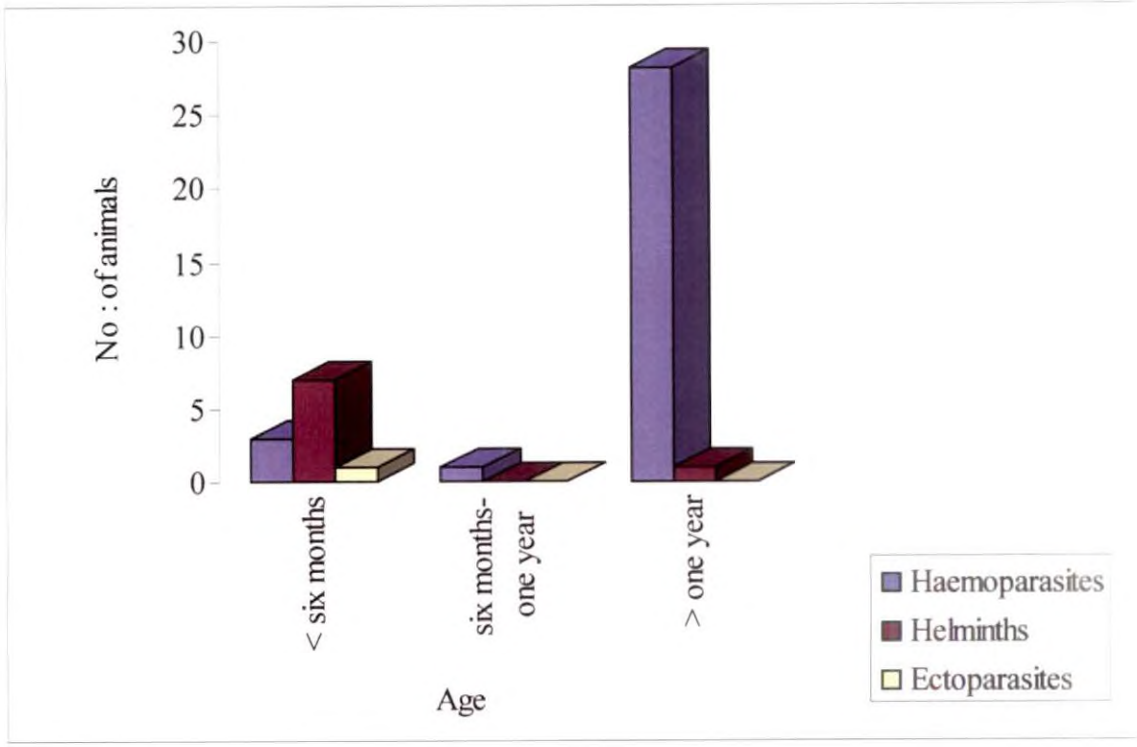


Fig 7: Age wise distribution of animals positive for parasites.

4.3. CLINICAL MANIFESTATIONS

In the majority of cases the striking features observed were pallor of visible mucous membranes and exaggerated respiration. The clinical manifestations observed varied with the etiology. The important clinical symptoms noted in haemoparasite infection were pale icteric mucous membranes, fever, enlargement of superficial lymph nodes, respiratory difficulty, anorexia, drastic reduction in milk yield in case of milch animals, nasal discharge and digestive disturbances. Anaemia was the most pronounced in cases of anaplasmosis which was evident from the pale mucous membranes and clinical pathology. Marked lymph node enlargement and icterus was also observed in anaplasmosis and theileriosis. Babesiosis presented the additional unique symptom of haemoglobinuria. But a single case of babesiosis where haemoglobinuria was absent was observed in the study.

In animals infected with intestinal parasites, the important observations noted were emaciation, rough hair coat, anorexia, diarrhoea and weakness. A single case of anaemia due to tick infestation was recorded. The animal was in the recumbent stage. Heavy tick infestation was noticed. The animal was not taking milk properly and succumbed to the infection on the second day in spite of treatment.

4.4. CLINICAL PATHOLOGY

4.4.1. Haematology

The comparison of the haemogram values between the control and the diseased groups before and after treatment are presented in the tables 3 and 4. The comparison of the leucogram between the control and the diseased groups before and after treatment are presented in the tables 5 and 6.

4.4.1.1. Packed Cell Volume (PCV)

There was statistically significant variation in the mean values of PCV of group I (18.8±0.68 per cent) and group II (18.88±1.39 per cent) with that of control group (29.95±1.60 per cent) before treatment. The mean values did not vary much between the infected groups. The value recorded in the group III, which consisted of a single case, was 10 per cent. After treatment the mean values for PCV in group I and group II increased to 25.96±0.64 per cent and 24.86±1.55 per cent respectively which were less when compared to the control group.

4.4.1.2. Haemoglobin (Hb)

The mean haemoglobin concentration of the control and diseased groups before and after treatment varied significantly. The mean value for haemoglobin concentration in the control group was 9.97±0.50g/dl. No significant difference was observed between group I (6.03±0.22 g/dl) and group II (6.05±0.44 g/dl) before treatment. The mean values observed in the diseased groups after treatment was within in the normal range and were recorded as 8.33±0.18 g/dl (group I) and 8.06±0.55 g/dl (group II). The haemoglobin concentration in tick-infested animal (group III) was 3g/dl.

4.4.1.3. Total Erythrocyte Count (TEC)

Statistically significant difference was noticed in the mean total erythrocyte count of group I, group II and control animals before and after treatment. Animals of group I (3.19±0.16 $x10^6/mm^3$) and group II (3.58±0.30 $x10^6/mm^3$) had mean values without much appreciable difference before treatment. The mean value obtained for TEC in the control group was 5.49±0.35$x10^6/mm^3$. After treatment TEC increased to 4.53±0.16 $x10^6/mm^3$ (group I) and 4.26±0.43 $x10^6/mm^3$ (group II). An erythrocyte count of $1.8x10^6/mm^3$ was recorded in tick-infested animal (group III).

4.4.1.4. Erythrocytic Indices (MCV, MCH, MCHC)

No significant differences were recorded between the control group and diseased group for erythrocytic indices before and after treatment. In all cases the mean values for erythrocytic indices ranged within the normal levels. The mean values for MCV in group I, group II and control group before treatment were 60.45+1.40 fl, 54.14+2.81 fl and 56.27+3.24 fl respectively. The corresponding values for MCH were 19.50±0.57 pg, 17.31±1.14 pg, and 18.22±1.31 pg, and those for MCHC were 32.20±0.49 per cent, 31.94±0.99 per cent and 33.86±1.14 per cent.

4.4.1.5. Total Leukocyte Count (TLC)

The mean values of TLC recorded in different groups were within the normal range with no significant difference from the control group (6.16±0.92 $x10^3/mm^3$). The highest mean value for TLC was recorded for group II (7.68±0.79 x $10^3/mm^3$). The mean TLC observed in group I before treatment was 6.76±0.40 x $10^3/mm^3$. After treatment the mean values observed were 7.53±0.41 $x10^3/mm^3$ and 7.09±0.46 $x10^3/mm^3$ for group I and group II respectively. In group III the TLC was recorded as 4.75x $10^3/mm^3$.

4.4.1.6. Thrombocyte Count

Thrombocyte count in all cases did not show any significant reduction from the mean value of control animals (5.38±0.52 $x10^5/mm^3$). The mean values recorded for group I (4.63±0.23 $x10^5/mm^3$) and group II (4.90±0.45 $x10^5/mm^3$) before treatment were within the normal limits. After treatment the mean values for group I and group II were 4.99±0.17 $x10^5/mm^3$ and 5.06±0.39 $x10^5/mm^3$ respectively. The thrombocyte count in group III was $3.01x10^5/mm^3$.

4.4.1.7 Differential Count

Mean values of lymphocyte and eosinophil percentages varied significantly while all other cells remained in the normal range before and after treatment in all groups. Group I varied significantly from the control group while

group Π animals did not present any significant difference. Mean value of lymphocyte per cent in Group I was found to be decreased (66.56±0.69 per cent) when compared to the control (70.67±1.60 per cent) and group Π (70.00±1.38 per cent) before treatment. Increased eosinophil count was also recorded in this group (4.56±0.35 per cent) when compared to the control (2.17±0.80 per cent) and group Π (2.25±0.69 per cent) animals. After treatment these values came to normal range with no apparent significant difference from the control values. In group III a mild degree of eosinophilia (seven per cent) was recorded.

