Haematological and biochemical alteration in theileriosis affected cattle

Suresh Chandra Behera

Adm. No. 02VPath/14

DEPARTMENT OF VETERINARY PATHOLOGY
COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY
ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
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By

Suresh Chandra Behera

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ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
DEPARTMENT OF VETERINARY PATHOLOGY
COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY

Dr. S.K. Panda
Professor and Head
Department of Veterinary Pathology
College of Veterinary Science and Animal Husbandry
Orissa University of Agriculture and Technology
Bhubaneswar-751003, Odisha

Bhubaneswar
Date :

CERTIFICATE-I

This is to certify that the thesis entitled **"Haematological and biochemical alteration in theileriosis affected cattle"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science (Veterinary Pathology)** to the Orissa University of Agriculture and Technology is faithful record of bonafide and original research work carried out by **Suresh Chandra Behera** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him from various sources during the course of investigation has been duly acknowledged.

CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **"Haematological and biochemical alteration in theileriosis affected cattle"** submitted by **Suresh Chandra Behera** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Veterinary Pathology)** has been approved/disapproved by the students' advisory committee and the external examiner.

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Dr. S.K. Panda
Professor and Head
Department of Veterinary Pathology
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   Department of Animal Breeding and Genetics
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   C.V.Sc. and A.H, O.U.A.T., Bhubaneswar _____

3. **Dr. P.K. Rath**
   Assistant Professor
   Department of Veterinary Pathology
   C.V.Sc. and A.H, O.U.A.T., Bhubaneswar _____

External Examiner _____
(Name & Designation)

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Bhubaneswar **Suresh Chandra Behera**

Date :

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LIST OF ABBREVATIONS

%	:	Percentage
µL	:	Microlitre
B	:	Basophil
BTT	:	Bovine Tropical Theileriosis
E	:	Eosinophil
Hb	:	Haemoglobin
L	:	Lymphocytes
M	:	Monocytes
Mg	:	Milligram
Mm	:	Millimole
N	:	Neutrophil
P	:	Phosphorus
PCV	:	Packed cell volume
TEC	:	Total erythrocyte count
TLC	:	Total leucocyte count

ABSTRACT

In our country incidence of theileriosis is rapidly increasing, causing production loss as well as life threatening for the crossbred cattle population. Prompt diagnosis of the disease is very much essential for which study of hemato-biochemical changes is one of the most important procedure. Present work was undertaken to study the haematological and biochemical alteration in theileriosis affected cattle for a period of twelve months from July 2015 to June 2016.A total of 1280 suspected cases with complaint of anorexia, non-remitting pyrexia, drop in milk yield, enlarged superficial lymph nodes, pale conjuctival mucous membrane, haemoglobinuria, nasal discharge and coughing which were suspected for theileriosis and screened for theileriosis on the basis of blood smear examination. Out of 1280 cases were examined for theileriosis, 928(72.5%)cases were found positive with presence of Piroplasms inside RBCs in blood smears examination indicating *Theileria annulata.* Adult animals (70.25%) are more exposed to theileriosis followed by 27.47% of 2-3 years of age and 2.58% of less than 1 year. Females are the most sufferers than its male counterpart. Crossbred Jersey seems to be more often affected. Mixed infection in theileriosis affected cattle are to be considered. The most important clinical signs observed during the study were reduced appetite, anorexia, non remittent fever, enlargement of superficial lymph nodes, salivation, lacrimation, recumbence, dropped milk yield, diarrhea, red eye which can be considered for diagnosis of the disease. Hemato-biochemical estimation can be utilised for diagnosis, prognosis and for better management of metabolic health status of the animal during the disease. The haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) values were distinctly lower in theileria positive animals than apparently healthy animals. The haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) values were distinctly lower in theileria positive animals than apparently healthy animals. Serum biochemical parameters like glucose, cholesterol, total protein, albumin, and globulin showed decreased values in the affected cattle than apparently healthy cattle. On the other hand average values of some other serum biochemical parameters like BUN, creatinine, AST, ALT, total bilirubin and direct bilirubin of affected cattle showed increased values than apparently healthy cattle. 56% of the urine samples showing positive result for ketosis indicates that animals affected with theileriosis are much more prone to ketosis than the healthier animals. This may be due to the metabolic disturbances caused by theileriosis.

CHAPTER I

INTRODUCTION

The cattle population contributes to the production of milk, making the country largest producer of milk in the whole world. But the average milk production per animal is very low with main reasons being low production capacity, poor nutrition and management issues including lack of proper tick control in the country.

Indian cattle industry to suffer from arthropod borne parasitic diseases as the climate favours easy maintenance and multiplication of vectors in this region (Dhar *et al*., 1987). Most of the haemoprotozoan diseases like theileriosis, babesiosis, anaplasmosis and trypanosomiasis are tick borne and of great economic importance. Among the tick borne diseases theileriosis is a big obstacle to livestock production.

Theileriosis is caused by the parasite belonging to the Genus *Theileriae* under Family *Theileridae*. Theileria are obligate intracellular protozoan parasites that infect both wild and domestic bovines throughout much of the world along with some species of small ruminants. Theileria parasites can be classified into pathogenic and nonpathogenic species based on their ability to induce lymphoproliferation in the infected host (Omer *et al*., 2003). *Theileria annulata* and *Theileria parva* are considered to be most pathogenic to cattle causing lymphoproliferation. *Theileria annulata* causes mediterranean coast fever or bovine tropical theileriosis or simply tropical theileriosis. The disease is common among the cattle population of North Africa, Southern Europe and Asia including India. *Theileria parva* causes East coast fever which is prevalent in eastern, central and southern Africa. Other theilerial parasites like *T. orientalis*, *T. buffeli* and *T. sergenti* are generally non-lymphoproliferative and thus nonpathogenic.

The severity of outcome is influenced by breed, age, immune status of the animal. It is also dependent on the number of sporozoites inoculated by feeding ticks. The pathological progression of the disease in a typical acute, often fatal, infection occurs through three stages each spanning about one week. The first stage comprises an incubation period of roughly around 7-8 days where neither parasite nor lesions can be detected. Following the incubation period, the second phase also of around a

week is marked by lymphoid hyperplasia, initially in the region of lymph node draining the area and later the whole body. This phase of infection is manifested by the onset of clinical signs including enlarged superficial lymph nodes, persistent fever (41^{0}– 42^{0}C), anorexia, congested mucous membranes, corneal opacity, emaciation, unthriftiness, infertility, tachycardia and tachypnea (Radostits *et al*., 2010). During the advanced third stage there is lymphoid depletion and disorganization associated with massive lymphocytolysis and depressed leucopoiesis causing severe leukopenia. Due to widespread destruction of the immune system, the animal shows dyspnoea, recumbency and finally death. Additionally, *T.annulata* infection is associated with profound changes in haematological and biochemical profiles and investigations of peripheral blood may reveal severe anaemia, leukopenia, lymphocytopenia, hypocalcaemia and hypoproteinemia. Many animals undergo a long period of convalescence and die entailing economic loss. A characteristic feature of these vector transmitted haemoparasitic diseases is that, the animals which recover from acute infection, become carriers, creating a potential source of infection to healthy susceptible population.

Diagnosis of theileriosis is mostly based on history, non-specific clinical signs such as pyrexia, anaemia and lymphadenopathy etc, along with laboratory detection of piroplasmosis in erythrocytes, identification of parasitic stages in the blood or infected organs and schizonts in lymphocytes of lymph node smear and WBC of blood smear. This method is fast, easy to do and most economical method of diagnosis.

The breeds of cattle reared in India are low producers. Hence, large numbers of pure bred high-yielding exotic cattle are being imported under various integrated development projects throughout the country. The crossbred cattle are more susceptible to tick infestations and tick-borne protozoan diseases than the indigenous cattle. Thus millions of high milk-yielding cattle are at risk of exposure to protozoan diseases.

Other factors like stress due to hot climate, vaccination, transportation, intercurrent diseases, parturition, lactation etc. also markedly influence the progression and outcome of the disease. Stress in cows occurs during parturition and early lactational period due to hormonal changes, physiological condition and

physical activity. Stress is also caused by conditions like milk fever, postparturient hemoglobinuria, ketosis, displaced abomasum, metritis, endometritis, pyometra etc. which are seen during this period. All these stressors make the animal fall prey easily to many diseases and theileriosis is one of them. Thus the incidence of theileriosis is more in postpartum cows followed by later lactation and pregnant animals. Early diagnosis of disease is of prime importance now a days to control the disease. Increased prevalence, lack of awareness among the farmers about the disease, late presentation of the case poses a great problem in diagnosis of the disease.

As there is alternation in haemato-biochemical status of the affected animal haematological and serum biochemical parameters were to be studied in theileriosis affected cattle which would help in better clinical management and decides the survival of the affected cows.

The current work is designed with the following objectives:

- To study incidence of theileriosis in cattle.
- To screen theileriosis affected cattle through history, clinical sign like rise of body temp., loss of appetite, swelling of pre scapular lymph nodes, weakness, paler of mucus membrane, drop in milk production etc along with blood smear examination.
- To study haematological changes in theileria affected cattle.
- To study serum bio-chemical alterations in theileria affected cattle.

CHAPTER II

REVIEW OF LITERATURE

Available literature pertaining to the clinicopathology of theileriosis in cattle has been briefly reviewed. Various factors affecting the incidence and severity of the disease as well as their haematological, biochemical and tissue alterations are included in this review.

1. Prevalence

Mallick *et al*. (1987) reported on the occurrence of haemoprotozoan infections in rural livestock and found that 20.45% and 4.72% of crossbred and indigenous cattle respectively were infected with *Theileria annulata.*

In an epidemiological study, Sahoo (1991) observed the annual average incidence of bovine tropical theileriosis in Bhubaneswar to be 4.87%; the highest (11.34%) being in the month of June and the lowest (1.01%) in the month of January. Jersey breed of cattle were more susceptible (7.24%) to T. annulata infection as compared to crossbred (5.11%) and indigenous cattle (3.04%).Theileriosis occurred more frequently in adult cattle (5.85%) of exotic breed than the calves (3.96%) below one year of age. He further observed that the infection was higher in females (5.19%) than the males (4.17%).The overall incidence of theileriosis recorded in cattle was highest in summer season (7.65%), moderate in rainy season (5.44%) and lowest in winter season (1.86%).The pathology of tropical theileriosis varied depending upon the duration of illness, susceptibility, age and breed of animals, he stated.

Aulakh *et al*. (2003) examined 101 bovines including 72 cows and 29 buffaloes in Punjab state and reported 6.94% prevalence of *Theileria annulata* in cattle. All the buffaloes were found to be negative for theileriosis.

Rakha and Sharma (2003) carried out epidemiological, clinical and serological study, in which they evaluated that, about 37% of crossbred animals showing pyrexia were positive for *T.annulata* and 43% of animals with pyrexia were found serologically positive by ELISA.

Dumanli *et al*. (2005) carried out the study to determine the prevalence and distribution of tropical theileriosis in cattle in eastern Turkey by microscopic, serological and molecular methods. They examined 1483 blood smears and found piroplasms of theileria spp. in 19.7% cases by microscopical examination. They detected antibodies against *T. annulata* by indirect fluroscence antibody test (IFAT) in 34.9% cases from 1505 serum samples. Also they found 37.8% prevalence of *Theileria annulata* by PCR from 1561 whole blood samples.

Harish *et al*. (2006) studied a total of 11755 blood samples from cattle, sheep, goats, buffaloes, dogs, horses and wild animals from various parts of Karnataka from March 1997-April 2002 were screened for haemoprotozoan parasites. The number of cases found positive for theileriosis, babesiosis, anaplasmosis and trypanosomiasis were 1918 (16.31%), 205 (1.74%), 776 (6.60%) and 263 (2.23%) respectively. This study suggests the endemic condition of theileriosis among crossbred cattle population and sporadic nature of other haemoprotozoan diseases in different geoclimatic conditions of the state.

