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PI Ganesan

Professor and Head, Department of Veterinary Medicine, Apollo College of Veterinary Medicine, Jaipur, Rajasthan, India

Pratipal Singh Kaurav

Assistant Professors, Department of Livestock Farm Complex, Apollo College of Veterinary Medicine, Jaipur, Rajasthan, India

Mahendra Kumar Meena

Assistant Professors, Department of Livestock Farm Complex, Apollo College of Veterinary Medicine, Jaipur, Rajasthan, India

CS Sharma

Dean, Apollo College of Veterinary Medicine, Jaipur, Rajasthan, India

Corresponding Author:

PI Ganesan

Professor and Head, Department of Veterinary Medicine, Apollo College of Veterinary Medicine, Jaipur, Rajasthan, India

Factors associated with clinical and Hemato-biochemical changes in *Theileria* infected Gir cows

PI Ganesan, Pratipal Singh Kaurav, Mahendra Kumar Meena and CS Sharma

Abstract

Gir cows in an organized livestock farm in Jamdoli, Jaipur were confirmed with the clinical signs such as anorexia for more than three months, gradual reduction in milk yield, mild lymphadenopathy, conjunctivitis and mild nasal discharge. Blood smear examination of these animals confirmed *Theileria annulata* infection. Hematological studies in Gir cows revealed significant low RBC count, Hb content, lymphocytes count. Bio-chemical studies in these cows revealed significant high values of, total protein, total bilirubin, globulin, & phosphorus with insignificant low values of Albumin: Globulin ratio & Calcium level. The restricted macro-schizont replication in theileria infected lymphoid tissues, more white blood cell production in native cattle due to less pronounced bone marrow suppression activities, more activated macrophages, low production of pro-inflammatory cytokines, removal of causative organisms and the restoration of tissue homeostasis, were the attributed factors associated with less pronounced clinical and hemato-biochemical parameter variations in theileria infected native Gir cows.

Keywords: Gir-clinical, hemato-bio-chemical-Theileria

Introduction

Theileriae are obligate intracellular protozoan parasites that infect both wild and domestic bovidae throughout the world. The *ixodid* ticks are playing major role in transmission of the disease (OIE, 2014) [21]. There are a number of species of theileria that infect cattle; the two most pathogenic and economically important parasites are *T. annulata* and *T. parva* (Bhatnagar, C.S., *et al.* 2015; Demessie, Y and S. Derso 2015; Gul. N., *et al.* 2015; OIE, 2014) [6, 7, 13, 21]. *T. annulata* occurs in Southern Europe as well as North Africa and Asia (OIE, 2014) [21]. Traditional diagnosis of bovine theileriosis is mainly based on the microscopic examination of blood smears for the presence of the merozoites of theileria (Junlong, L., *et al.* 2015) [15].

Breed differences in susceptibility to *T. annulata* infection

Different breeds of cattle are different in their susceptibility to theileriosis. Saeed, Z *et al.* (2016) [24] reported that the exotic and cross bred cattle are highly susceptible, while indigenous cattle are relatively resistant to tropical theileriosis. Exotic cattle breeds are extremely susceptible to tropical theileriosis caused by *T. annulata*. The infection induces severe and often fatal diseases in susceptible *Bos-taurus* and cross-bred cattle. *Bos-indicus* animals manage the infection better and develop a solid immunity, presumably due to the activation of innate immunity. (Spooner RL, *et al.* 1991) [30]. Bakheit and Latif (2002) [4] carried out studies to assess the innate resistance of the indigenous Kenana breed of cattle in Sudan to tropical theileriosis and found that the percentage of schizont parasitosis in the Kenana cattle was reduced by 70% in comparison with Friesian calves. The percentage of piroplasm parasitaemia also significantly lower in the Kenana calves. The rate of WBC reduction was significantly greater in Friesian calves. Of the Kenana cattle, 78% recovered spontaneously, and only 22% required treatment compared to 100% mortality in the Friesian controls. Kenana cattle able to limit the macro-schizont replication and it results in less severe damage to the lymphoid tissues, during the acute phase of the disease. (Glass EJ *et al.* 2005) [10].

The macrophages in *Bos-indicus* breed are able to control the cytokine storm, where it is devastating in *Bos-taurus* breeds. (Glass EJ *et al.* 2007) ^[11]. Evaluated the resistance to *T. annulata* of indigenous Iranian cattle compared to Holstein cattle and reported lower parasitaemia rate, milder clinical signs, and lower levels of acute –phase proteins. Rehman, Z *et al.* (2014) ^[23] reported Sahiwal as an excellent breed for internal and external parasitic resistance. Sajid M.S *et al.* (2009) ^[26] reported low prevalence of parasite in Sahiwal cattle than European breeds suggesting that Sahiwal cattle are more resistant to tick infestation.