4.4.2. Serum Biochemistry

Table 7 and 8 summarizes the comparison of the serum biochemical changes in the control and the infected group.

4.4.2.1 Total Serum Protein

Mean values of serum total protein decreased significantly in the diseased animals before treatment. Between the diseased groups no significant difference was recorded. The values observed for group I, group Π and control groups were 6.21±0.14 g/dl, 5.98±0.27 g/dl and 7.10±0.32 g/dl respectively. The lowest mean value was observed in group Π (5.98±0.27 g/dl) .After treatment, the mean values in group I and group Π returned to normal range which were recorded as 6.90±0.11g/dl and 6.61±0.16 g/dl respectively.

The total protein value recorded for the tick-infested animal (group III) was 4g/dl.

4.4.2.2 Serum Albumin

The mean values of serum albumin in group I (2.85±0.07 g/dl) and group Π (2.88±0.15 g/dl) varied significantly before treatment from the control group (3.48±0.17 g/dl). After treatment the mean values increased to (3.29±0.06 g/dl) and (3.37±0.25 g/dl) which were not significantly different from control group. In group III, serum albumin value was 2g/dl.

4.4.2.3 Serum Globulin

No variation was observed in the mean values of the serum globulin between the affected group and control group. Control group recorded a mean value of 3.63± 0.26 g/dl while in group I and group II the values were 3.36±0.11 g/dl and 3.11±0.22 g/dl before treatment. In group III the serum globulin concentration was 2g/dl. After the treatment period, a slight, but not statistically significant increase in serum globulin was recorded. The values recorded for group I and group II were 3.61±0.10g/dl and 3.24±0.31g/dl respectively.

4.4.2.4 AG Ratio

There was no statistically significant difference in the ratio of albumin to globulin in the affected animals. The mean values ranged within the normal limits. The mean values recorded for group I, group II and control group were 0.88±0.03, 0.97±0.07 and 0.97±0.08 respectively before treatment. After treatment these values were recorded as 0.93±0.04 and 1.14±0.18 for group I and group II respectively. Group III recorded an AG ratio of 1.

4.4.2.5 Serum Glucose

The mean values in group I, group II and control group varied significantly from each other. The lowest value for serum glucose concentration was recorded for group I (37.19±1.44 mg/dl). After treatment also significant differences were obtained between the mean serum glucose values of group I (48.52±0.82 mg/dl), group II (54.14±3.87 mg/dl) and control group (54.00±3.32 mg/dl). In tick infested animal (group III), the serum glucose level was very low (29mg/dl).

4.4.2.6 Serum Bilirubin

A higher value of serum bilirubin was recorded in group I (0.88±0.08 mg/dl) before treatment. After treatment these values came down to normal level

(0.37±0.03 mg/dl) and no statistically significant differences were observed from that of the control group (0.22±0.18 mg/dl).

Group Π presented normal values for serum bilirubin with no significant difference from the control group before (0.48±0.15mg/dl) and after (0.39±0.05mg/dl) treatment. No increase in serum bilirubin level was recorded for group III (0.5mg/dl).

4.5 CLINICAL PATHOLOGY IN HAEMOPARASITE INFECTIONS

4.5.1 Haematology

The changes in the haematological parameters in the different haemoparasite infections are given in the table 9 and 10.

Though the haematological parameters were drastically reduced in all haemoparasite infections it was more severe in anaplasmosis. The anaplasmosis affected group recorded the lowest mean values for PCV, haemoglobin concentration and TEC. The mean values recorded for these parameters were 17.67±1.36 per cent, 5.87±0.38 g/dl and 3.07±0.28 $x10^6/mm^3$. The mean values observed for PCV, TEC and haemoglobin concentration in case of babesiosis affected group were 18.93±1.00 per cent,3.13±0.20 $x10^6/mm^3$and 5.92±0.20 g/dl. The theileriosis affected group recorded a mean PCV of 21.5±1.89 per cent, haemoglobin concentration of 6.8±0.40 g/dl and TEC of 3.68±0.62 $x10^6/mm^3$.

Regarding erythrocytic indices, the values obtained in all the four haemoparasite infections were within the normal range. The highest mean value for MCV was recorded in theilerial infections (61.70±6.61 fl) while the highest mean values for MCH (20.08±1.35 pg) and MCHC (33.62±0.99 per cent) were recorded in anaplasmosis. Thrombocyte count did not present any significant decrease in these infections. The thrombocyte count in the single recorded case of ehrlichiosis was $3.56x10^5/mm^3$. No significant alteration was observed in the TLC of the affected animals. The total leukocyte count recorded in anaplasmosis, theileriosis and babesiosis affected animals were 6.53±0.77 $x10^3/mm^3$,

6.89±1.27 $x10^3/mm^3$ and 7.05±0.63 $x10^3/mm^3$ respectively. The haemoparasite infections caused an eosinophilic response and the highest mean value for differential eosinophilic count was observed in babesiosis (5.27±0.44 per cent).

4.5.2 Serum Biochemistry

The serum biochemical alterations in the different haemoparasite diseases are presented in the table 11.

Both anaplasmosis (6.28±0.18g/dl) and babesiosis (6.00±0.22g/dl) recorded a considerable decrease in the serum total protein concentration while it was not that severe in theileriosis (6.62±0.40g/dl) affected group. The mean values for serum albumin concentration in anaplasmosis, babesiosis and theileriosis were 2.83±0.05g/dl, 2.89±0.12g/dl and 2.95±0.16g/dl respectively. Though a considerable increase in serum globulin concentration was not observed, the highest mean value was recorded in the theileria affected group (3.68±0.28 g/dl). AG ratios were within the normal range in all cases and were recorded as 0.84±0.04 for anaplasmosis, 0.95±0.05 for babesiosis and 0.81±0.05 for theileriosis.

Theilerial infection (33±3.58 mg/dl) recorded the lowest serum glucose concentration and the highest mean value for serum bilirubin concentration (0.99±0.52mg/dl).

Since only a single case was encountered in the study, the mean value for different parameters could not be calculated in case of ehrlichiosis.

4.6. TREATMENT

Suitable therapy was adopted depending on the etiological factors. Apart from the specific therapy, supportive therapy was adopted in appropriate cases. The supportive therapy included intravenous dextrose (five per cent) administration, oral or parenteral haematinics and vitamin supplements. Oral haematinics were used in 30 cases since it was most economical. Intramuscular

administration of iron dextran (imferon) @ 6ml /animal was attempted in ten cases. It was administered twice at three days interval.

Treatment response was evaluated by assessing the clinical response and improvement in the haematological and biochemical parameters by tenth day post treatment. Clinical response in subsequent days of treatment was recorded in all cases.

Administration of oxytetracyclines evoked desirable clinical response in all cases of anaplasmosis, theileriosis and ehrlichiosis. Fever subsided and feed intake improved by second day itself. But reduction in milk yield and respiratory distress still persisted in most cases. The colour of the mucous membranes remained towards the paler side. Gradual improvement was observed in the subsequent days of treatment. A complete clinical cure was observed by tenth day by which the haematobiochemical profile of the animals showed marked improvement in the condition. The mucous membranes was almost normal (pale pink) by this period and respiratory difficulties vanished.

In case of babesiosis the animals showed marked improvement after the single intramuscular injection of diminazene aceturate except for a case wherein the drug was repeated after 48 hours .Haemoglobinuria subsided and the animal started taking feed. There was gradual improvement in the general condition of the animal and haematobiochemical parameters were found to be normal by tenth day.

In group II animals the gastrointestinal disturbances lessened with the administration of anthelmintics. The dung was of normal consistency by third day of treatment. The feed intake improved and the general body condition of the animals improved in the subsequent days. By tenth day, a marked improvement in the condition and haematobiochemical parameters could be observed.

Thus the treatment regime adopted was found suitable in most cases and uneventful recovery occurred with the haematological and biochemical parameters returning to the normal range within ten days. However, a few

casualties were recorded (five cases in group I and one case each in group II and group III).

The death in group II was that of a six month old calf heavily positive for strongyles. The animal was passing blood mixed diarrhoeic faeces and was dehydrated. A packed cell volume of 18 per cent and haemoglobin concentration of 5.4 g/dl was recorded. Death occurred on the first day of clinical presentation.