Ram *et al*. (2006) assessed sero-prevalence of theileriosis in cattle of arid and semiarid regions of northwest India by using Indirect fluroscence antibody test (IFAT) and reported overall higher sero-prevalence in cattle of arid (western Rajasthan, 66%) than semi-arid (Haryana, 57%) region. Out of 401 cattle from western Rajastan, 167 (42%) showed moderate levels of antibodies while 98 (24%) were highly positive. In Haryana, out of 523 cattle, 235 (45%) showed moderate levels of antibodies while 62 (12%) were highly positive. Antibody titres in animals below one year of age were significantly lower ($P<0.05$) than the older cattle in both regions.

Soundarajan and Rajavelu (2006) studied prevalence of haemoprotozoan parasites in cattle and buffaloes reared in and around Madras. They recorded 28.2% and 8.0% prevalence of *Theileria annulata* in cattle and buffaloes respectively.

Durrani *et al*. (2008) recorded prevalence of Theileriosis in buffaloes from twenty one villages of District Lahore. Based on microscopic examination 39.9 (134/336) prevalence was recorded as compared to 53.3% (179/336) with polymerase chain reaction (PCR) test.

More (2008) reported the estimated annual losses due to Theileriosis were around $1470 million in 2003. In his study, of the total 1869 blood smears received in the department over a period of 5 years (2002-2007), from Satara and Pune districts of Maharashta State, 908 (48.58%) samples were found positive for Theileriosis (*T. annulata*). Theileriosis with non-specific acute bacterial infection was more common (52.86%) as compared to Theileriosis alone (47.14%).

Razmi *et al*. (2009) clinically examined and investigated a total of 160 dairy cattle from 78 farms for the presence of theileria spp., anaplasma spp. in blood smears in Mashhad area, in Iran from 2002 to 2003. The prevalence of *Theileria annulata* and *Anaplasma marginale* infection was 32 (20%) and 15 (9.38%) respectively.

Godara *et al*. (2010) reported that, in the Indian subcontinent, bovine tropical theileriosis (BTT) has been a persistently recognised major constraint to livestock improvement programmes. In enzootic areas, the disease accounts for high mortality up to 70% in dairy cattle, especially calves and over 200 million animals are at risk. They studied per-acute and fatal course of BTT in a three-day-old crossbred she calf, born in semi-arid Jaipur, India.

Ugalmugle *et al*. (2010) studied about prevalence of *Theileria annulata* from Ahmednagar district of Maharashtra. Animals had pyrexia & prevalence rate amongst pyrexia cases was 16.66% and level of parasitaemia was < 5%. Pyrexia (100%), presence of ticks on the body (100%), enlargement of prescapular and poplitial lymph nodes (92.30%), anorexia (76.92%), dyspnoea (69.23%) and anaemia (61.59%) were the predominant clinical features noted in the study.

Singh *et al*. (2011) reported a case of concurrent infection of *Babesia bigemina* and *Theileria annulata* diagnosed in Holstein Friesian cow presented at Large Animal Clinics, GADVASU, Ludhiana (Punjab) by routine Giemsa stained blood smear examination and was successfully treated with diminazene aceturate and halofuginone.

Sree Devi *et al*. (2011) studied about prevalence of concurrent tick-borne haemoprotozoon and rickettsial infections in Murrah buffalo brought to hospital with symptoms of anorexia, reduced milk yield, lymphadenopathy, exophthalmoses and

anaemia. Giemsa stained blood and lymphnode smears revealed intracytoplasmic inclusion bodies of *Ehrlichia bovis* in neutrophils and schizonts of *Theileria annulata* in lymphocytes. The differential leukocyte count indicated neutrophilia and lymphocytopenia.

Haque *et al*. (2012) asses the status of theileriosis using PCR based assay to detect *T. annulata* infection at low parasitemias and its comparison with blood smear examination in cattle in different locations of Punjab.

Vahora *et al.* (2012) studied the seasonal incidence of Haemoprotozoal diseases in crossbred cattle and buffalo in Kaira and Anand districts of Gujarat and has recorded higher incidence of haemoprotozoal diseases in crossbred cattle and buffalo. In both the species, higher incidence of theileriosis was recorded during monsoon season as compared to other protozoan diseases.

Chaudhri *et al*. (2013) examined stained blood smears from pyretic cross bred cows (3041) and buffaloes (3122) of Eastern Haryana from July, 2003 to June 2010 revealed significantly higher infection in cows (27.88%) than buffaloes (0.6%). The pyretic cross-bred cows had *Theileria annulata* (22.88%), *Trypanosoma evansi*(0.33%), *Babesia bigemina* (3.22%) and *Anaplasma marginale*(1.45%) whereas buffaloes (3122) had *T.evansi*(0.32%) and *B. bigemina*(0.32%) only. Percentage of pyretic cows detected positive for *T.annulata, B.bigemina* and *A.marginale* was high from 2006-09 (27.6 to 32.8%), 2007-09 (3.91 to 5.60%) and 2006-10 (0.87 to 2.70%) respectively.

Kohli *et al*. (2014) experimented on 301 blood samples each month from apparently healthy crossbred cattle from various locations of Dehradun district. Samples were tested using Giemsa staining technique and specific PCR test. Microscopic examination of blood smears revealed 27.2% (82) overall prevalence of theileriosis.

2. Clinical pathology

2.1 Blood smear & Lymphnode smear

Soulsby (1982) stated that *Theileria annulata* piroplasms in red blood cells are more or less indistinguishable from *T. parva* and more commonly occur as round, oval or ring shaped (0.5-1.3) forms and rod shaped, commas (1.6 µm) also found.

Panda *et al*. (2011) studied the prevalence and clinicopathological changes of theileriosis in bovine in coastal areas of Odisha. They observed that the seasonal incidence of theileriosis was 23% in rain, 35% in summer and 22% in winter. Young animals were most susceptible. On haematological examinations, the haemoglobin concentration in all the animals varied between 2.2 to 17.0 g/dl in positive cases. TEC varied from 1 million to 5.8 million/cmm of blood. Differential leucocyte count in positive samples, for neutrophils ranged between 6 to 84%, for lymphocyte ranged between 14 to 88%. Histopathologically, kidney showed degenerative changes, Bowman's space distended and bowman's capsules were thickened with fibrous hyperplasia. Liver showed fibrosis in the portal tract, disruption of hepatic cords, dissociation of hepatocytes and Kupffer cell proliferation. Lungs revealed areas of emphysema, atelectasis and thickened alveolar wall, and pneumonic changes. Lymph node were enlarged and showed severe depletion of lymphocytes

Bhatia *et al*. (2006) stated that, parasite from Genus Theileria are pleomorphic and occur as minute round, ovoid, rod like, comma shaped or irregular form in lymphocytes, histiocytes and erythrocytes of vertebrate hosts. Further they described different stages of *T. annulata*, *T. parva*, and *T. mutans* which were indistinguishable and with pleomorphism in all species. They noted that, *T. annulata* erythrocytic forms were indistinguishable from *T. parva* and 80% are round or annular (0.5-1.5 µm) and rest of are oval or comma shaped. They noticed 4-7 parasites in one erythrocyte in severe infection. Macro or microschizonts were observed in lymphocytes of spleen and lymphonodes.

Mandal (2006) described salient morphology of theileria parasite as small dot like, comma shaped, round and ring form in RBC whereas he observed parasite as schizont form i.e, Koch's blue bodies in cytoplasm of the lymphocytes. He also noted macroschizonts and microschizonts in the lymphocytes, merozoites, round and elongated bodies and ookinete as developmental stages of theileria spp.

More (2008) described clinical pathology of theileriosis in his study and revealed lymphocytosis with macroschizonts and microschizonts in mononuclear cells, and/or piroplasms in erythrocytes. In chronic cases treated with tetracycline and Diaminazine aceturate, lymphoblastoid cells without macroschizont, microschizonts were noticed in blood smears.

Ibrahim *et al.* (2009) studied clinical and laboratory examination of 54 cattle for infection by *Theileria* and *Babesia* piroplasmids. Blood smear stained with Giemsa stain revealed 12 (22%) positive cases for *Babesia spp.* and 7 (13%) for *Theileria.*

Rashid *et al*. (2010) revealed that, in theileria infection, the blood smear showed pleomorphic *T. annulata* piroplasms in the erythrocytes. Lymphnode smears revealed schizontal stages (Koch's blue bodies)) of *T. annulata* in lymphocytes.

Khan *et al*. (2011) confirmed 50 crossbred cattle positive for theileriosis by blood smear examination by detection of schizonts of *T. annulata* in blood cells.

Nair *et al*. (2011) cross-sectional study was conducted using 150 blood samples collected from apparently normal/ healthy crossbred cattle of Northern Kerala, South India, for detection of haemoprotozoan infections using staining techniques (Giemsa and Acridine Orange) and specific PCR. Examination of Giemsa stained smears revealed *theileria* like piroplasms in 61 samples and *B. bigemina* piroplasms in 4 samples. Various morphological appearances of theileria piroplasms were observed. They were thin and thick rod shaped or annular with light staining trailing cytoplasm.

Digraskar *et al*. (2012) in a case study found *Theileria annulata* infection in a 15 day old Holstein Friesian crossbred calf where presence of large number of Koch's blue bodies in lymphocytes.

Omer *et al*. (2012) carried out study on blood samples of 299 local breed female cattle in Northern Iraq in 2006 for detection of piroplasmosis. By direct blood smear examination, the rate of *Theileria annulata* alone was 45.1% while in mixed infections with Babesia was 11.7%. The total rate of theileria infection was 56.9%. Using enzyme linked immunosorbent assay technique (ELISA), the seropositivity of theileria was 77.9%, while babesia was 12.4%. The overall rate of seropositivity by ELISA was 90.3% for piroplasms while by blood smears examination the rate of infected animals was 56.9%.

Samanta and Dutta, (2012) revealed presence of dot shaped haemoprotozoan parasites in erythrocytes by microscopic examination of stained blood smears. In few

erythrocytes found some ring form of parasites. The parasite showed morphologically resemblance to *T. annulata* of cattle.

Mahajan *et al*. (2013) have seen schizonts in giemsa stained tissue section particularly in liver.

2.3 Clinical signs

Soulsby (1982) described three different forms of theileriosis viz. acute, sub-acute and chronic forms. Clinical signs described by him were increase in body temperature, swelling of superficial lymphnodes, emaciation, lacrimation, depression and loss in body weight.

Hussein *et al*. (2007) revealed clinically enlargement of superficial lymph nodes, fever, congested mucous membranes, corneal opacity and emaciation were found in cases of theileriosis.

Osman *et al*. (2007) reported variation in clinical signs of infected animals according to stage of infection. There was pyrexia, anorexia, ocular and nasal discharge, swollen lymph nodes, constipation, nervine symptoms, coughing and dyspnea in infectd animals.

Sangwan and sangwan (2007) during *Theileria annulata* infection indirect losses of essential body nutrients can be caused by their accelerated metabolism or excretion. Concentrations of copper in blood, plasma and tissues were estimated on Atomic Absorption Spectrophotometer. The infected animals showed clinical symptoms like fever, anorexia, respiratory distress and recumbancy. With the progression of disease, haemoglobin and haematocrit decreased significantly. However, the blood copper concentrations did not exhibit changes due to theileriosis.

Durrani *et al*. (2008) carried out study to detect *Theileria annulata*, the causative agent of theileriosis and *Babesia bovis*, the causative agent for babesiosis, in Friesian cattle by PCR and conventional blood smear examination. The disease manifestations observed clinically included high fever, swelling of sub mandibular and sub scapular lymph nodes, weakness, increased respiration and pulse, anorexia, loss of condition and rough hair coat. Neurological sign of incoordination was also

seen in weak animals. Signs of lacrimation, pale conjunctiva, diarrhoea, dyspnea and frothy nasal discharge were observed.