The prevalence studies on bovine theileriosis in indigenous animals and crossbred animals were 13.33% & 26.66% respectively for *T. annulata* infection in Rajasthan state. Ayadi, O *et al.* (2017) ^[3] reported that indigenous breeds are more resistant to *T. annulata* infection than exotic breeds such as Friesian and Holstein cattle (Gharbi *et al.* 2014; Saleem *et al.* 2014) ^[9, 25]. This difference in sensitivity is attributed to the difference in the immune response to produce pro-inflammatory cytokine which is higher in exotic breeds (Glass *et al.* 2005; Jensen *et al.* 2008) ^[10, 14]. Ayadi, O. *et al.* (2017) ^[3] reported two types of anemia, a microcytic hypochromic observed in Montbeliard- native breed cattle of North Algeria infected with *T. annulata*, and normocytic normochromic anemia in cross bred cattle. Genetic polymorphism in innate immune genes plays a role in disease resistance in various breeds of cattle and buffaloes. Reported that the indigenous breeds are quite resistant to infection and they act as reservoirs or carriers, and likely to transmit the organisms to other breeds of bovine. Shreya M. Patel *et al.* (2015) ^[31] studied innate immune response of Gir animals and reported that the activation of innate immunity initiates various types of reactions. This activation contribute defense against physical, microbial or chemical damage and help in damage repair, removal of causative organisms and in the restoration of tissue homeostasis.

Glass E. J *et al.* (2005) ^[10] reported more activated macrophages in *B. indicus* animals due to theilerial species infection. Ayadi O *et al.* (2017) ^[3] reported less production of pro-inflammatory cytokines in theileria infected *Bos-indicus* animals. Glass E J *et al.* (2007) ^[11] reported low level

macrophage pro-inflammatory cytokine dependent acute phase proteins in the tolerant breeds like *Bos-indicus*. The clinical manifestations and hemato-biochemical parameters get altered in theileria infected native cattle population to lesser extents because of the above factors. Keeping in view of the above facts this study was carried out to determine the clinical and hemato-biochemical parameter alterations under farm conditions for theileriosis in indigenous Gir cows

Materials and Methods

The study was carried out in a livestock farm in Jamdoli, Jaipur with six Gir cows. The clinical signs of these cows were recorded. Blood smear examinations of all the six Gir cows carried out using Giemsa staining as per standard procedure based on clinical signs. After confirmation for theileria infection these cows' blood samples were subjected to hemato-biochemical reactions.

Results and Discussion

1. Clinical signs of the Indian cattle with theileriosis

Bakheit and Latif (2002) ^[4] reported that the clinical signs are milder due to innate resistance in native cattle due to low level schizont parasitosis, reduced WBC, macrophages controlling activity on cytokines and low level production of acute phase proteins. Evaluated the resistance to *T. annulata* of indigenous Iranian cattle compared to Holstein cattle and reported lower parasitaemia rate, milder clinical signs. Nejash A. *et al.* (2016) ^[19] reported that *Bos-indicus* cattle are more resistant to ecto-parasites than *Bos-taurus* animals and claimed differences between these two breeds of cattle in regard to their susceptibility to parasitism by cattle ticks. Reported lower susceptibility of Tharparker to *T. annulata* infection from Pakistan. Shashi Choudhary *et al.* (2022) ^[29] reported that the indigenous cattle were less prone than cross bred cattle and upgraded Murrah buffaloes. These observations are in concurrence with the findings of the present studies.

2. Hematological studies in Gir cows with theileria infection (Table 1)

Table 1: Hematological values in Gir cows with theileriosis

Parameters	Gir-infected cows mean values	Gir normal cows (range values)
RBC ($10^6/UL$)	4.75	6.0-8.0
Hb (g/dl)	9.36	10.0-12.0
WBC ($10^3/UL$)	10.06	8.0-10.0
PLT ($10^3/UL$)	437.0	100-800
LYM (%)	45.00	50.0-60.0
Granulocytes (%)	22.6	15.0-45.0

The study revealed insignificant low values of RBCs, Hb and lymphocytes percentage levels in this Gir cows with theileria infection (Table 1) Nilima N *et al.* (2022) ^[20] reported decreased level of Hb, RBCs, and attributed that this might be due to the damage caused by the organisms inside the RBC's during their multiplication, lysis of RBCs and erythrophagocytosis by piroplasms, which infect and replicate. Spooner *et al.* (1991) ^[30] reported that the *Bos-indicus* animals manage the infections due to activation of the innate immunity. Swami S B *et al.* (2019) ^[32] reported that low level lymphocytes could be due to inflammatory conditions and reported that the hematological values from the Gir cows affected with theileria infection showed low level of RBC count, Hb content, lymphocytes followed by

significant raise in the total leucocytes.