The single case of tick infestation was recorded in a ten day old calf with a considerably low packed cell volume of 10 per cent and haemoglobin concentration of 3g/dl.The animal was not sucking milk and was in a recumbent stage. Death occurred on the day the case was presented.

In group I the death included two cases each of theileriosis and babesiosis and one case of anaplasmosis. The first case of babesiosis occurred in a ten day old calf. The animal was in recumbent stage and with a subnormal temperature. The packed cell volume and haemoglobin concentration was 10 per cent and 3 g/dl respectively. The second case of babesiosis was recorded in an adult cattle. The blood smear revealed a high per cent of parasitemia in this case. The packed cell volume was 16 per cent and haemoglobin concentration was 5g/dl. Death occurred by the first day of clinical presentation in these two cases.

The cases of theileriosis were recorded in cattle above three years of age. One case was heavily positive for the theilerial organisms. Peripheral smear revealed large numbers of schizont affected lymphocytes and intra erythrocytic piroplasms. The packed cell volume and haemoglobin concentration were 16 per cent and 5.4g/dl respectively. The animal died by next day. In the latter case only few intraerythrocytic piroplasms were observed. But the animal was very weak and did not respond well to the therapy. Death occurred by third day of treatment.

Death due to anaplasmosis was recorded in a 15 day old calf. A considerably low packed cell volume (13 per cent) and haemoglobin concentration (4.2g/dl) was recorded in this case. The calf died by next day of clinical presentation.

Table 3. Comparison of haemogram of control and clinical cases before treatment (Mean±SE).

PARAMETERS	CONTROL	GROUP I	GROUP II
PCV (per cent)	29.5±1.60[b]	18.8±0.69[a]	18.88±1.39[a]
Haemoglobin (g/dl)	9.97±0.50[b]	6.03±0.22[a]	6.05±0.44[a]
Total RBC count ($x10^6/mm^3$)	5.49±0.35[b]	3.19±0.16[a]	3.58±0.30[a]
MCV (fl)	56.27±3.24	60.45±1.40	54.14±2.81
MCH (pg)	18.22±1.31	19.50±0.57	17.31±1.14
MCHC (g/dl)	33.86±1.14	32.20±0.49	31.94±0.99
Platelet ($x10^5/mm^3$)	5.38±0.52	4.63±0.23	4.90±0.45

Means bearing the same superscript in a row do not differ significantly at $P< 0.05$

Table 4. Haemogram of control and clinical cases after treatment (Mean±SE)

PARAMETERS	CONTROL	GROUP I	GROUP II
PCV (per cent)	29.5±1.60[b]	25.96±0.64[a]	24.86±1.55[a]
Haemoglobin (g/dl)	9.97±0.50[b]	8.33±0.18[a]	8.06±0.55[a]
Total RBC count ($x10^6/mm^3$)	5.49±0.35[b]	4.53±0.16[a]	4.26±0.43[a]
MCV (fl)	56.27±3.24	57.86±0.96	60.61±3.77
MCH (pg)	18.22±1.31	18.62±0.40	19.54±1.06
MCHC (g/dl)	33.86±1.14	32.17±0.41	32.34±0.47
Platelet ($x10^5/mm^3$)	5.38±0.52	4.99±0.17	5.06±0.39

Means bearing the same superscript in a row do not differ significantly at $P< 0.05$

Table 5. Comparison of leucogram of control and clinical cases before treatment (Mean±SE)

PARAMETERS	CONTROL	GROUP I	GROUP II
Total WBC count ($x10^3/mm^3$)	6.16±0.92	6.76± 0.40	7.68±0.79
Neutrophil (per cent)	23.67±1.31	26.05±0.57	25.25±1.13
Lymphocyte (percent)	$70.67±1.60^b$	$66.56±0.69^a$	$70.00±1.38^b$
Monocyte (per cent)	3.17±0.56	2.81±0.24	2.25±0.49
Eosinophil (per cent)	$2.17±0.80^b$	$4.56±0.35^a$	$2.25±0.69^b$

Means bearing the same superscript in a row do not differ significantly at $P< 0.05$

Table 6. Leucogram of control and clinical cases after treatment (Mean ± SE)

PARAMETERS	CONTROL	GROUP I	GROUP II
TotalWBCcount($x10^3/mm^3$)	06.16±0.92	07.53±0.41	07.09±0.46
Neutrophil (per cent)	23.67±1.31	24.50±0.42	24.52±1.02
Lymphocyte (per cent)	70.67±1.60	69.52±0.55	70.29±0.57
Monocyte (per cent)	03.17±0.56	03.33±0.23	03.57±0.37
Eosinophil (per cent)	02.17±0.80	02.19±0.26	01.14±0.40

Means bearing the same superscript in a row do not differ significantly at $P< 0.05$

Table 7. Comparison of serum biochemistry of control and clinical cases before treatment (Mean±SE)

PARAMETERS	CONTROL	GROUPI	GROUP II
Total protein (g/dl)	7.10±0.32^{b}	6.21±0.14a	5.98±0.27a
Albumin (g/dl)	3.48±0.17^{b}	2.85±0.07^{a}	2.88±0.15^{a}
Globulin (g/dl)	3.63±0.26	3.36±0.11	3.11±0.22
AG ratio	0.97±0.08	0.88±0.03	0.97±0.07
Glucose (mg/dl)	54.00±3.32^{b}	37.19±1.44^{a}	44.00±2.87^{c}
Bilirubin (mg/dl)	0.22±0.18^{b}	0.88±0.08^{a}	0.48±0.15^{b}

Means bearing the same superscript in a row do not differ significantly at $P< 0.05$

Table 8. Serum biochemistry of control and clinical cases after treatment (Mean±SE)

PARAMETERS	CONTROL	GROUP I	GROUP II
Total protein (g/dl)	7.10±0.32	6.90±0.11	6.61±0.16
Albumin (g/dl)	3.48±0.17	3.29±0.06	3.37±0.25
Globulin (g/dl)	3.63±0.26	3.61±0.10	3.24±0.31
AG ratio	0.97±0.08	0.93±0.04	1.14±0.18
Glucose (mg/dl)	54.00±3.32^{b}	48.52±0.82^{a}	54.14±3.87^{b}
Bilirubin (mg/dl)	0.22±0.18	0.37±0.03	0.39±0.05

Means bearing the same superscript in a row do not differ significantly at $P< 0.05$

Table 9. Effect of haemoparasites on haemogram (Mean±SE)

PARAMETERS	Anaplasma	Babesia	Theileria
PCV (per cent)	17.67±1.36	18.93±1.00	21.5±1.89
Haemoglobin (g/dl)	5.87±0.38	5.92±0.20	6.8±0.40
Total RBC count ($x10^6/mm^3$)	3.07±0.28	3.13±0.20	3.68±0.62
MCV (fl)	59.26±2.59	61.3±1.72	61.70±6.61
MCH (pg)	20.08±1.35	19.02±0.47	19.83±2.70
MCHC (g/dl)	33.62±0.99	31.18±0.66	31.85±1.27
Platelet($x10^5/mm^3$)	4.37±0.29	5.12±0.35	3.21±0.40

Table 10. Effect of haemoparasites on leucogram (Mean±SE)

PARAMETERS	Anaplasma	Babesia	Theileria
Total WBC count ($x10^3/mm^3$)	6.53±0.77	7.05±0.63	6.89±1.27
Neutrophil (per cent)	26.33±0.86	25.50±0.90	27.25±0.65
Lymphocyte (per cent)	66.92±1.36	66.67±0.88	64.5±1.66
Monocyte (per cent)	3.17±0.42	2.53±0.27	3.25±0.85
Eosinophil (per cent)	3.58±0.48	5.27±0.44	5.00±1.73

Table 11. Effect of haemoparasites on serum biochemical values (Mean±SE)

PARAMETERS	Anaplasma	Babesia	Theileria
Total protein (g/dl)	6.28±0.18	6.00±0.22	6.62±0.40
Albumin(g/dl)	2.83±0.05	2.89±0.12	2.95±016
Globulin(g/dl)	3.45±0.17	3.12±0.14	3.68±0.28
AG ratio	0.84±0.04	0.95±0.05	0.81±0.05
Glucose (mg/dl)	37.92±1.22	36.73±1.76	33±3.58
Bilirubin (mg/dl)	0.97±0.10	0.76±0.11	0.99±0.52