Issi and Gul (2008) encountered a case of hematuria in a calf with prenatal tropical theileriosis and concluded that *Theileria annulata* related prenatal infections could be developed in calves and apparent haematuria could be seen in these cases.

Ibrahim *et al.* (2009) studied clinical and laboratory examination of 54 cattle for infection by *Theileria* and *Babesia* piroplasmids. Prevalence of the clinical signs revealed high percentage of the infected animals showed pale mucous membrane (42.59%) and fever (38.88%), while low percentage showed swelling of lymph nodes and hematuria (18.51%).

Masare *et al*. (2009) studied about, epidemiological and clinico-therapeutic studies made on fifteen clinical cases of theileriosis calves. Clinically, fever, tachycardia, polypnea, reduced appetite, dullness, pale to icteric mucous membrane and enlargement of lymph-nodes were observed. Haematological examination revealed anaemia. PM examination showed icterus, hepatomegaly and ulcers in abomasum.

Bhojne *et al*. (2010) studied about visible signs such as rise in body temperature (Avg. 105^0F) with palpable enlargement of the prescapular lymph nodes. Tachycardia also observed in the present investigation could be attributed to anemia. Among many drugs evaluated Buparvaquone (Butalex) was found very effective and highly specific for the treatment of clinical cases of bovine tropical theileriosis at an early as well as late stage of infection.

Khan *et al*. (2011) confirmed 50 crossbred cattle positive for theileriosis by blood smear examination by detecting of schizonts of *T. annulata* in blood cells. The clinical findings including high rise in rectal temperature (103-106^0 F), general debility, enlargement of prescapular lymphnodes, hemorrhages on mucosal membranes (conjuctival, nasal and oral), cachexia, dyspnea, lacrimation, conjunctivitis and eye ball protrusion were recorded.

Mahmmod *et al*. (2011) reported about, the clinical findings of examined cattle and buffaloes showed typical signs of tropical theileriosis: fever, enlargement of

the superficial lymph nodes, severe lacrimation, bilateral conjunctivitis, photophobia, and corneal opacity.

Dehkordi *et al*. (2012) reported about identification of the prevalence rate of *Theileria annulata*, by blood and lymph node biopsy smears and blood and lymph node PCR. In 174 out of 1202 blood samples (14.478%) and in 129 out of 1202 lymph node biopsy samples (10.73%), the piroplasm forms and macroschizonts of theileria were observed on blood and lymph node biopsy smears, respectively in Southwest Iran.

Digraskar *et al*. (2012) in a case study found *Theileria annulata* infection in a 15 day old Holstein Friesian crossbred calf where there was swollen (Tennis ball size) prescapular & prefemoral lymph node.

Sumathi and Veena (2012) revealed clinical signs of theileriosis in cattle such as rise of body temperatue (104-105^{0}F), dullness, dysponea, tachycardia, pallor mucous membrane and enlarged superficial lymphnodes.

Oryan *et al*. (2013) reported pathological features of a natural outbreak of tropical bovine theileriosis. *T. annulata* was confirmed by presence of piroplasms in the blood smears and PCR test. On necropsy, pale mucous membrane and ecchymotic haemorrhages in the mucosal and serosal surfaces with lymphoadenopathy were observed. Also friable, yellowish liver and punched out ulcers on abomasal mucous membrane were found.

Temiz *et al*. (2014) conducted study to determine the relationship between the degrees of anemia and blood gases in cattle with theileriosis. They found clinical signs of pyrexia, tachycardia, tachypnea, and swelling in superficial lymph nodes in all cattle with theileriosis. Paleness of the mucous membrane of conjunctiva was observed in the majority of infected animals, and hyperemia and petechial hemorrhages were observed in some animals. In addition, petechial hemorrhages in some of the animals were observed on the planum nasolabiale and perineum. There were also some general clinical findings such as dyspnea, coughing, decrease in rumen movements and rumination.

3. Haemato-Biochemical Profile

Sahu *et al.* (1996) investigated the haematological and biochemical alterations in theileria infected crossbred cattle. There was significant decline in Hb, PCV, TEC, TLC and non significant increase in ESR values in infected animals as compared to non infected animals reflecting anaemia. There was lymphocytosis and leucopenia due to significant decline in neutrophil %, eosinophil % and monocyte %. There was also significant decline in serum glucose, total protein and Ca and non significant decline in serum Mg and P in infected animals

Nasir (2000) studied the restoration of haematological profile after buparvaquone and oxytetracycline therapy in clinical theileriosis. They found that buparvaquone treatment was better as compared to oxytetracycline in restoration of haematological profile.

Singh *et al*. (2001) studied about some blood parameters of cross bred calves experimentally infected with *T. annulata* infection and found that, there was progressive decrease in Hb, PCV along with marked reticulocytosis. Serum analysis revealed a decrease in concentration of calcium, cholesterol and triglycerides, total serum protein, albumin, globulin, albumin:globulin ratio, While there was increase in concentration of BUN.

Omer *et al*. (2002) studied about haematological parameters in young and adult Friesian cattle. Changes in blood parameters in *T. annulata* infected cattle indicated severe macrocytic hypochromic anaemia, panleukopenia, lymphocytopenia, eosinopenia, neutropenia and thrombocytopenia but no reticulocytosis.

Omer *et al*. (2003) analysed biochemical parameters in adult and young Friesian cattle naturally infected with *Theileria annulata* in the Qassim Region, Saudi Arabia. 43 clinical cases of tropical theileriosis were studied, together with 40 clinically healthy Friesian cattle. Cattle clinically infected with *T. annulata* had significantly lower serum total protein, albumin, globulin, creatinine, calcium, phosphorus, magnesium, potassium, iron and copper concentrations and significantly higher AST activity and bilirubin concentration than the healthy cattle.

Ceylan *et al*. (2004) was conducted study to investigate whether the erythropoietin (Epo) level and some blood parameters (erythrocyte count, haemoglobin and packed cell volume) were affected in cattle suffering from theileriosis in Turkey. Blood samples were collected from 12 cattle with tropical theileriosis and 6 healthy cattle. Epo level increased in cattle with tropical theileriosis. The erythrocyte count, packed cell volume and haemoglobin decreased in cattle with theileriosis. It was concluded that Epo level increased as a result of anaemia that developed in cattle with tropical theileriosis.

Abou-El-Naga *et al*. (2005) evaluated infection of *T. annulata* on some blood constituents before and after treatment with buparvaquone. The prevalence of tropical theileriosis was 40.3% and 29.4 % in cross and native breed respectively using blood smear examination. Immunofluorescent antibody technique (IFAT) could identify *T. annulata* in 80.7 % of cross breed and 70.5 % of native cattle. In addition, there was seasonal variation in prevalence. Cattle clinically infected with *T. annulata* had significantly low levels of total proteins, albumin, magnesium, potassium and iron concentrations but AST, glutamyl transferase activities, total, direct and indirect bilirubin, creatinine levels were significantly high.

Muraleedharan *et al*. (2005) analysed haemogram of cattle naturally infected *Theileria annulata* and reported low TLC and Hb levels in 31.39% of cattle. The total leucocyte counts showed leukocytosis (25.09%) or an inclination towards leukopaenia (19.72%) and the DLC indicated lymphocytosis (44.94%) and neutrophilia (16.19%).

Aulakh and Singla (2006) stated that out of 101 suspected animals (72 cattle, 29 buffaloes) examined for *Theileria annulata* infection during July 2002 to June 2003, 6.94% of cattle were positive and all the buffaloes were negative for haeamoprotozoan infections. Haematological observations revealed normocytic hypochromic anaemia, neutrophilia, lymphopenia and leucocytosis. Biochemical changes revealed significant decrease in total plasma proteins, albumin and globulins while blood urea nitrogen (BUN) and circulating immune complexes were found to be increased significantly ($P<0.05$).

Col and Uslu (2006) conducted an experiment to study haematological changes in Holstein Friesian cattle naturally infected with *Theileria annulata*. They

recorded significant decreases in red blood cell count, haematocrit value, haemoglobin amount, mean corpuscular haemoglobin concentration, and white blood cell, lymphocyte, neutrophil, monocyte, eosinophil, and basophil counts as well as significant increases were seen in mean corpuscular volume and marked reticulocytosis. In the coagulation profile, activated partial thromboplastin time and prothrombin time were significantly prolonged and platelet counts were significantly less.

Col and Uslu (2007) studied about serum components to elucidate metabolic profile in cattle naturally infected with *T. annulata.* Statistically significant increases were observed in the mean serum activity of aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, bilirubin, creatinine, urea, and creatinine kinase, and statistically significant decreases were seen in the mean serum contents of glucose, total protein, albumin, triglycerides, cholesterol, calcium, and phosphorus, along with a nonsignificant decrease in iron level in infected animals.

Hussein *et al*. (2007) revealed haematological findings in cattle suffered from theileriosis showed normocytic hypochromic anemia. Biochemical findings showed decreased serum levels of albumin and total proteins with increased serum globulins. Cattle infected with theileriosis showed significant decrease in serum level of iron.

Altug *et al*. (2008) studied alteration in the haemato-biochemical profile in the cattle naturally infected with *Theileria annulata*. They recorded significant decrease in Hb, PCV and platelet count along with increase in the total leucocyte count. However, in the biochemical profile, they observed significantly increased activity of AST, ALT and ALP.

Hasanpour *et al*. (2008) investigated changes in selected blood and serum components and electrocardiography (ECG) in 20 adults (13 females and 7 males) of water buffaloes suffering from severe theileriosis. The age of all animals used in this study ranged 1.5-5 yr. Theileriosis was diagnosed by observation of parasites in the peripheral blood and the presence of schizonts in lymphocytes that were provided from swollen lymph nodes. Statistically significant decreases were observed in the means of RBC, WBC, and packed cell volume (PCV) in blood of infected animals. The mean levels of sodium, calcium, phosphorus, and potassium of infected animals

were lower than healthy animals, but only the decrease of potassium was significant. The mean serum activities of aspartate transferase and alanine aminotransferase were significantly higher than in uninfected animals.

Nazifi *et al*. (2008) study was carried out in two observational clinical studies on crossbred cattle naturally infected with *Theileria annulata* and Anaplasmosis. Infected animals were divided into 3 subgroups with different parasitaemia (<10%, 10–20% and 20–30%). In theileria infected cases significant negative correlation was observed between parasitaemia and superoxide dismutase (SOD) and positive correlations were observed between parasitaemia and lactate dehydrogenase (LDH) and mean corpuscular fragility (MCF). In Anaplasmosis cases positive correlations were observed among parasitaemia and MCF and LDH activity.

Saber *et al*. (2008) investigate variation of some blood biochemical in crossbred cattle naturally infected with *Theileria annulata* had significantly lower serum total protein, calcium, cholesterol and triglyceride concentration and significantly higher ALP, ALT, AST, phosphorus, sodium, potassium, bilirubin and BUN concentration than healthy cattle.

Ananda *et al*. (2009) in their study found that haematological values were adversely affected in theileriosis cases. The haemoglobin level was reduced from 8gm/100ml to 3gm/100ml in severly infected cases. The TEC and PCV were decreased to 2.3 million/cmm and 9% respectively. They concluded that this might be due to damage caused by the organisms inside RBC's during their multiplication. The highest prevalence was found in 4-6 year age group and in monsoon months.

