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Clinicopathological evaluation of oriental theileriosis in crossbred cattle of Hassan district, Karnataka

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Abstract
The present work has been carried out to study the clinico-pathological evaluation of Oriental theileriosis in crossbred cattle. Out of 100 suspected clinical samples, 54 samples were exclusively found positive for *Theileria orientalis* by using blood smear examination and polymerase chain reaction. Infected cattle have shown clinical signs like anorexia, reduced milk yield, pale conjuctival mucus membrane, lymphnode enlargement, severe tick infestation. There was significant increase in physiological parameters such as temperature, respiratory rate and heart rate in infected animals. Blood samples collected from positive animals were subjected to estimation of haemato-biochemical parameters. Haematological analysis revealed significant decrease in haemoglobin, total erythrocyte count (TEC), packed cell volume (PCV) and non-significant changes were noticed in erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). There was also slight decrease in leucocyte count with neutrophilia, lymphocytopenia and eosinophilia. Biochemical analysis revealed significant decrease in blood glucose level, total protein, albumin, globulin, cholesterol concentration and significant increase in blood urea nitrogen level (BUN), creatinine, alanine amino transferase (ALT), aspartate amino transferase (AST), total bilirubin and direct bilirubin in *T. orientalis* infected animals.

Keywords: *Theileria orientalis,* hemato-biochemical, erythrocytic indices, neutrophilia, bilirubin

1. Introduction
Oriental Theileriosis or Theileria-associated bovine anaemia is an important vector borne disease of cattle caused by a *Theileria orientalis*, a haemoprotozoan parasite of genus *Theileria*. Chitose and Ikeda genotypes are associated with severe disease. *T. orientalis* has a worldwide distribution and found in many countries such as Australia, New Zealand, Japan, Korea, China, Vietnam, Myanmar, Srilanka and India. In Asia, Australia and New Zealand the primary tick vector for *T. orientalis* is *Haemophysalis* spp. In the last decade or so, *T. orientalis* gaining lot of importance in Indian sub continent. The disease has been reported in many states such as Odisha (Sahoo *et al.,* 2017; Ahmed *et al.,* 2020) [20, 3], Assam (Brahma *et al.,* 2018) [4], Kerala (Sudhakar *et al.,* 2021) [27], Maharashtra (Patil, 2017) [19] and Karnataka (Madhukar, 2015) [15]. *Theileria orientalis* exerts its major effect due to its piroplasmic form causing destruction of erythrocytes resulting in severe anaemia, but the pathogenesis of anaemia consequent to infection is not clearly established and may be multifaceted (Stockham *et al.,* 2000) [26]. Studies of erythrocyte survival in infected animals demonstrated that both infected and uninfected erythrocytes have reduced survival (Yagi *et al.,* 1991) [30]. Studying the clinicopathological conditions associated oriental theileriosis helps in early diagnosis and appropriate treatment. Because of high treatment cost, reduced production and death of infected animals in severe cases makes this disease as one of the important economic disease of cattle in India.

2. Materials and Methods
2.1 Ethical animal handling
The study protocol was reviewed and approved by the Institutional Animal Ethical Committee, (IAEC), Veterinary College, Bengaluru. All the precautions were taken to avoid or minimize discomfort, distress and pain to the animals under study.

2.2 Hemato biochemical evaluation
One hundred cattle which were showing clinical signs suggestive of bovine theileriosis were

subjected to blood smear examination and polymerase chain reaction. Haematological parameters *viz*., Haemoglobin (Hb), Total erythrocyte count (TEC), Packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), Total leukocyte count (TLC) were estimated using Fully automatic blood cell counter (ERMA, Model: PCE-210) in the Department of Veterinary Medicine, Veterinary College, Hassan. Differential leucocyte count (%) was done by preparing a thin blood smear, staining with Giemsa stain and observing under oil immersion lens. For collection of serum, 5 ml of blood was drawn from each case aseptically using a clot activator vial. The vials were kept undisturbed for about 20 to 30 minutes, centrifuged and serum was collected in sterile serum vials. Serum biochemical parameters like glucose, ALT, AST, total protein, albumin, cholesterol, blood urea nitrogen, creatinine, total billirubin and direct billirubin were estimated by using biochemical kits supplied by Swemed Biomedicals Pvt Ltd (Semiautomatic Biochemical analyser Model: ARTOS ELITA) at Department of Veterinary clinical complex, Veterinary College, Hassan. Blood samples from ten apparently healthy cows of the locality were also collected for haemato-biochemical estimation and compared with that of affected cattle.