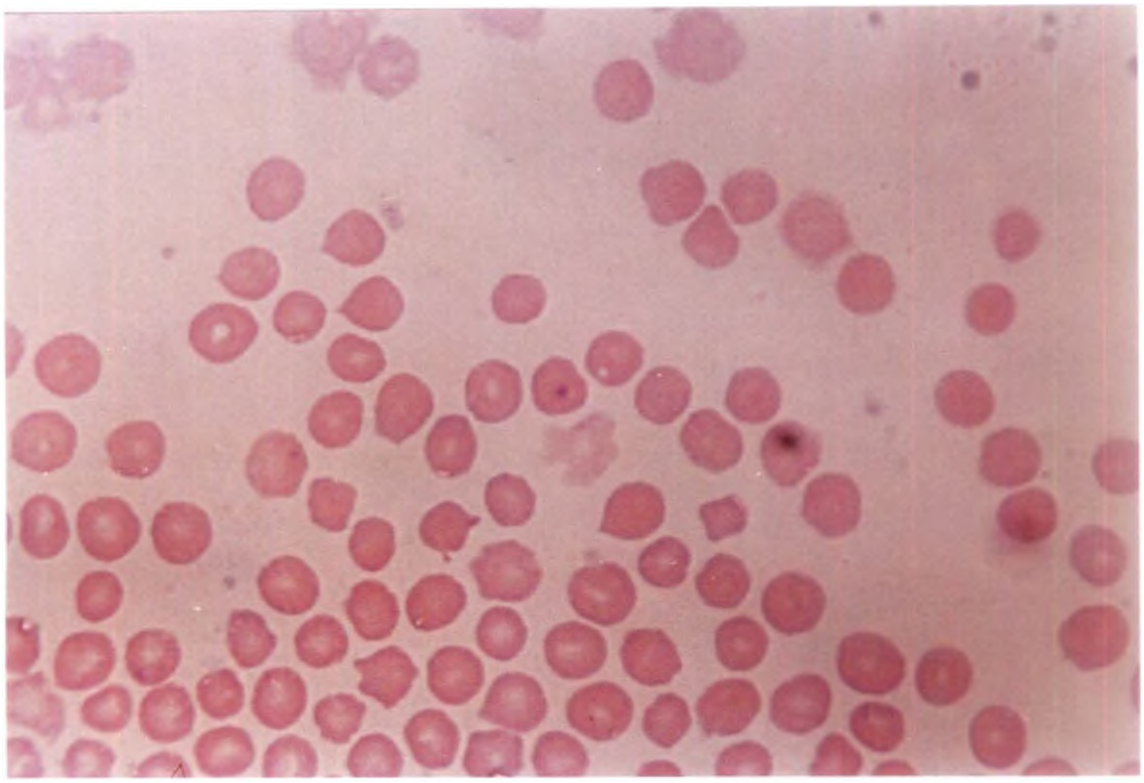


Fig. 2. Peripheral blood smear showing intraerythrocytic Anaplasma organism (Giemsa staining x1000)

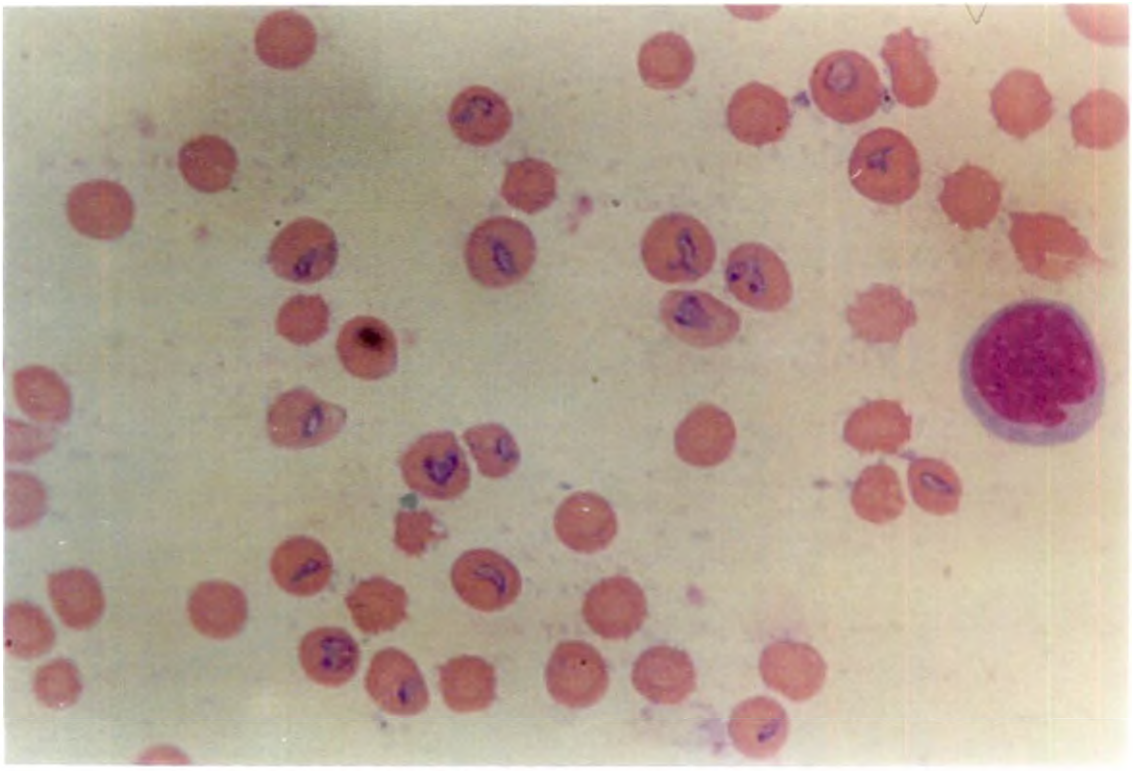
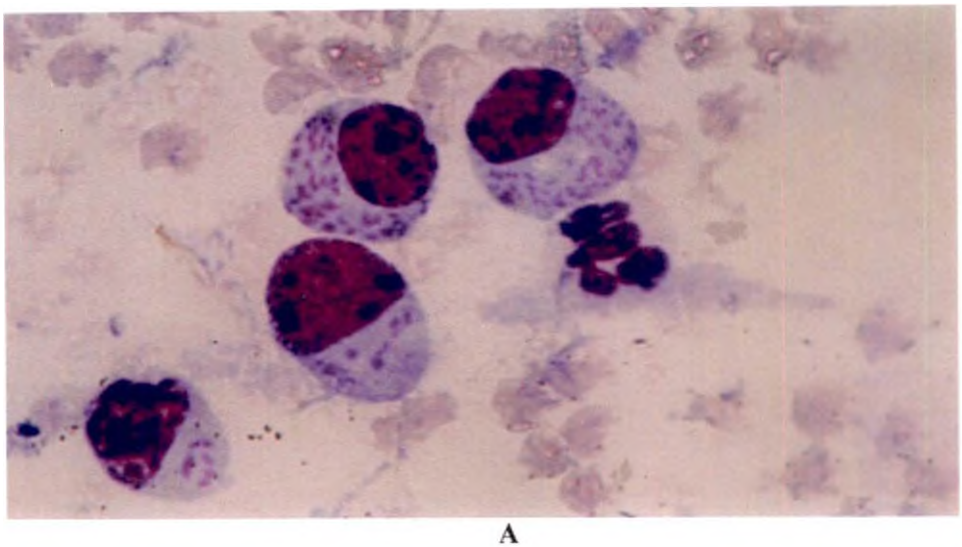
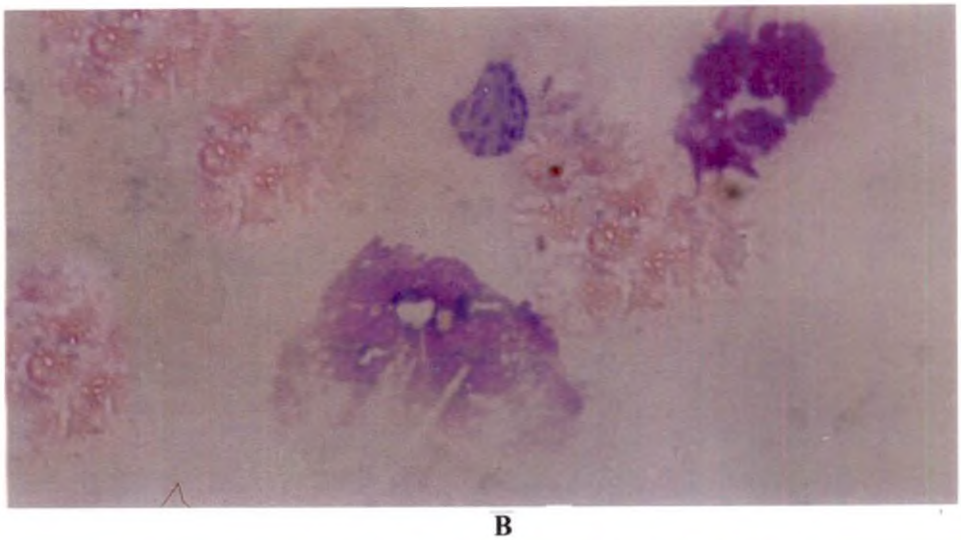