El-Deeb and Younis (2009) demonstrate the clinical picture associated with theileriosis in buffaloes with particular emphasis to the oxidative stress and ketosis. Blood and serum analysis revealed significant decrease in RBCs and Hb concentration in infected animals. Also significant increase in the levels of beta hydroxy butyric acid (BHBA) and non-esterified free fatty acid (NEFA) with a significant decrease in the levels of reduced glutathione (R.GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), nitric oxide (NO), glucose and glucose-6-phosphate dehydrogenase (G6PD) in infected animals compared to control ones. These results may be attributed to the abnormalities in

metabolism and anorexic state of affected buffaloes or may be due to the abnormalities in liver functions. There was a significant increase ($p \leq 0.05$) in the levels of NEFA and BHBA in *Theileria annulata* infected buffaloes, in comparison with healthy buffaloes, indicating the ketotic state of these cases.

Nazifi *et al*. (2009) assess the effect of the severity of *Theileria annulata* infection on some haematological parameters and antioxidant enzymes in naturally infected cattle. There were significant differences in red blood cell counts (RBCs), packed cell volume (PCV), concentration of haemoglobin and methaemoglobin and activities of SOD and GPX between healthy cattle and those infected with *Theileria annulata*. As parasitaemia increased in infected cattle, a significant decrease was observed in RBCs, PCV, haemoglobin concentration, and in SOD and GPX activities. In contrast, with increase in parasitaemia, a significant elevation in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and concentration of methaemoglobin was detected.

Vahora *et al*. (2009) investigate the effect of theileriosis on blood profiles in 28 clinically affected crossbred cows. The haematological estimations revealed decreased RBCs, haemoglobin and MCHC values, while MCH, MCV and PCV values were found normal. The blood smears revealed presence of schizonts and piroplasms in the lymphocytes and erythrocytes respectively.

Rashid *et al*. (2010) describes the haemato-biochemical alterations in cross-bred cattle naturally infected with theileriosis. There is decrease in Hb, PCV, TEC and TLC and also significant decrease in total protein, albumin and marked rise in BUN, total serum albumin.

Ugalmugle *et al*. (2010) haematological analysis revealed anaemia (70%) of normocytic normochromic, leukocytosis (40%) while leucocytopenia (20%) was observed. Differential leucocytic counts revealed either relative lymphocytosis associated with neutropenia or lymphocytopenia associated with neutrophilia. Biochemical analysis revealed bilirubinaemia, hypoproteinaemia primarily associated with hypoalbuminaemia, elevated levels of SGOT and SGPT were recorded particularly in severely affected cases indicating liver involvement.

Khan *et al*. (2011) in their study on 50 cross bred cattle positive for theileriosis reported significant ($p \leq 0.05$) decrease in erythrocyte counts, packed cell volume and haemoglobin concentration in infected cattle as compared to non-infected controls. In infected cattle, significant ($p \leq 0.05$) decrease in serum total proteins, albumins, globulins, glucose, calcium, phosphorous, cholesterol and triglycerides concentrations compared with negative controls were recorded. Significant ($p \leq 0.05$) increase was observed in serum bilirubin and alanine transaminase concentrations in infected cattle compared with non-infected control group. Non-significant difference was also observed in serum magnesium and uric acid concentrations compared with non-infected control group.

Mahmmod *et al*. (2011) reported haematological analysis which revealed a significant decrease in RBCS count, PCV%, haemoglobin amount, and WBCs in the infected animals when compared to the control group.

Ramin *et al*. (2011) in an evaluative study of the erythrocytes and leucocytes alterations in cows infected with *Theileria annulata* found that the mean concentrations for PCV, Hb, RBCs and MCHC were significantly ($p<0.05$) lower and MCV was higher than the control group. Among the indices under study, mean RBCs and Hb showed significant differences ($p<0.01$) in all types of anemia. The types of anemia were varied from normocytic hypochromic to macrocytic hypochromic.

Dua *et al*. (2012) estimated concentrations of few minerals in blood plasma of female cattle showing moderate to severe natural infection of *Theileria annulata.* Levels of calcium, copper, sodium and potassium were significantly ($P<0.01$) lower in diseased animals in comparison to control. Levels of phosphorus, magnesium and zinc were also apparently lower in infected animals non-significantly. Iron levels were non-significantly higher in infected animals.

Omer *et al*. (2012) carried out study on blood samples of 299 local breed female cattle in Northern Iraq in 2006 for detection of piroplasmosis. The haematological parameters of cattle infected with theileria alone were PCV=27%, RBC= 5.6 million/ cm3 and Hb 9.5 g/liter did not vary from non-infected ones. While in mixed infections (Theileria +Babesia) the blood picture values were decreased dramatically and were PCV=18%, RBC=4.08 million/ cmm and Hb 5.7 g/l.

Tehrani *et al*. (2013) dealt with biochemical and hematological changes in cattle naturally infected with *Theileria annulata.* In haematological examination revealed significant decreases ($p \leq 0.05$) in the Hb content, PCV%, RBCs, MCHC, basophils, lymphocytes counts in sick cattle, compared to the control ones. Neutropenia, eosinopenia, lymphopenia, monocytopenia with a significant increase ($p \leq 0.05$) in the numbers of thrombocytes were recorded. The infection caused significant increases in total urea and total nitrogen in infected cattle compared to the control ($P<0.05$). Depressed phosphorus, calcium, sodium, chloride and iron concentrations were observed when compared to the control ($P<0.05$). No significant differences were observed in manganese, zinc and glucose value.

Dede *et al*. (2014) studied serum biochemical profile and serum protein fractions in cattle infected with *T. annulata*. The ALT (alanine amino transferase) and ALP (alkaline phosphatase) activities and the concentrations of bilirubin and urea were markedly increased in sera from infected animals whereas the concentrations of glucose, minerals (Na, K and Ca), total proteins, albumin and of α-globulins and the albumin/globulin (A/G) ratios were significantly depressed compared to the controls. These results show that liver and kidney failures occur during theileriosis leading to a global protein deficiency.

CHAPTER III

MATERIALS AND METHODS

Source of animals

The present study was conducted for a period of twelve months from July 2015 to June 2016 in and around Bhubaneswar. The blood samples received at Teaching Veterinary Clinical Complex, C.V.Sc., OUAT. Blood samples were also received from field veterinarians of different districts of Odisha and examined in the Department of Veterinary Pathology. Animals with complain of anorexia, non-remitting pyrexia, decreased milk yield, swollen superficial lymph nodes, pale conjuctival mucous membrane, haemoglobinuria, nasal discharge, coughing etc were suspected for theileriosis. From the owners the patient data regarding age, sex, lactational history, parturition history, clinical signs etc. were also recorded. Initial screening of the theileriosis positive cases was done from the blood smear examination. A total of 1280 cases were screened, out of which 928 cases were found positive for theileriosis. Selected 153 positive cases in postpartum cows were selected for further haematological examination and 25 serum samples were collected for biochemical studies. The positive cases were followed up with the cattle owner/ treating physician for any casuality.

Prevalence study

Prevalence of the disease among cattle was analysed on basis of age, sex, breed and season. Data pertaining to diagnosis of theileriosis by blood smear examination in pathological laboratory, at Teaching Veterinary Clinical Complex, C.V.Sc. and A.H., OUAT were collected for a period of one year i.e, from July 2015 to June 2016.

Microscopic examination

After collection of blood thin blood smears on slide were prepared immediately as quick as possible. These smears were allowed to air-dry and fixed with methanol for about 3-5min followed by staining with Giemsa stain. The stain was diluted down with distilled water in ratio 1:10 and kept for about 35-50min. After

washing the slides were air-dried and examined with microscope using oil immersion lens at 100X magnification.

Haematological examination

Blood samples were received in 5ml EDTA vials at pathological laboratory, Teaching Veterinary Clinical Complex, C.V.Sc & A.H., OUAT. The samples were examined for estimation of Hb%, PCV, TLC, TEC, DC etc. by following methods (Schalm, 1965).

- Estimation of haemoglobin percentage (g/dl) by Sahli's acid hematin method using N/10 HCl.
- Wintrobe's haematocrit method was used for PCV estimation.
- Total Leucocyte Count (thousands/cmm) was done by using Thomas fluid as diluent in haemocytometer.
- Total Erythrocyte Count (million/cmm) was done by using Haem's fluid as diluent in haemocytometer.
- Differential Leucocyte Count (%) was done by preparing thin blood smear, staining with Giemsa stain and observing under oil immersion lens.

Serum biochemical analysis:

5ml of blood was collected aseptically using clot activator vial from each of the 25 theileria positive cows. The vials were kept undisturbed for about 45-60 minutes and then serum was collected in sterile serum vials. The serum samples were stored at -20°C until further tests. Serum biochemical parameters like glucose, total protein, albumin, globulin, urea, creatinine, SGOT, SGPT, and Total billirubin were estimated by using biochemical kits supplied by Crest Biosystems, Goa.

Chemical examination of Urine Sample

The urine samples were collected from 25 animals found theileria positive in haematological examination and whose serum samples were examined in biochemical analysis. Urine specimens were collected by urethral voided urine. In voluntary voiding utmost care was taken to collect the urine aseptically in a sterile container without any external contamination which may hamper the quality of the urine. Urine

was collected aseptically in a sterile container. All the samples were tested for presence of sugar, protein, ketone bodies and bile salt.

1. Glucose

Bendict's test indicates presence of glucose in the urine. Eight drops of urine was added to 5 ml of Bendict's reagent. Then it was boiled by means of spirit lamp. The presence of glucose was indicated by presence of a range of precipitate ranging from green to yellow.

2. Ketone bodies

Presence of Ketone bodies in the urine was detected by Rothera's test. In this test 3 ml of Rothera reagent was taken to which equal amount of urine was added. Strong ammonia solution was added slowly. A permanganate coloured ring at the junction indicated presence of ketone.

3. Bile salts (Hay's Sulphur powder test)

10 ml of urine was taken in a test tube. A pinch of sulphur powder was added to it. Sinking of sulphur powder to bottom was indicative of presence of bile salt in urine.

4. Protein

Robert's test was followed for detection of albumin in urine. In this test 5 ml of Robert's reagent was taken in a test tube and the tube was inclined. Then by means of a dropper the urine was allowed to flow slowly down the side, so that two fluids are not mixed. . A white coloured ring at the junction indicated the presence of protein.

CHAPTER IV

RESULTS

The present research work was conducted to study the haematological and biochemical alteration in theileriosis affected cattle over a period of twelve months from July 2015 to June 2016. A total of 1280 numbers of blood samples were received at Teaching Veterinary Clinical Complex and Department of Veterinary Pathology, C.V.Sc. and A.H., OUAT with complaint of anorexia, non-remitting pyrexia, drop in milk yield, enlarged superficial lymph nodes, pale conjuctival mucous membrane, haemoglobinuria, nasal discharge and coughing which were suspected for theileriosis. Patient data regarding age, sex, breed and clinical signs etc. were also recoded. The blood samples were screened for theileria parasite by blood smear examination. Out of 1280 cases were examined for theileriosis, 928 cases were found positive for theileria parasite. Total 153 positive cases were selected for further research pertaining to haematological examination and 25 serum samples were collected from theileria positive cases for biochemical studies.

Epiemiological study

Prevalence

A total of 1280 suspected blood samples were screened for theileriosis by blood smear examination. There were 928 (72.5%) positive and 352(27.5%) negative cases (Chart:1). Examination of blood smear revealed the presence of piroplasms in different forms as small dot like ovoid, round, irregular cocci, racquet shaped, rod, bacillar shaped, signet ring and pear shaped inside RBCs in different samples indicating presence of *Theileria annulata* **(Fig. 1).** Koch's blue bodies inside lymphocytes are also found in few cases **(Fig. 2).**

The prevalence study of the theileria positive cases is made more specific according to age, sex, breed and season.

Age wise prevalence

Agewise, the cases were grouped into three categories namely, calves within 1 year of age, heifers (2-3 years), and adults (above 3 years). The highest population

affected by theileriosis were adults 652 (70.25%) followed by 255 (27.47%) of 2-3 years of age and 21 (2.58%) of less than 1 year (Chart:2).