2.3 Blood smear examination
Blood smear was made using blood collected from the peripheral circulation such as ear vein, fixed with absolute methanol (5mins), stained with 10% Giemsa stain (30mins) and examined under oil immersion microscope to observe intraerythrocytic forms of *Theileria orientalis* (Zafar *et al*., 2006) [32]. The parasites were identified according to the characters described by Soulsby (1982) [25].

2.4 DNA extraction and PCR
The DNA from all the blood samples was isolated using the method described by Martin *et al*. (2010) [16] and was subjected to PCR assay for amplification of specific genes. PCR assay was performed for confirmation by using *Theileria orientalis* (MPSP gene) specific primers (Ahmed *et al.,* 2020) [3].

2.5 Statistical analysis
Statistical analysis was carried out by use of SPSS software version. The average value of haematological and biochemical parameters of the affected cattle were compared with average values of apparently healthy cattle by student t test and expressed as Mean ± SE, with P<0.05 considered statistically significant.

3. Results
Major clinical signs seen in *Theileria orientalis* infections were anorexia/inappetence, reduced milk yield, pale conjuctival mucus membrane (Fig.1), suspended rumination, history of tick infestation (Fig.2), lymph node enlargement (Fig.3a&3b), recumbence/general weakness, reduced rumeno reticular motility, fever, tachycardia, respiratory distress, lacrimation /salivation, nervous signs and diarrhoea. Examination of Giemsa stained blood smear revealed presence of *Theileria* piroplasms in 54 out of 100 cattle selected for the study. The piroplasms appeared as rod shaped in the cytoplasm (Fig. 4a&4b). The DNA extracted from all the infected cattle was subjected to species specific PCR for molecular identification. The species specific PCR with MPSP-F and MPSP-R primer pair yielded 776bp specific for *T. orientalis*. The mean, standard error and P value (One way ANOVA) for physiological, hematological and biochemical parameters are depicted in Table-1, 2 &3 respectively. There was a statistically significant increase in physiological parameters such as temperature, respiratory rate and heart rate in cattle affected with *T. orientalis* compared with control group. There was statistically significant decrease in haemoglobin, total erythrocyte count, PCV in infected animals compared with healthy control group. With respect to erythrocytic indices, there was statistically significant increase noticed in MCV, MCHC and non significant increase in MCH when compared with control healthy group. There was slight decrease in leukocyte count with neutrophilia, lymphocytopenia and eosinophilia. There was significant decrease in blood glucose level, total protein, albumin, globulin, cholesterol and significant increase in blood urea nitrogen, creatinine, SGPT, SGOT, total bilirubin and direct bilirubin in *T. orientalis* affected animals compared with healthy control group.

Table 1: Physiological parameters of Oriental theileriosis (Mean ± SE)

Sl. No	Parameter	Control Group (N:10)	*T. orientalis* (N: 54)	P Value
1.	Temperature (°F)	101.23 ± 0.19[a]	102.99 ± 0.08[b]	< 0.001
2.	RR (per Minute)	37.60 ± 1.01[a]	53.48 ± 0.43[b]	< 0.001
3.	HR (per minute	76.70 ±1.97[a]	91.26 ± 0.85[b]	< 0.001

The value with different superscript differ significantly at 5 % level of significance (P < 0.05)

Table 2: Haematological & Biochemical parameters of Oriental theileriosis (Mean ± SE)