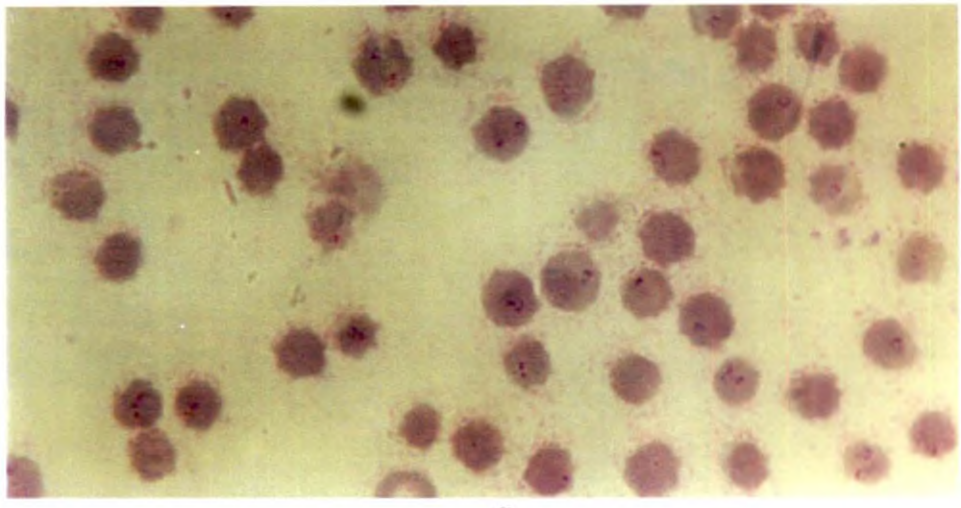
Fig. 3. Paired Babesia piroplasms inside the erythrocytes (Giemsa staining, x1000)



A



B



C

Plate 1. Blood smear (Giemsa staining, x1000)

A- Koch Blue Bodies inside the lymphocytes, B-macroschizonts outside the cells, C- intraerythrocytic Theilerial piroplasms

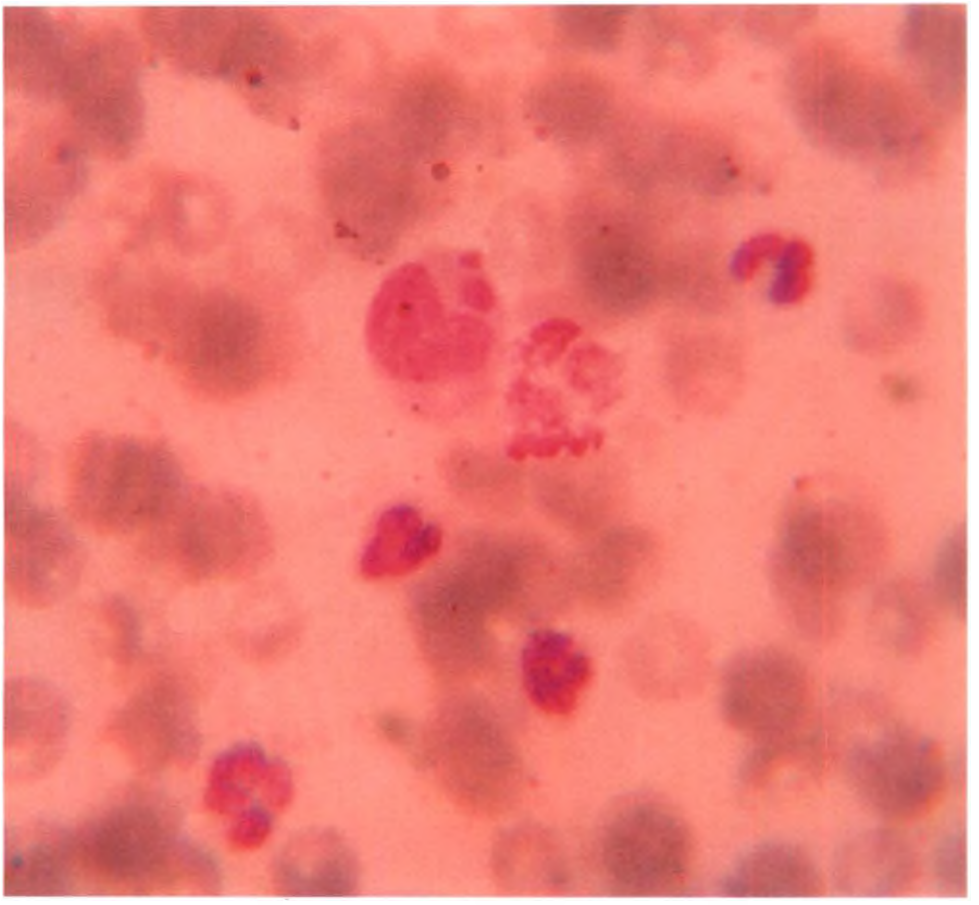


Fig. 4. *Ehrlichia bovis* morula inside the monocyte.

Discussion

5. DISCUSSION

Anaemia is one of the important clinical manifestations seen in animals under field conditions (Katoch and Mandial, 2003). Though a number of possible causes have been suggested for anaemia, parasitic causes require special mention. The present study envisages an insight into parasitic anaemia in cattle and studying the different probable etiological factors and adoption of a suitable therapeutic regimen for the same.

5.1. INCIDENCE OF PARASITIC ANAEMIA

A total of fifty anaemic cattle were screened for the presence of parasites out of which 41 turned to be positive. Thus with a higher incidence of 85 percentage, parasites turned to be the prime factor in the etiology of anaemia in cattle.

Parasites as an important etiological factor for anaemia has been highlighted by many authors (Schalm, 1972). Similar observations were recorded by Samanta *et al.* (1995) who recorded an incidence rate of 79 per cent for parasitic anaemia in cattle.

The parasitic etiology in the present study included haemoparasites, intestinal helminths and ectoparasites. Haemoparasites were the predominant infection with an incidence rate of 78 per cent followed by intestinal helminths (19 per cent) and ectoparasites (2.5 per cent).This was in agreement with the findings of Metenawy (2000) who also recorded a higher prevalence rate of haemoparasites in a screening study in cattle (77 per cent).

Anaemia due to ectoparasites was found to be least significant as the cause of anaemia in cattle. Only a single case was recorded. Literature regarding anaemia due to ectoparasites was scanty. Otter *et al.* (2003) recorded anaemia and mortality due to lice infestation in calves. But Jonsson *et al.* (1998) could not find any haematological alteration in cattle infested with lice. Jennings (1976) opined

that ectoparasites could cause anaemia only if the infestations were severe enough.

5.2. EFFECT OF AGE

Age of the animal was found to be an important factor in determining the susceptibility to various parasitic infections. It was significant to observe that calves had more helminth infection than adults. Increased incidence of nematode infection in calves has been documented by many authors (Gupta and Chabra, 1990; Borthakur and Das, 1998; Rao *et al.*, 2000 ; Pal *et al.*, 2001). The single case of amphistomosis was recorded in a three year old cattle. Manna *et al.* (1994) reported higher incidence rate of amphistomiasis in adult cattle. The acquired immunity in adult cattle plays an important role in warding off most nematode infection (Radostits *et al.*, 2000).