Sex wise prevalence

In general farmers do not prefer to rear males for their less utility and also there is mechanization of farming and thus males are no more required for drafting or pulling bullock carts. In other hand the demand of milk production is very much high enhancing the rearing of the females. So number of female cases presented was very high in comparison to males. Out of total 928 positive cases of both the sexes, 907 (97.73%) were females which was much more higher than males 21 (2.27%) (Chart:3).

Breed wise prevalence

Out of all the 928 cases, there were 795 (85.66%) cross bred Jersey (CBJ), followed by deshi or local breeds 34 (3.66%) and 99(10.66%) were of different breeds like Red Sindhi, Holstein Friesian and its crosses, Hariana and mixed breeds (Chart: 4).

Season wise prevalence

The whole year is divided into 3 seasons as summer, rainy and winter. Seasonwise, the incidence of theileriosis was highest in rainy season 422(45.47%) followed by summer 337(36.31%) and winter 169(18.21%). (Chart:5).

Haematological examination

The haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) values were distinctly lower in theileria positive animals than apparently healthy animals. Hb in average varies from 10.6 gm% in apparently healthy animals to 8.5 gm% in the positive cases. PCV, accordingly, varied from 32.2% in apparently healthy cases to 24.8% in positive instances. Likewise, the TEC values varied from 5.9 million per cubic milli metre (million/cu. mm.) in apparently healthy animals to 4.1 million/cu. mm. in theileia positive cases. (Chart:6).

Also, the TLC (Total Leucocyte Count) and absolute neutrophil count (Abs N) and absolute lymphocyte count (Abs L) values also showed a similar trend. The average of TLC was 8.4 thousand/cu. mm. in apparently healthy animals which was reduced to 6.6 thousand/cu. mm. in theileria positive animals. Average Abs N count was also reduced from 4.4 thousand to 2.7 thousand in apparently healthy animals to positive animals. Likewise, the average Abs L count was also marginally reduced from 3.7 thousand in apparently healthy animals to 3.5thousand in positive animals. (Chart:7)

Serum biochemical analysis

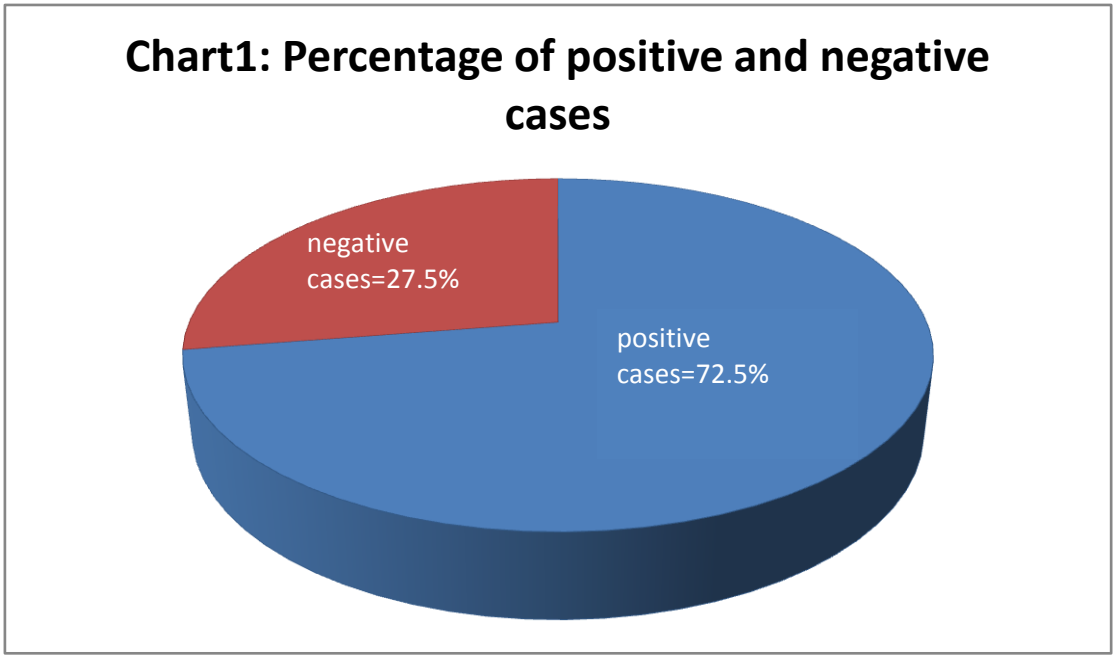
Serum samples were collected from 25 selected theileria positive cases for estimation of Serum parameters like glucose, cholesterol, total protein, albumin, globulin, Blood urea nitrogen, creatinine, SGOT, SGPT, total billirubin. These parameters were estimated by using the kits from CREST BIOSYSTEMS, a division of Coral Clinical Systems, Goa, India. The analysis was done as per the instruction manual supplied by the kit. The average values of the serum biochemical parameters of overall positive and apparently healthy animals are given in Table 1. (Chart:8)

Table 1: Average values of serum biochemical parameters in affected and apparently healthy cattle

Parameters	Theileria positive cases	Apparently healthy animals
Glucose(g/dl)	52.42	82.14
Cholesterol (mg/dl)	90.17	126.21
Protein (g/dl)	5.94	7.67
Albumin (g/dl)	2.98	3.76
Globulin (g/dl)	3.05	3.84
BUN (mg/dl)	24.88	17.47
Creatinine (mg/dl)	2.38	1.77
AST (IU/L)	212.9	102.6
ALT (IU/L)	30.93	25.38
Total billirubin(mg/dl)	2.41	1.82

Urine analysis

Urine samples from those animals (theileria positive cases) were collected whose serum samples were examined in biochemical analysis. All the 25 samples were tested for presence of sugar, protein, ketone bodies and bile salt. It was observed that sugar was detected in 5 urine samples, protein in 6 urine samples and bile salt in 8 urine samples. Ketone bodies were detected highest number of urine samples i.e. 14. (Chart:9) **(Fig. 3 and 4).**



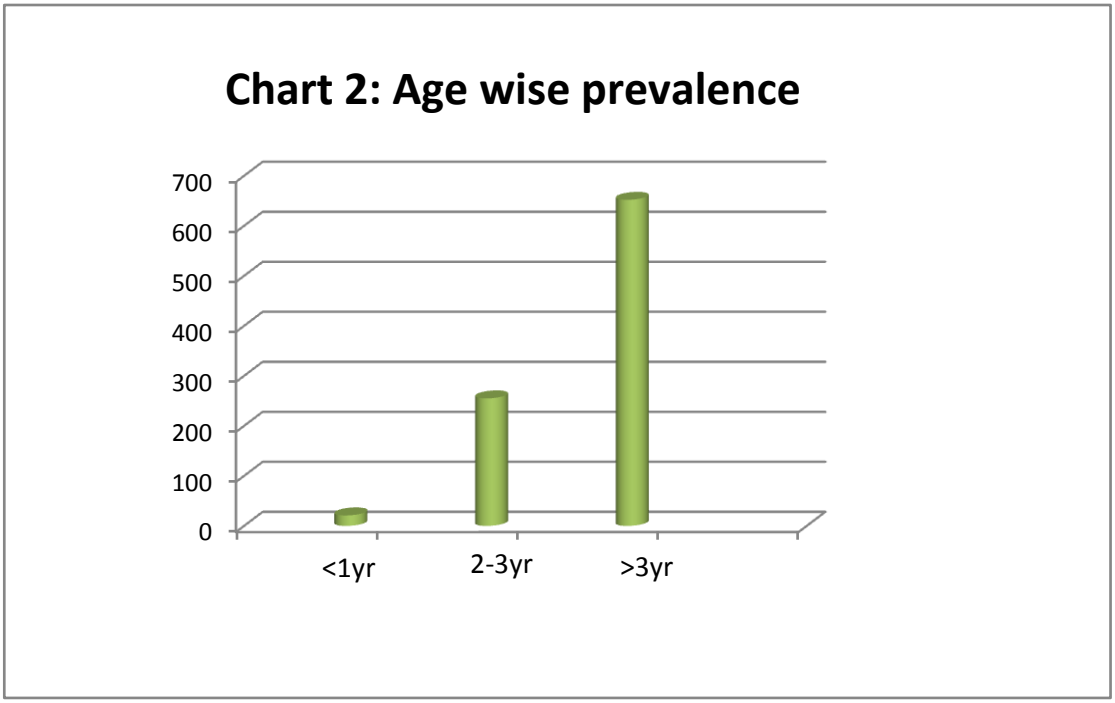


Chart 3: Prevalence in males and females

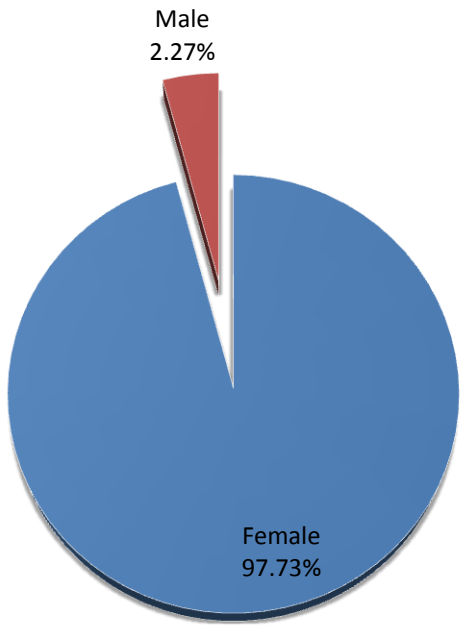


Chart 4: Breed wise prevalence

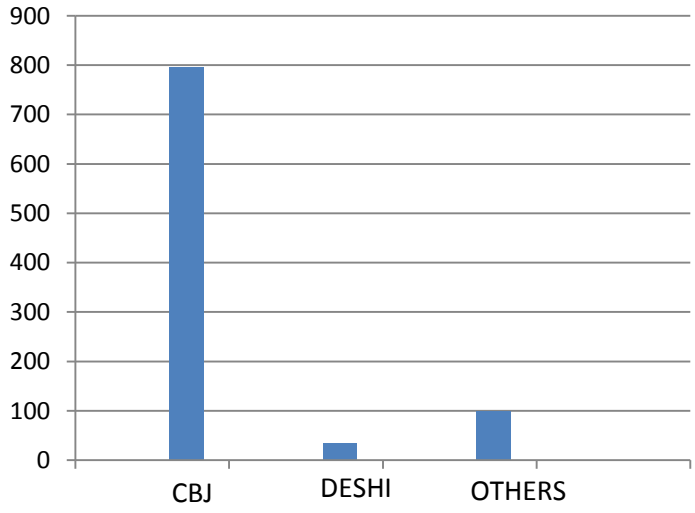


Chart 5 : Seasonwise prevalence

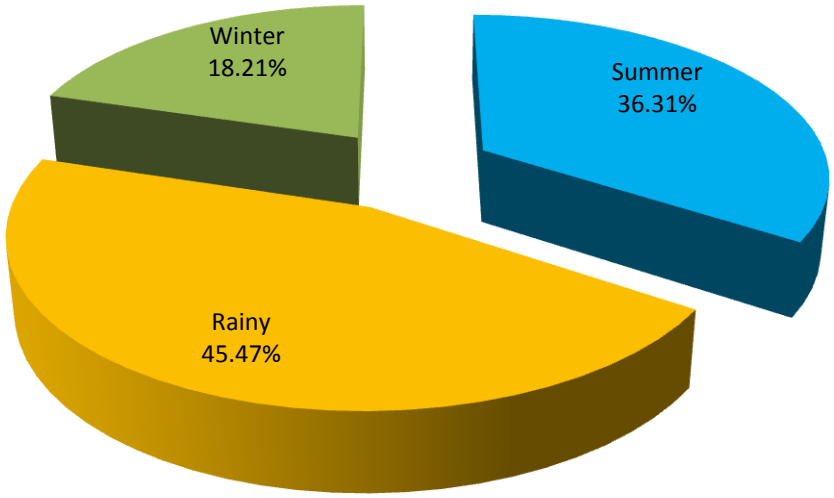


Chart 6: Average values of TEC, Hb% and PCV in theileria positive cases and apparently healthy animals