Sl. No.	Parameter	Healthy Control Group (N:10)	*T. orientalis* (N: 54)	P Value
1.	Haemoglobin (g/dL)	11.6 ± 0.21[a]	6.63 ± 0.09[b]	< 0.001
2.	TEC (X10^6/µ)	6.36 ± 0.16[a]	3.54 ± 0.07[b]	< 0.001
3.	PCV %	38.23 ± 0.94[a]	22.40 ± 0.40.[b]	< 0.001
4.	MCV (fL)	60.14 ± 3.35[a]	64.31 ± 1.44[b]	0.005
5.	MCH (pg)	18.25 ± 0.88[a]	19.01 ± 0.38[a]	0.062
6.	MCHC %	30.34 ± 0.85[a]	32.75 ± 0.37[b]	< 0.001
7.	TLC (X10^3/µ)	8.11 ± 0.27[a]	7.80 ± 0.12[a]	0.269
8.	Neutrophils %	30.50 ± 1.38[a]	37.93 ± 0.59[b]	< 0.001
9.	Lymphocytes %	64.50 ± 1.69[a]	54.96 ± 0.72[b]	< 0.001
10.	Eosinophils %	2.10 ± 0.45[a]	3.85 ± 0.19[b]	0.003
11.	Monocytes %	2.10 ± 0.37	2.69 ± 0.16	0.303
12.	Basophils %	0.80 ± 0.21	0.57 ± 0.09	0.343

The value with different superscript differ significantly at 5 % level of significance (P < 0.05)

Table 3: Biochemical parameters of Oriental theileriosis (Mean ± SE)

Sl. No.	Parameter	Healthy Control Group (N:10)	*T. orientalis* (N: 54)	P Value
1.	Glucose (mg/dl)	74.50 ± 2.03[a]	58.20 ± 0.87[b]	< 0.001
2.	Total Protein (g/dl)	7.70 ± 0.18[a]	5.55 ± 0.08[b]	< 0.001
3.	Albumin (g/dl)	4.23 ± 0.13[a]	2.79 ± 0.06[b]	< 0.001
4.	Globulin (g/dl)	3.47 ± 0.13[a]	2.76 ± 0.06[b]	< 0.001
5.	Cholesterol (mg/dl)	116.30 ± 4.07[a]	87.85 ± 1.75[b]	< 0.001
6.	BUN (mg/dl)	20.20 ± 1.75[a]	25.39 ± 0.75[b]	0.005
7.	SGPT (U/L)	24.30 ± 1.76[a]	32.50 ± 0.76[b]	< 0.001
8.	SGOT (U/L)	74.40 ± 9.12[a]	106.61 ± 3.93[b]	< 0.001
9.	Creatinine (mg/dl)	0.84 ± 0.08[a]	1.13 ± 0.03[b]	< 0.001
10.	Total Bilirubin (mg/dl)	1.51 ± 0.15[a]	2.02 ± 0.06[b]	<0.001
11.	Direct Bilirubin (mg/dl)	0.35 ± 0.08[a]	0.87 ± 0.03[b]	<0.001

The value with different superscript differ significantly at 5 % level of significance (P < 0.05)

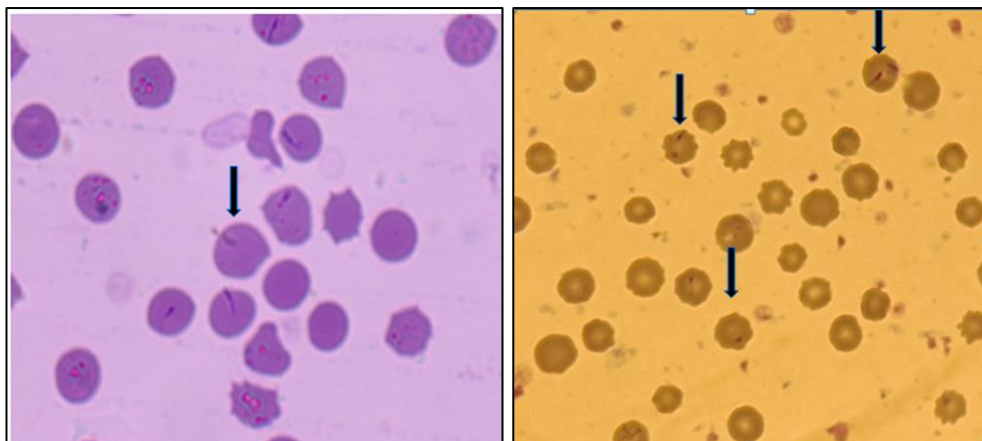**Fig 1:** Pale conjuctival mucus membrane