Regarding haemoparasites majority of cases were observed in cattle above one year of age. Only three cases were recorded in calves below six months of age. Calves are considered to be least susceptible to the infection by Babesia and Anaplasma (Roby *et al.*, 1961; Jones and Brock, 1966; Radostits *et al.*, 2000 ; Latif *et al.*, 1979). But no such immunity has been suggested in case of Theileria. All animals were susceptible to theileriosis with higher mortality in young animals (Al –Atya *et al.*, 1991).

In spite of the stated immunity, one case each of anaplasma and babesia was recorded in calves below fifteen days of age. This could be due to transplacental transmission and such incidences have been reported by Zaugg (1985), Potgieter and Rensburg (1987) and Ganesan *et al.* (1995). Mallick *et al.* (1980) reported the rare occurrence of babesiosis in a ten day old calf while Roychoudhury *et al.* (1976) reported clinical cases of babesiosis in calves below thirty days of age. In the present study since the dams were not available presence of organisms in dams could not be confirmed.

Tick infestation can occur in animals irrespective of age. But it is supposed to be pathogenic mostly in young animals since adult animals can resist

the harmful effects. In the present study a single case of anaemia due to ectoparasites was observed in a ten day old calf. Otter *et al.* (2003) described cases of anaemia and mortality in calves infested with *L. vituli*. The results described indicated that ectoparasites may be of more pathogenic significance when it is present in large numbers in young calves.

5.3. CLINICAL MANIFESTATION

In general, anaemic animals showed pallor of visible mucous membranes, exaggeration of respiration, anorexia, weakness and reduced milk production in dairy animals. Katoch and Mandial (2003) recorded similar findings in anaemic animals. Depending on the etiologic factors specific symptoms varied in affected animals.

In haemoparasite infections, fever, anorexia, enlargement of superficial lymph nodes, nasal discharge and increased salivation were noticed. Similar clinical findings in haemoparasite infections have been recorded before by Mallick *et al.* (1980), Mbassa *et al.* (1994), Devada *et al.* (1996), Chitravel *et al.* (1998), Soodan *et al.* (2000) and Patel *et al.* (2001).

Marked lymph node enlargement and icterus were observed in cases of anaplasmosis and theileriosis. Jones and Brock (1966) opined that in anaplasmosis, the increased destruction of erythrocytes in the reticuloendothelial system resulted in the enlargement of lymphatic glands, spleen and liver. Similar observations in theileriosis have been reported by Maxie *et al.* (1981) and Mbassa *et al.* (1994).

The unique symptom of haemoglobinuria was observed in all 15 cases of babesiosis except for a single case. All other typical symptoms of babesiosis infection were present and blood smears on microscopical examination revealed intra erythrocytic Babesia piroplasms. Radostits *et al.* (2000) described a sub acute syndrome of babesiosis where the haemoglobinuria was absent.

Helminth infection in calves was often evident by the pallor of the mucous membranes, weakness, rough hair coat, diarrhoea or constipation and pot bellied appearance (Pandey and Misra, 1985; Devi *et al.*, 2000; Panda *et al.*, 2002). The findings in the present study were in consistent with these authors.

In ectoparasite infestations, the calf was in recumbent stage and was not sucking. The poor management together with the heavy infestation and young age might have predisposed to such a poor condition.

5.4. CLINICAL PATHOLOGY

5.4.1. Haematology

The haematological results were consistent with anaemia as defined by Kramer *et al.* (2000).

5.4.1.1. Packed cell volume, haemoglobin and total erythrocyte count

PCV, haemoglobin and TEC are the three important parameters to assess the anaemic status in an animal.

The mean values of PCV, haemoglobin concentration and TEC were significantly decreased in all three groups. A reduction in these parameters in group I can be attributed to the increased destruction of erythrocytes as suggested by Jatkar and Krier (1970) , Sandhu *et al.* (1998) and Singh *et al.*(2001) or depression of erythropoiesis as suggested by Pandey and Misra (1987) and Maxie *et al.*(1981).

In helminth infection, the development of anaemia involves a number of mechanisms. Baker and Douglas (1957) opined that blood sucking activities of the parasites and abomasal haemorrhage contributed to the anaemia in strongyle infections. Mulligan *et al.* (1963) suggested that low plane of nutrition together with defective intestinal absorption resulted in anaemia in trichostrongyle infections. A suppression of the erythropoiesis was suggested in fascioliasis by Sinclair (1965).

The mean values for packed cell volume, haemoglobin concentration and total erythrocyte count were significantly decreased in the case of tick infestation. The heavy burden of the ticks together with the poor nutritional status might have predisposed to anaemia. Otter *et al.* (2003) reported anaemia and mortality in calves infested with *L. vituli.*

After treatment even though the values raised to the normal range significant difference was observed from the control animals in group I and group II. The severity of the anaemia in the animal warrants a lag period for the normalization of these parameters.

5.4.1.2. Erythrocytic indices (MCV, MCH, MCHC)

The mean values for MCV, MCH and MCHC did not present any significant alterations in any of the groups under consideration. Even though the packed cell volume, haemoglobin concentration and total erythrocyte count was reduced significantly, the erythrocytic indices ranged within the normal limits. This indicates that normocytic normochromic anaemia occurred in all three groups. Sandhu *et al.* (1998) and Mbassa *et al.* (1994) described normocytic normochromic anaemia in cases of theileriosis. Pandey and Misra (1985) described normocytic normochromic anaemia in calves infected with *N. vitulorum*. Pandey and Misra (1987) reported normocytic normochromic anaemia in cases of babesiosis in cattle.

5.4.1.3. Total Leukocyte Count

Significant leucocyte responses were not recorded in the diseased animals under study.

5.4.1.4. Thrombocyte Count

Thrombocyte count in all cases did not present any significant reduction from the mean value of control animals. The mean values recorded for the group I ($4.63\pm0.23 \times 10^5/mm^3$) and group II ($4.90\pm0.45 \times 10^5/mm^3$) were within the normal limits.

Thrombocytopenia has been suggested in case of ehrlichiosis. But in the present study, the single case of ehrlichiosis recorded a thrombocyte count of $3.56x\ 10^5/mm^3$ which was within the normal limits.

5.4.1.5. Differential Count

The type of cells that varied significantly in the study was lymphocytes and eosinophils and the variation was recorded in the haemoparasite infected group. No significant difference was recorded for the mean values of differential count in group II.

Parasitic infections are known to trigger an eosinophilic response in the body. This is more pronounced in the case of haemoparasite infections (Radostits *et al.*, 2000). Decrease in lymphocyte count could be attributed to the stress that prevailed during infections.

5.4.2. Serum Biochemistry

5.4.2.1. Serum Total Protein

Significant reduction in serum total protein levels was recorded in all three groups. Reduction in the serum albumin concentration is often reflected as the reduction in serum total protein. In helminth infection, loss of protein through damaged mucosa and hindered intestinal absorption can lead to reduced serum protein levels (Pandey and Misra, 1985; Bandyopadhyay and Dasgupta, 2000). Bharti and Prasad (2001) opined that disturbances in liver function due to tissue damage caused by Paramphistomum and Fasciola predisposed to hypoproteinaemia.

In haemoparasite infections, the fall in the serum total protein occurs due to reduced albumin synthesis because of the functional affection of the liver. The increased destruction of erythrocytes in haemoparasite infections causes stress to the normal functioning of the liver. Shastri *et al.* (1991) has described inflammatory and degenerative changes in the liver of cattle died due to babesiosis. Sandhu *et al.* (1998) described that *T. annulata* infection caused

hepatic tissue damage that included coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the periportal areas indicating severe damage to hepatobiliary system due to hypoxia resulting from anaemia and jaundice. Allen *et al.* (1981b) opined that in anaplasmosis decreased albumin concentration resulted from negative nitrogen balance due to the anorexic status of the animal. Similar findings were reported by Singh *et al.* (2001) and Patel *et al.* (2001).

The single case of ectoparasite infestation recorded a serum protein concentration of 4g/dl. Vatsya (2003) has recorded a low serum protein concentration in experimental Boophilus infestation in calves. The continous loss of blood due to the feeding ticks can lead to hypoproteinemia.

5.4.2.2. Serum Albumin

Serum albumin concentration levels were significantly reduced in all the three affected groups. The reduction in the serum albumin could be attributed to reduced production by the affected liver. The increased destruction of erythrocytes and the hypoxia will lead to degenerative changes in the liver. This is in agreement with the findings of many authors (Sandhu *et al.,* 1998 and Shastri *et al.,* 1991). Reduced feed intake due to the anorexia in the affected animals also added to hypoproteinemia (Singh *et al.,* 2001).