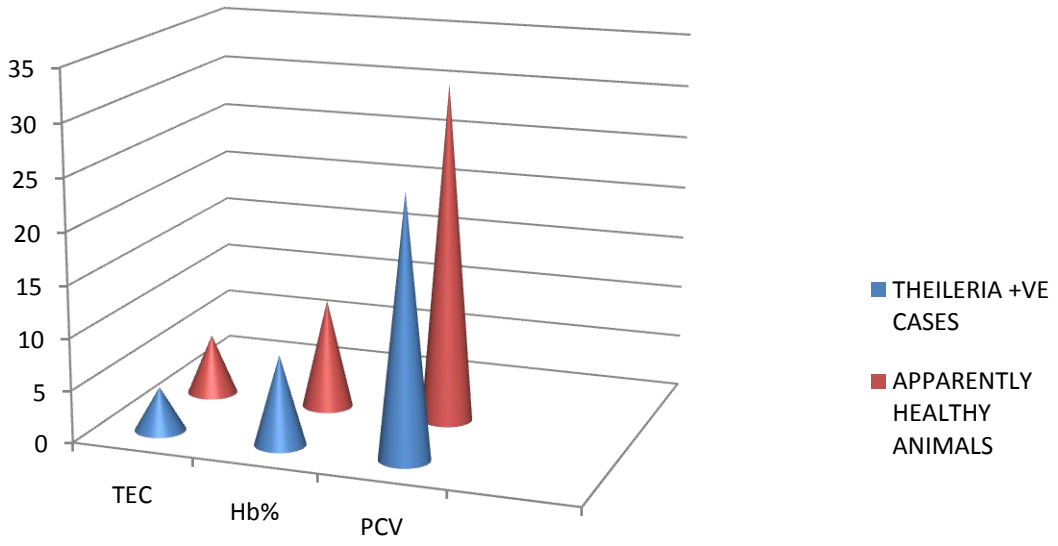


Chart 7: Average values of TLC, Abs N and Abs L in theileria positive cases and apparently healthy animals

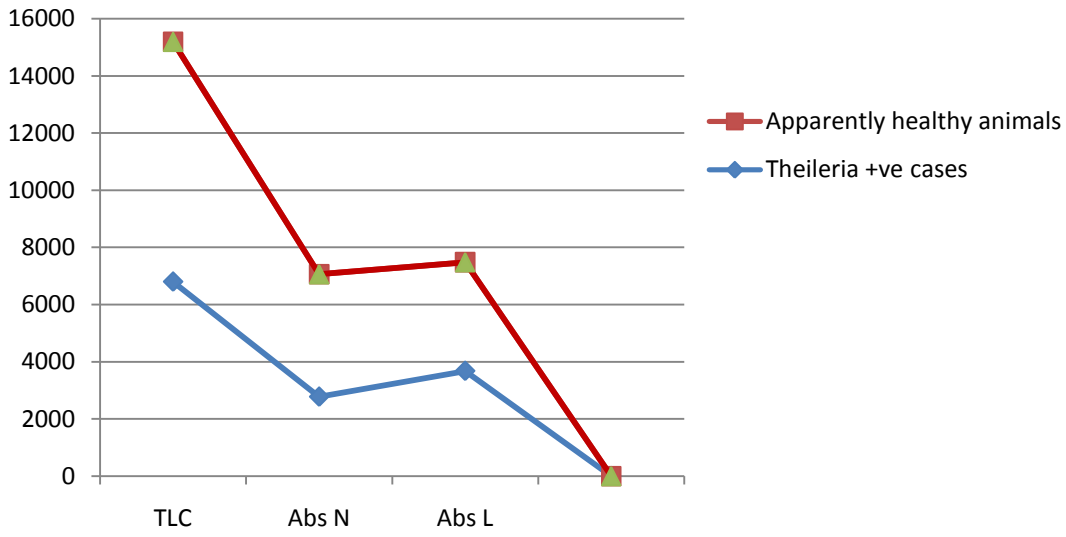
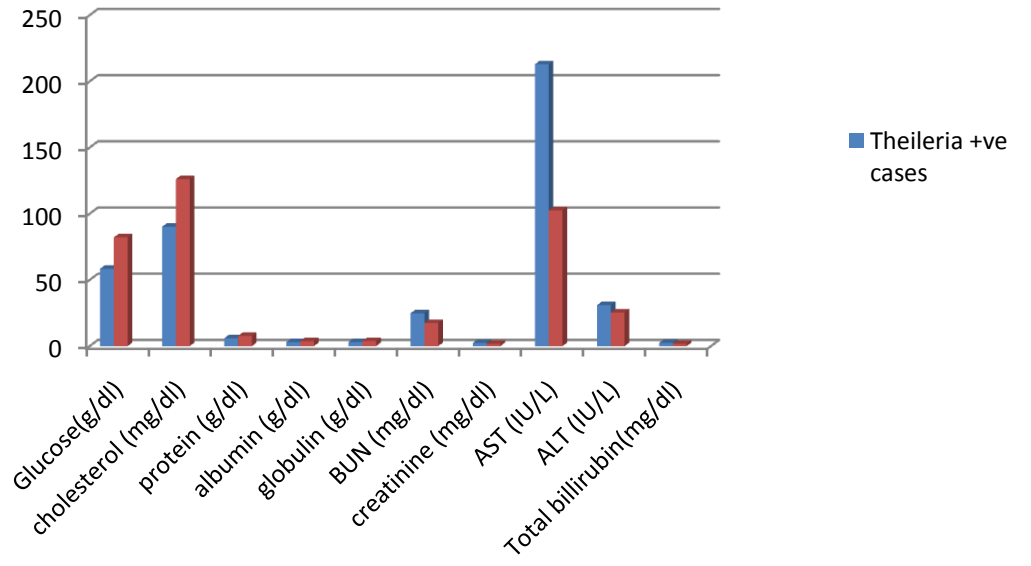


Chart 8: Average values of different serum biochemical parameters in theileria positive cases and apparently healthy animals



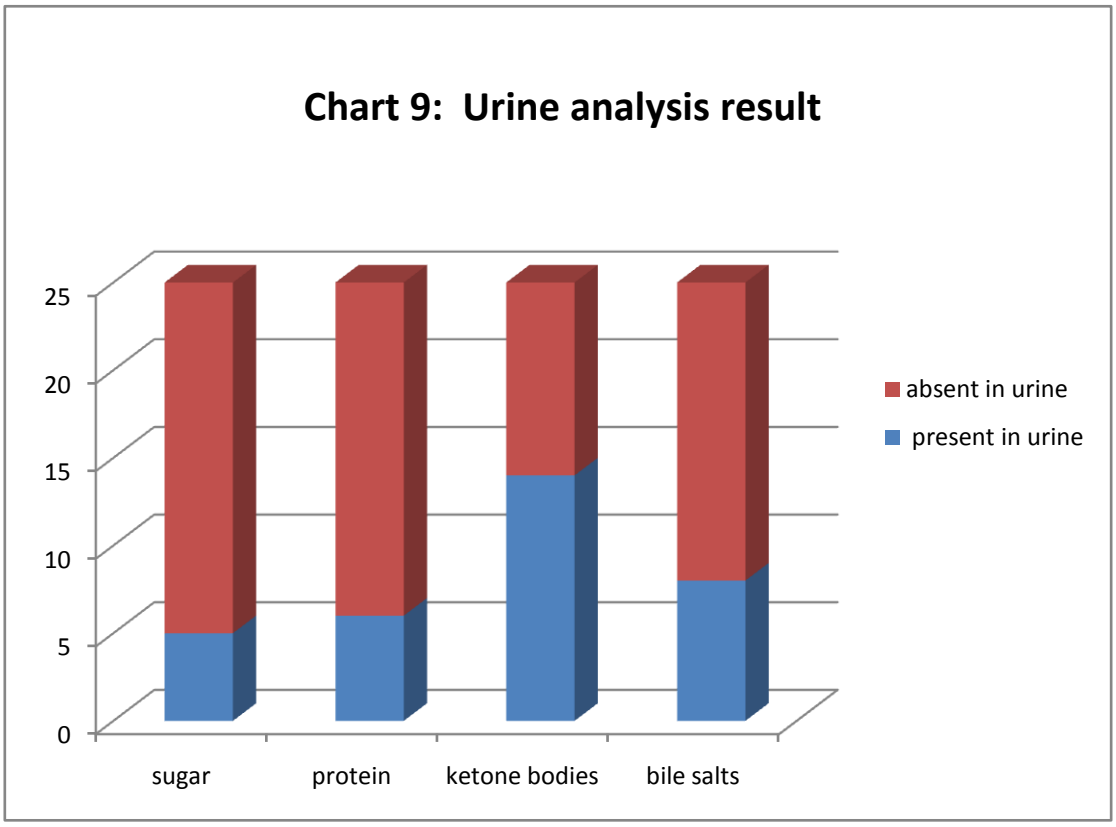


Chart 9: Urine analysis result
25
20
15
10
5
0
sugar
protein
ketone bodies
bile salts
absent in urine
present in urine