This study observed insignificant low level changes in the WBC count in these Gir animals. Bakheit and Latif (2002) ^[4] reported that the rate of WBC reduction was significantly lower in Kenana cattle breed of Sudan due to their capacity to limit the macro-schizont replication which occurs in the theileria infected lymphoid tissues. The level of WBC is more in native cattle due to less pronounced bone marrow suppression, which is in agreement with this part of the study. The platelet counts (PLT) were maintained in its range in this theileria infected Gir cows. Mahmoud Rushdie Abd Ellah *et al.* (2015) ^[17] reported significant low level of PLT values which was attributed to the thrombocytopenia together with normocytic normochromic anemia and partially attributed

to depression of the bone marrow. Omer OH *et al.* (2002) [22] and Thomas JS (2010) [33] reported thrombocytopenia in theileriosis infected pure bred cattle due to bone marrow hypoplasia. Agina *et al.* (2021) [1] reported no significant difference in the mean values of platelets of theileria species

infected Malaysian cattle groups, which is in agreement with this study.

3. Bio-chemical studies in Gir cows with theileriosis (Table 2)

Table 2: Biochemical values of Gir cows with theileriosis

Parameters	Infected Gir cows (Mean values)	Healthy Gir cows (range values)
AST (UL)	105.96	100.0-102
Creatinine (mg/dl)	1.81	1.0-2.0
Urea (mg/dl)	30.84	20-30
Total Bilirubin (mg/dl)	1.101	0.47±0.16
Total protein (g/dl)	9.516	6.82±0.02
Albumin (g/dl)	3.171	3.45±0.01
Globulin (g/dl)	6.34	3.36±0.05
A: G ratio	0.510	1.02±0.01
Calcium (mg/dl)	4.03	9.7±12.4
Phosphorus(mg/dl)	12.87	5.6-6.5

In this study the infected Gir cows showed increased values of AST. Nilima N *et al.* (2022) [20] reported elevated values of AST due to liver damage in these cattle. Reported an increased level of AST due to liver damage and muscular necrosis.

In this study the creatinine and the urea levels observed in normal levels. Nilima N *et al.* (2022) [20] reported insignificant increase in the creatinine, values with increased BUN level. Swami *et al.* (2019) [32] reported creatinine level doesn't showing significance in the theileria infected Gir cows which is in agreement with this study. The study showed a significant high level of total bilirubin and total protein values in this Gir cows. Sandhu *et al.* (1998) [27] and Singh *et al.* (2001) [28] reported elevated bilirubin values due to hepatic dysfunction and hemolysis in diseased cows due to theileria. Agina *et al.* (2021) [1] reported increased level of total protein in *T. sinensis* infected Malaysian cattle population due to an on-going inflammatory condition and dehydration, which is in agreement with the findings of this study. In this study, the albumin level was observed in an insignificant low level.

Emad A.H *et al.* (2020) [8]; Ghosh *et al.* 2007 [12]; Reported significant low serum albumin level in cattle due to impaired liver function. In this study highly elevated globulin level was observed. Serum globulin levels were elevated in these cases due to the body immune response for theileria infection. In this study the obtained A: G ratio values were low. Agina *et al.* (2021) [1] reported *T. sinensis* affected cattle with decreased A: G ratio. Nilima N *et al.* (2022) [20] reported decreased A: G ratio due to liver damage. The calcium level in this study is at low level and was insignificant. Mahmoud Rushdi Abd Ellah *et al.* (2015) [17] reported significant decreases in the calcium, due to hepatic and kidney functions. In the above study the theileria infected cattle showed hypophosphatemia due to renal tubular defects and hypocalcemia was attributed to hypo-albuminaemia. The phosphorus level was significantly high in this study. Agina *et al.* (2021) [1] reported an elevated level of inorganic phosphate in Malaysian native cattle infected with *T. sinensis* which is in agreement with the observation of this study.

Conclusion

The infected Gir cows showed the clinical signs for a period of 3 weeks in milder ways. i.e anorexia, mild nasal discharge, gradual decrease in milk yield and mild conjunctivitis. The animals suffered with *T. annulata* infection and the infection created low level changes in clinical and hemato- biochemical

parameters due to the presence of innate immunity in Gir animals for longer period. The changes varies from one region to another and the parameters getting influenced by the characteristics such as the innate immune response, the dose and virulence of the strain of *T. annulata* in each country. The observations presented in this paper can be taken as criteria for diagnosis and interpretation of results of *T. annulata* infection in native cows like Gir, Sahiwal, Tharparker, Ongole, Kangayam and some more cattle breeds of India.

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