5.4.2.3. Serum Globulin

No significant variation was observed in the serum globulin levels between the diseased groups and control groups. The serum globulin levels are supposed to increase because of the immune response against the specific etiological agent. But an increase in the serum globulin level cannot be expected within few days post infection since the body takes considerable time to put a humoral response. This might have contributed to the normal serum globulin level in the study.

5.4.2.4. AG Ratio

No significant variation was observed for the AG ratio. The values recorded ranged within the normal levels.

5.4.2.5. Serum Glucose

Serum glucose was reduced drastically in group I animals (37.19±1.44).

Yadav and Sharma (1986) observed rapid fall in blood glucose in theilerial infection. They opined that this could be due to utilization of glucose by parasites since it occurred during the period of rapid growth of both the schizonts and erythrocytic forms of *T. annulata.* After treatment although serum glucose level came to normal level in group II animals, a significant decrease still persisted in group I animals. This may be due to the severity of infection and would return to desired level with time.

5.4.2.6. Serum Bilirubin

Higher serum bilirubin values were recorded in haemoparasite infections. The heme from the lysed erythrocytes is converted into bilirubin by specific enzymes (Benjamin, 2001). Sandhu *et al.* (1998) suggested that increased destruction of erythrocytes in theileriosis resulted in increased levels of bilirubin. Dhar and Gautam (1977) suggested that rapid destruction of erythrocytes resulted in increased bilirubin production in acute infections of theileriosis. This was not being excreted quickly in bile due to liver damage leading to increased blood levels. These findings were in agreement with Bhikane *et al.* (2001) and Patel *et al.* (2001)

5.5. CLINICAL PATHOLOGY IN HAEMOAPARASITE INFECTIONS

5.5.1 Haematology

Anaplasmosis affected group recorded the lowest mean values for PCV (17.67±1.36 per cent), TEC (3.07±0.28 $x10^6/mm^3$) and haemoglobin concentration (5.87±0.38 g/dl). Various mechanisms have been suggested for the

pathogenesis of anaemia in anaplasmosis. Hansard and Foote (1959) had substantiated from ferrokinetic studies and histopathologic observations that a considerable depression of the haemopoietic system occurred in anaplasmosis. Baker *et al.* (1961) reported that an increased destruction of erythrocytes occurred in anaplasmosis that contributed to the severe anaemia. Production of antierythrocytic antibodies leading to anaemia in anaplasmosis has been recorded by Ristic (1961).

In case of babesiosis, the anaemia coincides with the lysis of erythrocytes that occurs during the release of the piroplasms from the infected cells (Pandey and Misra, 1987). Maxie *et al.* (1981) opined that a toxic depression of the erythropoietic system occurred in theileriosis which predisposed to anaemia. Similar observations have been made by Mbassa *et al.* (1994). Thus an increased severity of anaemia could be expected in anaplasmosis and theileriosis since the haemopoietic system is affected. In babesiosis an adequate regenerative response from bone marrow can compensate for the increased intravascular erythrolysis. These factors explain the drastically reduced haematological parameters in anaplasmosis in the present study. The haematological parameters in the theileriosis affected group were not severe as expected. This could be due to the considerably less number of cases in the study.

The mean values for erythrocytic indices were within the normal range in all the haemoparasite infections. This suggested a normochromic normocytic anaemia. Sandhu *et al.* (1998) and Mbassa *et al.* (1994) reported normocytic normochromic anaemia in theileriosis. Similar observations on babesiosis has been suggested by Pandey and Misra (1987).

Significant changes in total leucocyte count and thrombocyte count were not recorded in the haemoparasite infections in the study. Slight eosinophilia was observed in babesiosis which is in agreement with Radostits *et al.* (2000).

5.5.2 Serum Biochemistry

Decrease in serum protein levels was seen in all the infections, the most severe being in babesiosis. The defective liver function resulting from the anoxia due to anaemia can affect the normal production of protein by liver. This could be reflected as the reduction in the serum protein and albumin. The observations made by many authors confirm this (Singh *et al.*, 2001; Patel *et al.*, 2001 ; Allen *et al.*, 1981b). No considerable changes in the serum globulin levels and AG ratio were observed.

Theileriosis affected group recorded the lowest value in serum glucose and this is in agreement with the findings of Yadav and Sharma (1986). Serum bilirubin levels were the highest in theileriosis infections and similar observations were made by Dhar and Gautam (1977).

5.6 TREATMENT

Specific therapy was adopted depending on the etiological agent. Oxytetracycline was given at the dose rate of 10 mg/ kg bodyweight intravenously for five days for treating cases of anaplasmosis, theileriosis and ehrlichiosis. Oxytetracycline at the dose rate of 10mg/kg body weight has been suggested by Radostits *et al.* (2000) for treating anaplsmosis and ehrlichiosis. Singh *et al.* (1980) has reported that oxytetracycline at the dose rate of 10-15 mg/kg body weight for four to six days was effective in treating *T. annulata* infection. Cases of babesiosis were treated by single intramuscular injection of diminazene aceturate which is in accordance with Mallick *et al.* (1980) and Bhikane *et al.* (2001).

Two subcutaneous administration of ivermectin @ 200µg /kg bodyweight two weeks apart was done in treatment of strongyloides. This is in agreement with the findings of Islam *et al.* (2003) who recommended the same for the treatment of gastro intestinal nematodes. Oxyclosanide @ 10mg/kg bodyweight was used in treatment of amphistomiasis as suggested by Manna *et al.* (1994). Albenbazole was used successfully @ 5mg/kg bodyweight in treating cases of

strongyles and whipworms. This is in accordance with the findings of Sanyal *et al.* (1998).

Apart from the specific therapy, supportive therapy was adopted in appropriate cases which included intravenous fluids, haematinics and vitamin supplements. Oral haematinics was tried in most cases because of the economic aspect and also produced good results. Rajora *et al.* (1995) opined that oral preparations of haematinics were economic, easy to administer and produced no adverse effects.

Success of treatment was confirmed by noting daily clinical response and improvement of haematobiochemical parameters by tenth day. The treatment was found appropriate in most cases. But parameters like PCV, haemoglobin concentration and TEC were significantly decreased from the control group even by tenth day of treatment. This can be explained in terms of the lag period which occurs for the normalization of these parameters in a severely affected animal.

However, the few deaths that were recorded (five cases in group I and one case in group II and one case in group III) might be due to the severity of the disease and worse condition of the animal. The severity of the affection of the animals was apparent from the reduced haematological parameters. The packed cell volume was 10 per cent or nearly ten per cent in few cases and in such cases increased fatality could be expected. Palmer and Lincoln (2002) opined that a packed cell volume less than ten per cent in anaplasmosis resulted in the death of the animal.

Zaugg (2002) stated that in babesiosis, PCV values dropped rapidly from 35 per cent to below 10 per cent in a week after the onset of clinical signs. They opined that acute cases with PCV values above 12 per cent usually responded well to treatment and the prognosis was decreased for cases with PCV values below 10 per cent. But, the adult cattle cattle died due to babesisosis recorded a packed cell volume of 16 per cent. This could be due to high parasitemia that occurred. On microscopical examination, a large number of erythrocytes per field

were infected. Thus the acute nature of the disease might have resulted in the death of the animal. Similar was the case of theileriosis which also recorded a high per cent of parasitemia. The schizonts could be detected even in the peripheral blood smear. This clearly explains the acute nature of the disease. In the second case of theileriosis which recorded only lower parasitemia, the animal was in a very bad condition. Increased icterus was observed in this case which indicated the extensive liver damage and hence might have resulted in the death of the animal.

Although the case of strongylosis recorded a packed cell volume of 18 per cent, the calf was severely dehydrated and very weak. The severe condition of the animal might have led to death.

Summary

6. SUMMARY

Anaemia is one of the important clinical abnormalities seen in animals under field conditions. It results either from an increased rate of destruction or loss of erythrocytes or from a decreased rate of their production. A number of possible causes have been suggested for anaemia and parasitic etiology requires special mention. In the present study titled "Clinical investigations on parasitic anaemia in cattle", 50 cattle showing clinical signs of anaemia were subjected to detailed screening for the presence of parasites. The animals that turned out to be positive were then subjected to detailed clinical examination and all parameters under study such as signalment, history, physical examination, haematology, serum biochemistry, adoption of suitable therapy and treatment response were carried out. Six apparently healthy cattle that were brought to the AI centre for the purpose of insemination were selected as control for studying the normal haematological parameters.