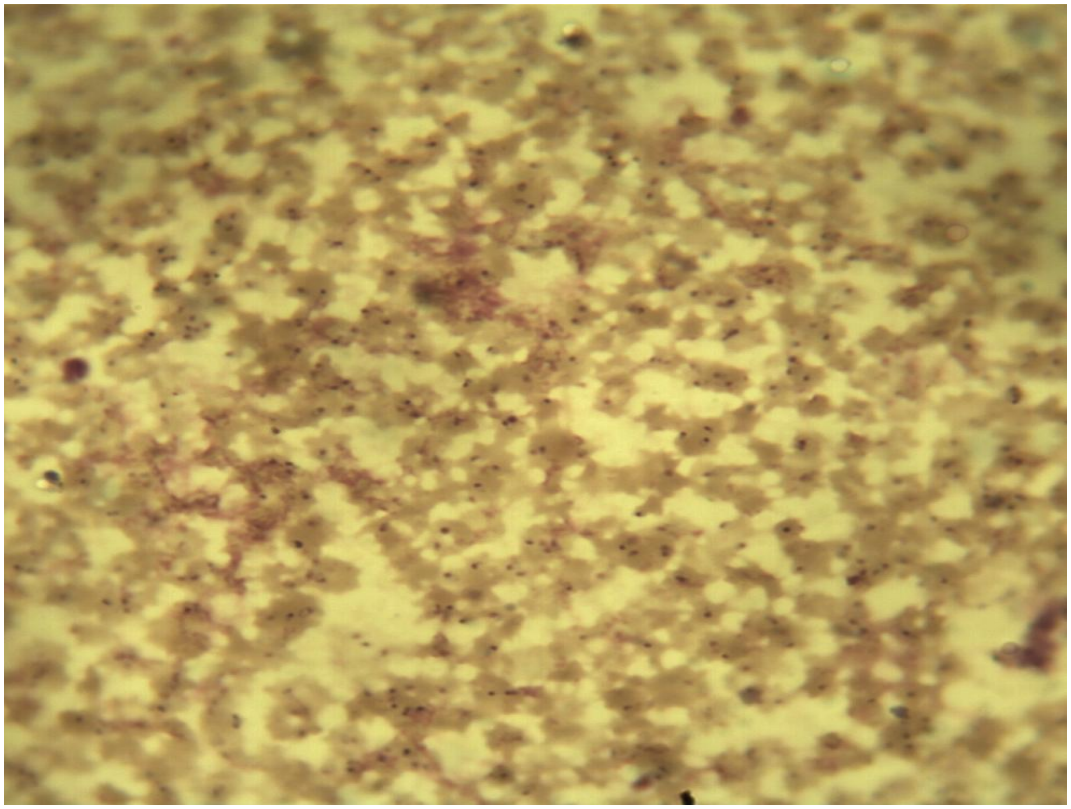


Fig. 1 RBC containing piroplasms

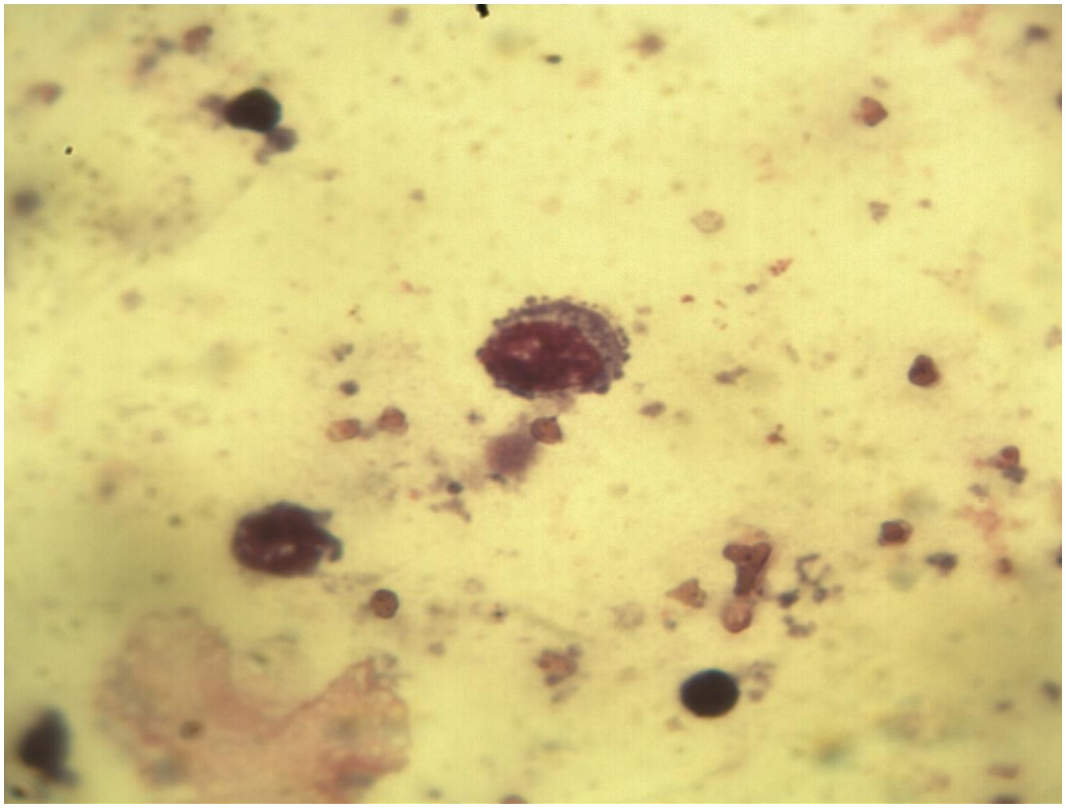


Fig. 2 Koch's blue body inside the cytoplasm of lymphoblasts

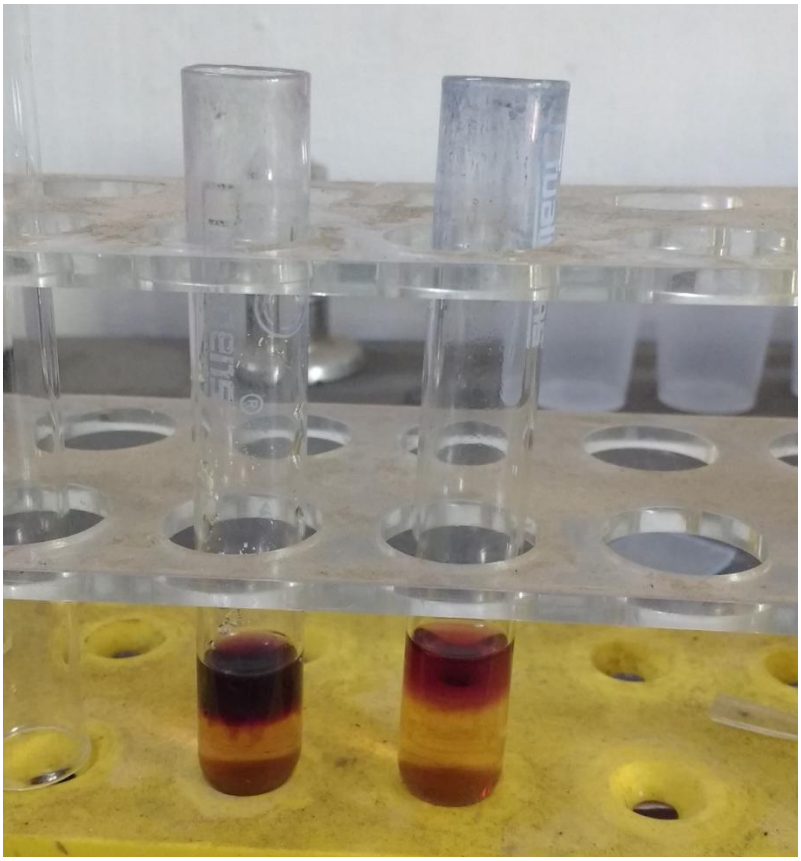


Fig. 3 Urine positive for Rothera test

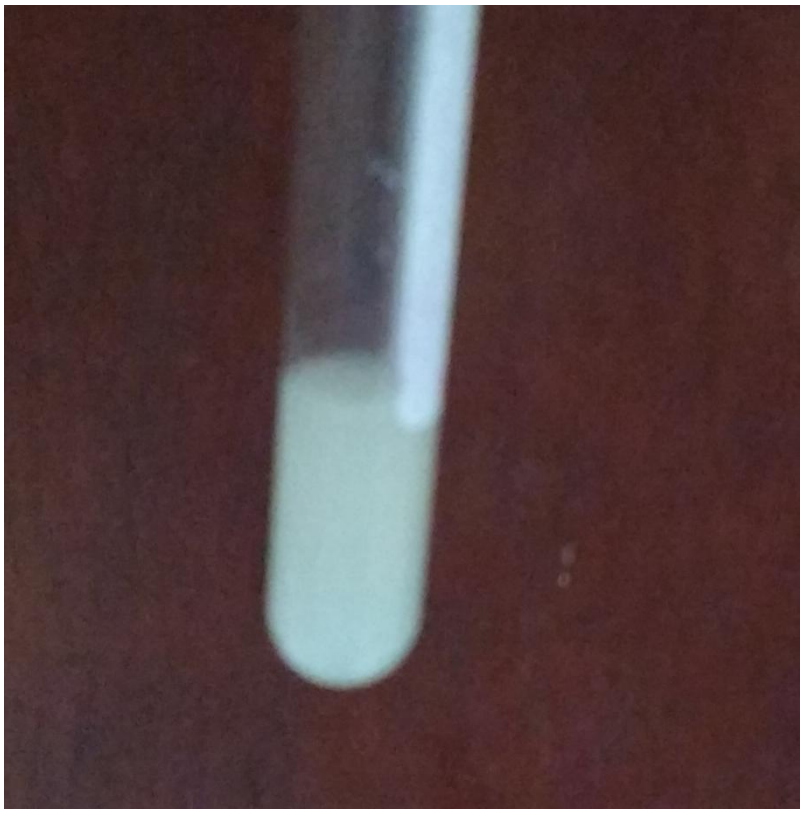


Fig. 4 Urine positive for Salkowich test

CHAPTER V

DISCUSSION

The rapidly increasing incidence of theileriosis in our country is causing production loss as well as life threatening for the crossbred cattle population. Prompt diagnosis of the disease is required for which studying hemato-biochemical changes is very much essential. Present work was undertaken to study the haematological and biochemical alteration in theileriosis affected cattle for a period of twelve months from July 2015 to June 2016.

A total of 1280 suspected cases with complaint of anorexia, non-remitting pyrexia, drop in milk yield, enlarged superficial lymph nodes, pale conjuctival mucous membrane, haemoglobinuria, nasal discharge and coughing which were suspected for theileriosis and screened for theileriosis on the basis of blood smear examination. Out of 1280 cases were examined for theileriosis, 928(72.5%) cases were found positive with presence of Piroplasms inside RBCs in blood smears examination indicating *Theileria annulata.* Many previous workers have reported prevalence of theileriosis by examining blood smears and biochemical alterations. Aulakh *et al*., (2003) reported 6.94% prevalence of *Theileria annulata* in cattle in Punjab state. Dumanli *et al*., (2005) carried out the study to determine the prevalence and distribution of tropical theileriosis and found piroplasms of theileria spp. in 19.7% cases by microscopical examination. Ram *et al*., (2006) assessed 66% sero-prevalence of theileriosis in cattle of arid regions i.e. western Rajasthan and 57% in semiarid regions of northwest India i.e. Haryana by using Indirect fluroscence antibody test (IFAT). Soundarajan and Rajavelu, (2006) studied prevalence of haemoprotozoan parasites in cattle and buffaloes reared in and around Madras. They recorded 28.2% and 8.0% prevalence of *Theileria annulata* in cattle and buffaloes respectively. Durrani *et al*., (2008) recorded 39.9% prevalence of Theileriosis based on microscopic examination in buffaloes in Lahore. Razmi *et al*., (2009) clinically examined and investigated a total of 160 dairy cattle in Iran and reported 20% prevalence of *Theileria annulata.* Chaudhri *et al*., (2013) examined stained blood smears from pyretic cross bred cows and found 27.88% *Theileria* affected cases. Kohli *et al*., (2014) experimented on prevalence of theileriosis from apparently

healthy crossbred cattle from various locations of Dehradun district and found 27.2% positive cases from microscopic examination of Giemsa stained blood smears.

Patient data regarding age, sex, stage of lactation, parturition history, clinical signs etc. were also recorded. The disease occurrence pattern varied in different age groups revealed highest incidence of theileriosis was in the adults 652 (70.25%) followed by 255 (27.47%) of 2-3 years of age and 21 (2.58%) of less than 1 year. The adults are very important as they are in both productive and reproductive period which may be hampred due to theileriosis in cows. In an epidemiological study, Sahoo (1991) observed that theileriosis occurred more frequently in adult cattle (5.85%) of exotic breed than the calves (3.96%) below one year of age. A striking feature was noticed where calves of less than 3 months being affected which may be regarded as juvenile theileriosis. Panda *et al*. (2011) observed that young animals were most susceptible. Digraskar *et al.* (2012) in a case study found *Theirelia annulata* infection in a 15 day old Holstein Friesian crossbred calf where there were swollen (Tennis ball size) prescapular & prefemoral lymph node. Issi and Gul (2008) encountered a case of hematuria in a calf with prenatal tropical theileriosis and concluded that *Theileria annulata* related prenatal infections could be developed in calves and apparent haematuria could be seen in these cases. Ananda *et al*., (2009) in their study found highest prevalence of theileriosis affected cases in 4-6 year age group.

Our study showed 907 (97.73%) were females and 21 (2.27%) were males. As the population of female cases are high in dairy farming. So the ratio of females were much more higher than males. Due to mechanization of farming, farmers also do not prefer to rear males for their less utility and thus males are no more required for drafting or pulling bullock carts. Sahoo, (1991) and Panda *et al.* (2011) observed that the infection of bovine tropical theileriosis in Bhubaneswar was higher in females (5.19%) than the males (4.17 %).