Fig 3a: Enlarged Prefemoral lymph node

Fig 2: Severe tick infestation

Fig 3b: Enlarged prescapular lymphnode

Fig 4a & 4b: Rod shaped intraerythrocytic piroplasm in Giemsa stained Blood smear examination

4. Discussion
Oriental Theileriosis is a tick borne disease of mainly cattle and it has emerged as one of the major constraints affecting livestock population (Kolte *et al.,* 2017) [12]. In the present study, major clinical signs found in cattles affected with oriental theileriosis are anorexia, reduced MY, pale CMM, fever, tachycardia, respiratory distress, LN enlargement. Similar signs were also noticed by many workers (Sudhakar *et al.,* 2021; Ahmed *et al.,* 2020; Patil, 2017 and Madhukar, 2015) [27, 3, 19, 15]. In the present study there was significant increase in body temperature, respiratory rate and heart rate compared to healthy group. Increased respiratory rate and heart rate could be attributed to severe anaemia, decreased oxygen carrying capacity and anoxia resulting in compensatory increase in respiratory rate and heart rate. Present results are in accordance with so many previous authors (Madhukar, 2015 and Sudhakar *et al.,* 2021) [15, 27]. Haematlogical evaluation revealed that *T. orientalis* affected cattle have shown severe anaemia with reduced haemoglobin, total erythrocyte count and PCV. Present results are in accordance with Sudhakar *et al.,* (2021) [27], Ahmed *et al.,* (2020) [3], Sivakumar *et al.*, (2017) [24]. The pathogenesis of anaemia in *T. orientalis* infected animals was not clearly established and it may be multifaceted. Increased osmotic fragility of erythrocytes leading to abnormal morphology, reduced survival rate of both infected and uninfected erythrocytes (Yagi *et al.*, 1991) [30] and oxidative damage and lipid peroxidation of erythrocytes (Yagi *et al.*, 2002) [31] were considered to play important role in the development of anaemia in *T. orientalis* infected animals. Shiono *et al.* (2001) [23] reported increased methaemoglobin concentration leading to production of free radicals and resulting in oxidative damage to RBC membranes in *T. orientalis* infected animals. The infected animals have shown significant increase in MCV, MCHC and insignificant increase in MCH values compared to control group suggesting macrocytic normochromic anaemia. Present study results are in accordance with Kim *et al.* (2017) [11] and Jackson (2018) [10] who have reported significant increase in MCV, MCH and MCHC values in *T. Orientalis* infected animals. Omer *et al.* (2002) [18] and Col and Uslu (2006) [7] have reported lower MCV, MCH and MCHC values in comparison to healthy animals. The erythrocytic indices are directly related to Hb, PCV and TEC. Whenever Hb, PCV and TEC are affected in theileriosis, MCV, MCH and MCHC are also affected. This might be due to host specific responses to haemolysis that determine the development of anaemia in infected animals (Sivakumar *et al.*, 2017) [24]. In the present study, there was slight decrease in leucocyte count with neutrophilia, lymphocytopenia and eosinophilia. The lower TLC values might be due to heavy destruction of theileria infected lymphocytes by the cell mediated immunity. Decrease values of TLC could also be related to destruction of lymphocytes in lymphoid organs and infiltration of these cells into various organs (Clark *et al.*, 1986) [6]. The histopathological finding of depletion of lymphocytes within spleen and lymph node in a study conducted by Patil, (2017) [19] supports leucopenia in Theileria infected animals. Several workers such as Sumathi and Veena (2012), Aditya (2015), Kumar *et al.,* (2015), Acharya (2016) [1] and Patil (2017) [19] have reported leucopenia in theileria infected animals. Contrary to this some authors like Kim *et al.* (2017) [11] and Sudhakar *et al.