Out of the 50 anaemic animals screened, 41 turned to be positive for parasites. Remaining nine cases were excluded from the study as no parasitic etiology could be detected. Based on the diagnosis the clinical cases of parasitic anaemia were divided into three groups:

Group I (n=32) - Consisted of animals positive for haemoparasites. The various haemoparasites observed in the study included twelve cases of anaplasmosis, fifteen cases of babesiosis, four cases of theileriosis and a single case of ehrlichiosis.

Group II (n= 8) - Only eight cases of anaemia due to helminth infections were recorded which included four cases of trichuris, two cases of strongyloides and one case each of amphistome and strongyles

Group III (n=1) - A single case of tick infestation leading to anaemia was recorded in a ten day old calf.

Thus haemoparasites were the predominant infection with an incidence rate of 78 per cent followed by intestinal helminthes (19 per cent) and ectoparasites (2.5 per cent).

Age of the animal was found to be an important factor in determining the susceptibility to various parasitic infections. Incidence of nematode infection was observed more in calves. Majority of cases of haemoparasites were observed in cattle above one year of age. Transplacental transmission could be suggested in two calves below 15 days of age. Anaemia due to ectoparasites was seen in a ten day old calf and it indicated that ectoparasites could be of pathogenic significance when present in large numbers in young calves.

Clinical signs characteristic of anaemia were observed that included pallor of visible mucous membranes, exaggeration of respiration, anorexia, weakness and drastic reduction in milk yield in dairy animals. In haemoparasite infections fever, anorexia enlargement of superficial lymph nodes, nasal discharge, increased salivation and icterus were noticed. Anaemia and lymph node enlargement was most pronounced in cases of anaplasmosis and theileriosis. Haemoglobinuria was observed in most cases of babesiosis except for a single case. Helminth infections were characterized by anaemia, weakness, rough hair coat and digestive disturbances.

The clinical pathological studies revealed a decrease in packed cell volume, haemoglobin concentration and total erythrocyte count in all affected cases. But the erythrocyte indices (MCV, MCH, and MCHC) remained normal indicating normocytic normochromic anaemia. Mean values of thrombocyte count and total leukocyte count did not show any significant variation. Although thrombocytopenia has been suggested in ehrlichiosis, in the present study no thrombocytopenia was observed. The mean values for eosinophil (4.56±0.35 per cent) were increased in haemoparasites infections while the mean values for lymphocyte was decreased (66.56±0.69 per cent).In group II these remained similar to control group.

Significant reduction in serum total protein was recorded in all three groups. The mean values observed for group I, group II and control groups were 6.21±0.14 g/dl, 5.98±0.27 g/dl and 7.10±0.32 g/dl respectively. The total protein value recorded for the tick-infested animal was 4g/dl. The mean values of serum albumin in group I and group II and control group before treatment were 2.85±0.07 g/dl, 2.88±0.15 g/dl and 3.48±0.17 g/dl respectively. No significant changes in serum globulin and AG ratio were observed. The serum glucose levels was decreased in group I and group II the lowest being recorded in group I. In tick infested animal the serum glucose level was very low (29mg/dl).Increased serum bilirubin level was recorded in group I which was attributed to the enhanced destruction of erythrocytes and hepatic cell insufficiency.

Suitable therapy was adopted depending on the etiological factors. Intravenous administration of oxytetracyclines @ 10mg /kg bodyweight for a period of five days was done in cases of anaplasmosis, theileriosis and ehrlichiosis. Intramuscular injection of diminazene aceturate at the dose rate of 8mg/kg bodyweight was indicated in cases of babesiosis. Repetition of the drug was done after 48 hours in a single case. Two Subcutaneous administrations of ivermectin at the dose rate of 200 µg/kg bodyweight one week apart was found convincing in the treatment of strongyloides. Single oral administration of oxyclosanide was found to produce a suitable clinical cure in amphistome infection. In case of strongyle infections oral administration of albendazole at the dose rate of 5mg/kg body weight orally produced better clinical cure.

Apart from the specific therapy, supportive therapy was adopted in appropriate cases, which included intravenous fluids, oral and parenteral haematinics and vitamin supplements .Oral haematinics were used in most cases since it was most economical and produced good effects. Intramuscular administration of iron dextran (imferon) at the dose rate of 6ml /animal was attempted in ten cases. Treatment was found to be effective and good clinical response was obtained .However few deaths were recorded which was considered

to be due to the severity of infection as evident from the drastically decreased haematological parameters.

Thus in the present study it was concluded that:

1. Parasites especially haemoparasites could be considered as most important etiological factor in development of anaemia in cattle. Among haemoparasites infections, cases of babesiosis, anaplasmosis, theileriosis and ehrlichiosis were observed in the decreasing order of prevalence. The severity of anaemia was more in anaplasmosis followed by babesiosis, theileriosis and ehrlichiosis.

2. Clinical manifestations like pale mucous membranes and lymph node enlargement was markedly observed in anaplasmosis and theileriosis and this could be used as a diagnostic tool in the diagnosis of these diseases in field condition.

3. Age of the animal is important in determining susceptibility to infection. Helminth infection is more important in young cattle while haemoparasite is a threat in adult animals. Ectoparasites as a cause of anaemia is only less significant

4. Oral haematinics are economical and effective in supportive therapy of anaemia.

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CLINICAL INVESTIGATIONS ON PARASITIC ANAEMIA IN CATTLE

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ABSTRACT

The study "Clinical investigations on parasitic anaemia in cattle" was conducted in 50 cattle with clinical signs suggestive of anaemia. Out of the 50 animals screened 41 turned to be positive for parasites thus recording an incidence of 85 per cent for parasitic anemia. The parasitic etiology included haemoparasites, intestinal helminths and ectoparasites.

Haemoparasites recorded the highest incidence rate of 78 per cent followed by intestinal helminthes (19 per cent) and ectoparasites (2.5 per cent). Anaemia due to ectoparasites was found to be least significant and was found to be pathogenic only in calves if present in large numbers.

All animals were subjected to detailed clinical examination and all parameters under study viz; signalment, history, physical examination, haematology, serum biochemistry and treatment response were carried out. The data obtained were analyzed statistically. Anaemia due to intestinal helminthes was observed mostly in young calves while haemoparasites infection dominated in adult animals. Cases of anaplasmosis, and babesiosis were observed in calves below 15 days of age indicating possibility of transplacental transmission. Ectoparasites were found to cause anaemia in young calves when present in large numbers

Clinical manifestations of anaemia included pallor of visible mucous membranes, exaggeration of respiration, anorexia, production depression in milch animals etc. Clinical pathology revealed reduction of packed cell volume, haemoglobin concentration and total erythrocyte count. The erythrocyte indices were within the normal range indicating normocytic normochromic anaemia. No significant changes were recorded in the mean values of thrombocyte count and total leukocyte count. Differential count revealed eosinophilic response in haemoparasite

infection. Reduction in lymphocyte count was also recorded in haemoparasites infection.

Serum total protein and serum albumin levels recorded a considerable decrease in the affected groups. Serum globulin and AG ratio did not show any significant changes. Serum glucose level was decreased drastically especially in group I animals. Serum bilirubin levels were significantly increased in group I animals which was due to increased erythrocyte destruction.

Oxytetracyclines were used in treatment of anaplasmosis, theileriosis and ehrlichiosis. Diminazene aceturate was indicated in cases of babesiosis. Cases of strongyloides were treated by ivermectin administration. Albendazole was found effective in trichuris infection and oxyclosanide produced better clinical cure in amphistome infection. Apart from specific drugs, supportive therapy in the form of intravenous fluids, haematinics and vitamin supplements were given. Oral haematinics were found to be economical and effective.

After treatment although the haematological values came to normal levels, significant decrease from control group was observed in packed cell volume, haemoglobin concentration and total erythrocyte count. This was attributed to the lag period occurring for the normalization of these parameters. Thus treatment was found to be effective with good clinical response and haematobiochemical parameters returning to normal levels. But few deaths were recorded. This could be due to the severity of infection which was evident from the drastic reduction in the haematological parameters.