Amongst all the 928 cases, breed wise study revealed that there was a higher incidence of 795 (85.66%) theileria positive cases in cross bred Jersey cattle, followed by deshi or local breeds 34 (3.66%) and 99(10.66%) were of different breeds like Red Sindhi, Holstein Friesian and its crosses, Hariana and mixed breeds.. Cross bred Jersey cattle population was higher in this region of study which might

have influenced the result besides the breed susceptibility. Mallick *et al*. (1987) reported on the occurrence of haemoprotozoan infections 20.45% and 4.72% of crossbred and indigenous cattle respectively were infected with *Theileria annulata.* Sahoo (1991) observed T. annulata infection in crossbred (5.11%) as compared to indigenous cattle (3.04%). Rakha and Sharma, (2003) carried out epidemiological, clinical and serological study, in which they evaluated that, about 37% of crossbred animals showing pyrexia were positive for *T.annulata* and 43% of animals with pyrexia were found serologically positive by ELISA.

Season wise, there was highet incidence of theileriosis is during rainy season 422(45.47%) followed by summer 337(36.31%) and winter 169(18.21%). The result of our study is in accordance with Vahora *et al.* (2012) who recorded higher incidence during monsoon season. Panda *et al*. (2011) studied the prevalence and clinicopathological changes of theileriosis in bovine in coastal areas of Odisha. They observed that the seasonal incidence of theileriosis was 23% in rain, 35% in summer and 22% in winter. The occurrence of theileriosis in hot and humid climate may be highest as this type of climate favors the development of the parasite inside the ticks. Ananda *et al*., (2009) in their study found highest prevalence of theileriosis cases in monsoon months.

The haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) values were distinctly lower in theileria positive animals than apparently healthy animals. Sahu *et al.* (1996) investigated the haematological and biochemical alterations in theileria infected crossbred cattle. There was significant decline in Hb, PCV, TEC, TLC and non significant increase in ESR values in infected animals as compared to non infected animals reflecting anaemia. Panda *et al*. (2011) studied the prevalence and clinicopathological changes of theileriosis in bovine in coastal areas of Orissa and found haemoglobin concentration in positive cases varied between 2.2 to 17.0 g/dl and TEC varied from 1 million to 5.8 million/cmm of blood. However there was haemoglobin level less than 5g/dl in indicating destruction of blood constituents which more particularly renders the animal to a critical anaemic stage by reducing the erythrocyte and haemoglobin values and with requirement of a special care for improving haematological status and more than 13g/dl in which indicated dehydration and requirement of adequate fluid therapy. Omer *et al.* (2002), Muraleedharan *et al.*

(2005), Aulakh and Singla (2006), Col and Uslu (2006), Altug *et al.* (2008), Ananda *et al.* (2009), and Khan *et al.* (2011) also observed the decreased haemoglobin values. Aulakh *et al.* (1998), Col and Uslu (2006), Altug *et al.* (2008), Hasanpour *et al.* (2008), Ananda *et al.* (2009) and Khan *et al.* (2011) observed reduced PCV values due to theileriosis in comparison to healthy animals.

The average of TLC was 8.4 thousand/cu. mm. in apparently healthy animals which was reduced to 6.6 thousand/cu.mm. in theileria positive animals. Average Abs N count was also reduced from 4.4 thousand to 2.7 thousand in apparently healthy animals to positive animals. Likewise, the average Abs L count was also marginally reduced from 3.7thousand in apparently healthy animals to 3.5 thousand in positive animals. The leucopenia with leukocyte count less than 4000 per cumm indicating probable immunodeficiency and poor prognosis. However there was leukocytosis in few cases with leukocyte count above 10 thousand/cu. mm indicating a mixed bacterial infection. Several workers have reported leucopenia Aulakh *et al.* (1998), Omer *et al.* (2002), Muraleedharan *et al.* (2005), Col and Uslu (2006), Altug *et al.* (2008), Hasanpour *et al.* (2008) and Khan *et al.* (2011). The lower TLC values might be due to heavy destruction of theileria infected lymphocytes by the cell mediated immunity. All the positive cases of theileriosis showed leucopenia, lymphopenia and relative mild neutrophilia.

The RBC of theileriosis infected cattle revealed pathomorphologic alterations like anisocytosis, poikilocytosis, microcytosis, hypochromasia indicating degenerating anaemia, polychromasia and basophilic stippling indicating regenerative anaemia and crenation indicating parasitic toxaemia. The leucocytes were larger in size and pleomorphic in shape having moderately bluish violet stained larger nucleus of various shape and pale stained pleomorphic cytoplasm, as compared to the uninfected lymphocytes. The lymphocytes which contained Koch Blue bodies were also larger in size. Sharma and Gautam (1973), Dhar and Gautam (1979) and Venugopal (1983), Branco *et al.* (2010) and Sahoo (2014) had recorded different shapes of the piroplasm inside erythrocytes. Vahora *et al*., (2009) investigate the effect of theileriosis on blood profiles and found the presence of schizonts and piroplasms in the lymphocytes and erythrocytes respectively. Omer *et al*., (2002) studied about haematological parameters in young and adult Friesian cattle. Changes

in blood parameters in *T. annulata* infected cattle indicated severe macrocytic hypochromic anaemia, panleukopenia, lymphocytopenia, eosinopenia, neutropenia and thrombocytopenia but no reticulocytosis.

Serum biochemical parameters like glucose, cholesterol, total protein, albumin, and globulin showed decreased values in the affected cattle than apparently healthy cattle. For better clinical management during theileriosis, the correction of hypoglycaemia was of prime importance. Liver dysfunction possibly caused by inflammatory condition of liver initiated by trapping of damaged infected erythrocytes and lymphocytes interstitially in liver might cause decrease in total serum protein values. As destruction of lymphocytes by the parasites resulted lymphopenia this might cause Decrease in total serum globulin values.

On the other hand average values of some other serum biochemical parameters like BUN, creatinine, AST, ALT, total bilirubin and direct bilirubin of affected cattle showed increased values than apparently healthy cattle.

The significant increased levels of BUN in the present study indicated kidney damage, possibly caused due to trapping of agglutinated damaged infected erythrocytes and lymphocytes in the glomeruli, resulting into glomerulo nephritis.

Increase in the AST and ALT values in the present study indicated liver damage viz., primary or secondary liver necrosis and muscle necrosis (Benjamin, 2001). The extensive erythrocyte damage caused by infestation of parasite (merozoite) result into severe anemia leading to generalized hypoxia. This hypoxia could be one of the reasons for degeneration leading to necrosis. Furthermore, prolonged recumbency in theileriosis also aid in muscle degeneration and necrosis.

The rise in bilirubin levels might be due to due to increased destruction of parasitized erythrocytes by erythrophagocytosis in the spleen, lymphnodes, and other organs of the reticuloendothelial system. Another cause for increase in bilirubin levels could be hepatic dysfunction and hemolytic anemia.

Sahu *et al.* (1996) investigated the haematological and biochemical alterations in theileria infected crossbred cattle and found significant decline in serum glucose,

total protein and Ca and non significant decline in serum Mg and P in infected animals. Serum analysis by Singh *et al*., (2001) revealed decrease in concentration of calcium, cholesterol and triglycerides, total serum protein, albumin, globulin, albumin : globulin ratio, While there was increase in concentration of BUN. Omer *et al*., (2003) analysed biochemical parameters in adult and young Friesian cattle naturally infected with *Theileria annulata* and they had significantly lower serum total protein, albumin, globulin, creatinine, calcium, phosphorus, magnesium, potassium, iron and copper concentrations and significantly higher AST activity and bilirubin concentration than the healthy cattle. Abou-El-Naga *et al*., (2005) evaluated infection of *T. annulata* and affected cattle had significantly low levels of total proteins, albumin, magnesium, potassium and iron concentrations but AST, glutamyl transferase activities, total, direct and indirect bilirubin, creatinine levels were significantly high. Similar findings were also observed by Aulakh and Singla, (2006), Col and Uslu, (2007), Hussein *et al*., (2007), Altug *et al*., (2008), Hasanpour *et al*., (2008), Saber *et al*., (2008), Rashid *et al*., Ugalmugle *et al*. (2010), Khan *et al*., (2011) and Dede *et al*., (2014).

The findings of urine samples examination revealed presence of sugar in 5, protein in 6 bile salt in 8 and Ketone bodies in 14 urine samples, out of the collected 25 urine samples. The result of the urine analysis was indicating systemic dysfunction along with metabolic disturbances. 56% of the urine samples showing positive result for ketosis indicates that animals affected with theileriosis are much more prone to ketosis than the healthier animals. This may be due to the metabolic disturbances caused by theileriosis. El-Deeb and Younis, (2009) demonstrate the clinical picture associated with theileriosis in buffaloes with particular emphasis to the oxidative stress and ketosis. In their study they found that there was a significant increase ($p \leq 0.05$) in the levels of NEFA and BHBA in *Theileria annulata* infected buffaloes, in comparison with healthy buffaloes, indicating the ketotic state of these cases.

CHAPTER VI

SUMMARY AND CONCLUSION

In our country incidence of theileriosis is rapidly increasing, causing production loss as well as life threatening for the crossbred cattle population. Prompt diagnosis of the disease is very much essential for which study of hemato-biochemical changes is one of the most important procedure. Present work was undertaken to study the haematological and biochemical alteration in theileriosis affected cattle for a period of twelve months from July 2015 to June 2016.

A total of 1280 suspected cases with complaint of anorexia, non-remitting pyrexia, drop in milk yield, enlarged superficial lymph nodes, pale conjuctival mucous membrane, haemoglobinuria, nasal discharge and coughing which were suspected for theileriosis and screened for theileriosis on the basis of blood smear examination. Out of 1280 cases were examined for theileriosis, 928(72.5%)cases were found positive with presence of Piroplasms inside RBCs in blood smears examination indicating *Theileria annulata.*

Patient data regarding age, sex, stage of lactation, parturition history, clinical signs etc. were also recorded. The disease occurrence pattern varied in different age groups revealed highest incidence of theileriosis was in the adults 652 (70.25%) followed by 255 (27.47%) of 2-3 years of age and 21 (2.58%) of less than 1 year. The adults are very important as they are in both productive and reproductive period which may be hampred due to theileriosis in cows.

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- Out of 1280 cases were examined for theileriosis, 928(72.5%) cases were found positive with presence of Piroplasms inside RBCs in blood smears examination indicating *Theileria annulata.*
- Adult animals (70.25%) are more exposed to theileriosis followed by 27.47% of 2-3 years of age and 2.58% of less than 1 year.
- Females are the most sufferers than its male counterpart.
- Crossbred Jersey seems to be more often affected.
- Mixed infection in theileriosis affected cattle are to be considered.
- The most important clinical signs observed during the study were reduced appetite, anorexia, non remittent fever, enlargement of superficial lymph nodes, salivation, lacrimation, recumbence, dropped milk yield, diarrhea, red eye which can be considered for diagnosis of the disease. Hemato-biochemical estimation can be utilised for diagnosis, prognosis and for better management of metabolic health status of the animal during the disease.
- The haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) values were distinctly lower in theileria positive animals than apparently healthy animals.
- The haemoglobin (Hb), packed cell volume (PCV) and total erythrocyte count (TEC) values were distinctly lower in theileria positive animals than apparently healthy animals.
- Serum biochemical parameters like glucose, cholesterol, total protein, albumin, and globulin showed decreased values in the affected cattle than apparently healthy cattle.
- On the other hand average values of some other serum biochemical parameters like BUN, creatinine, AST, ALT, total bilirubin and direct bilirubin of affected cattle showed increased values than apparently healthy cattle.
- 56% of the urine samples showing positive result for ketosis indicates that animals affected with theileriosis are much more prone to ketosis than the healthier animals. This may be due to the metabolic disturbances caused by theileriosis.

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