* (2021) [27] have reported slightly elevated leucocyte count probably due to secondary bacterial infection in theileria infected animals which might be immune compramised. With respect to differential leucocyte count, the present study results are in accordance with several authors such as Adithya (2015) [2], Acharya (2016) [1], Patil (2017) [19] and Sudhakar *et al.* (2021) [27]. However some of the authors have reported slight differently with respect to some of the leucocytes. Muraleedharan *et al.,* (2005) [17] and Madhukar (2015) [15] reported neutropenia, lymphopenia, monocytosis, eosinopenia, basophilia in theileria infected animals. In the present study, there was significant decrease in blood glucose level, total protein, albumin, globulin, cholesterol and significant increase in blood urea nitrogen, creatinine, SGPT, SGOT, total bilirubin and direct bilirubin in *T. orientalis* affected animals compared with healthy control group. Significant decrease in total protein, albumin and globulin might be due to impairment in protein synthesis, extensive degradation and increased albumin excretion due to renal damage, in infected animals. Hypoproteinaemia and hypoalbuminaemia might be due to extravascular accumulation of proteinaceous fluid in body cavities (Stockham *et al.*, 2000) [26]. In contrary to our result of decreased globulin level, Izzo *et al.* (2010) [9] reported increased mean globulins compared to control group. This might be due to immune response to the *T. orientalis* infection. Increased BUN, creatinine compared to control group in the present study could be attributed to increased turnover of proteins and renal damage associated with increased catabolism of haemoglobin in infected animals (Dude *et al.,* 2014) [8]. Our findings are in accordance with Col and Uslu (2007) [7], Adithya (2015) [2], Acharya (2016) [1] and Patil (2017) [19] with respect to decreased cholesterol levels. In contrast to our findings, Yadav and Sharma (1986) [29] reported increase in cholesterol levels in calves infected with theileriosis and according to them this could be due to liver damage that results in a concurrent increase in the level of fats with the reduction of sugar and protein. The present findings of significantly increased AST and ALT values in theileriosis infected animals were in accordance with those reported by Sandhu *et al.* (1998) [21], Omer *et al.* (2003) [18], Adithya (2015) [2], Acharya (2016) [1] and Patil (2017) [19]. Increase in the AST and ALT values in the present study indicated slight liver damage *viz.*, primary or secondary liver necrosis and muscle necrosis (Benjamin, 2001) [5]. The extensive erythrocyte damage caused by infestation of parasite (merozoite) results into severe anaemia leading to generalized hypoxia. This hypoxia could be one of the reasons for degeneration leading to necrosis. Furthermore, prolonged recumbency in *T. orienatis* affected animals also aid in muscle degeneration and necrosis which could be the reason for elevated AST values. In contrary to our result Lawrence *et al.* (2018) [14] reported no significant change in AST values. There is a significant increase in total bilirubin and direct bilirubin in the present study. These results are in accordance with Sandhu *et al.* (1998) [21], Omer *et al.* (2003) [18], Adithya (2015) [2], Acharya (2016) [1], Patil (2017) [19] and Sudhakar *et al.* (2021) [27] who have reported significant increase bilirubin as compared to healthy control. 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 haemolytic anaemia.

4. Conclusion
Oriental theileriosis is highly prevalent in and around Hassan, Karnataka area and emerging as one of the major threat to economy status of livestock owner. Present study enabled to know the cliniopathological status of oriental theileriosis which will help in early diagnosis and to take necessary strategies to prevent and control the oriental theileriosis.

5. Acknowledgement

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