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RESEARCH ARTICLE

Clinico-pathological studies on Theileriosis in cattle

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ABSTRACT

The present study was conducted to study the clinical profile, hemato-biochemical, post mortem and histopathological changes along with molecular detection of theileriosis in cattle. Out of 290 suspected cases, 40 (13.79%) cattle were found to be positive for theileriosis. Age-wise prevalence was higher in animals above 12 months (65%) of age. Breed-wise and sex-wise prevalence was highest (45%) in Holstein Friesian females. The important clinical signs observed in theileriosis affected cattle were fever, pale, papery white or icteric mucous membranes, swollen lymph nodes and tick infestation. Hemato-biochemical changes were marked anaemia, thrombocytopenia, elevated levels of serum hepatic and renal biochemical parameters in affected animals. Blood smear examination of affected cattle revealed the presence of pleomorphic intra-erythrocytic piroplasm stages of *Theileria annulata* and *Theileria orientalis* including mixed infection (*T. annulata* + *T. orientalis*) in few cases. These cases were also confirmed by PCR. The characteristic post-mortem lesions were pale, icteric or papery white mucous membranes, swollen oedematous or haemorrhagic lymph nodes, splenomegaly, punched out ulcers in abomasum and hepatomegaly with icteric discoloration. Histopathology revealed multiple haemorrhagic ulcerations and necrosis of mucosal epithelium of abomasum, lymphoid depletion in lymph nodes and hepatocellular degeneration. Overall percent positivity of theileriosis was 13.79 % among cattle population in and around of Udgir city of Maharashtra involving either alone one pathogen or mixed infections of *Theileria annulata* and *Theileria orientalis*.

Key Words: Cattle, molecular detection, pathological lesions, Theileriosis, *Theileria annulata, Theileria orientalis*

INTRODUCTION

Tick-borne haemoprotozoan diseases poses a significant risk to the health and management of domesticated cattle in tropical and subtropical climates[1]. Due to tropical environment, India's climate is particularly conducive to the survival and growth of tick vectors, which facilitates the transmission of haemoparasitic diseases such as theileriosis, babesiosis and anaplasmosis[2].

Haemoprotozoan diseases, particularly babesiosis, tropical theileriosis, trypanosomiasis and anaplasmosis are considered significant barriers to the health and productivity of cattle[3]. Among all tick-borne diseases (TBD), theileriosis is one of the most economically detrimental illnesses affecting livestock globally. It is estimated that around 250 million cattle across the globe are susceptible to theileriosis[4].

Several tools are available for the diagnosis of theileriosis in cattle, which includes blood or lymph node biopsy smear examination, post-mortem findings, polymerase chain reaction (PCR) and serological evaluation. Although traditional diagnostic procedures lack sensitivity and specificity, they are still widely used and valuable aids for clinical and epidemiological investigations[5]. Among these methods, the PCR assay was found to be more sensitive and accurate than traditional microscopic examination because it enables the accurate detection and differentiation of various *Theileria* species, even in carrier animals with very low parasitemia that might be missed by microscopy[6].

Considering the economic importance of the disease and the need to develop effective therapeutic and control strategies, it is essential to investigate the clinico-pathological alterations and employ molecular techniques for species level detection of etiology of theileriosis in cattle. Therefore, the present study was undertaken to evaluate the clinical profile, haemato-biochemical and pathological alterations, along with the molecular diagnosis of theileriosis in cattle.

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MATERIALS AND METHODS

Selection of Animals

The present study was conducted at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences (COVAS), Udgir, Maharashtra on two hundred and ninety (n=290) cases of cattle with a

history of fever, pale or icteric mucous membranes, swollen lymph nodes and tick infestation. These cases were admitted to Teaching Veterinary Clinical Complex, COVAS, Udgir and reported from areas surrounding to Udgir during period May 2024 to March 2025. For obtaining control data of hematological, biochemical and clinical parameters, the blood samples were collected from healthy cattle (n=17) housed at the Livestock Farm Complex of the college.

Ethical Approval for Animal Studies

The present study was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Udgir and Committee for the Control and Supervision of Experiments on Animals (CCSEA) (Permission Letter No. VCU/IAEC/CPCSEA/XI/24). The blood samples from suspected cattle were collected with the consent of the farm/animal owners in the clinical case admission form during admission of case to college clinic. All the procedures were performed as per the ethical guidelines provided by CCSEA/IAEC and Maharashtra Animal and Fishery Sciences University, Nagpur.

Blood Smear Preparation, Staining and Screening of Theileriosis

Thin blood smears were prepared from a blood collected from suspected cattle by puncturing jugular vein with 18-gauge needle. These smears were air dried, fixed with methanol and subjected to Giemsa staining as per routine protocol[7]. Positive samples were selected or diagnosed on the basis of the presence of intra-erythrocytic rod or bayonet for *Theileria orientalis*[7]and signet ring, pyriform and dot shaped for *Theileria annulata*[8].

Haematological Estimations

The blood samples for analysing various haematological and biochemical parameters were collected under the aseptic condition from the jugular vein in K_2EDTA vacutainers (K_2EDTA concentration: 3.6 mg, 2 ml capacity) and clot activator vacutainers (Clot activator, silicone coated, 4 ml capacity) respectively (Becton, Dickinson and Company, USA). The haematological parameters such as total erythrocyte count (TEC), haemoglobin concentration (Hb), packed cell volume (PCV), MCV, MCH, MCHC, platelet count, total leucocyte count (TLC), absolute granulocyte, lymphocyte and monocyte count were estimated by using fully automated haematology analyser (Make: Nihon Kohden Corporation, Japan, Model: MEK-6550K- 4 Part Vet Haematology Analyser).

Biochemical Estimations

Serum samples separated and used to estimate various biochemical parameters such as alkaline phosphatase (ALP), aspartate transaminase (AST/SGOT), alanine transaminase (ALT/SGPT), total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, blood urea nitrogen (BUN), creatinine, calcium (Ca), phosphorus (P), magnesium (Mg), creatine kinase-MB (CK-MB), creatine kinase-NAC (CK-NAC) and Adenosine Deaminase (ADA) on semi-automated biochemical analyser (Make: Erba Mannheim GmbH, Germany, Model: Chem-7) by using standard commercial biochemical kits (Make: Erba Mannheim GmbH, Germany).

Pathological Examination

A detailed post-mortem examination of seven (n=7) cattle suspected to have died due to theileriosis was conducted, and the gross lesions observed in various organs and body systems were recorded. During the necropsy, the tissue samples measuring about 0.5 cm thickness were collected in 10% neutral buffered formalin. The formalin fixed tissue samples were processed and stained using haematoxylin and eosin staining technique for histopathology as per standard routine protocol[9].

DNA Extraction, Primer Designing and PCR Cycling Conditions

The blood samples of theileriosis suspected cattle for PCR detection were collected under aseptic condition from the jugular vein in commercial K_2EDTA vacutainers (Becton, Dickinson and Company, USA) and stored at -20°C. The genomic DNA was extracted from 250 µl of whole blood by using Phenol-Chloroform-Isoamyl alcohol method[10]. The extracted DNA samples were stored at -20°C for further analysis. The species-specific primer sets for detection of *T. annulata* {Merozoite-Piroplasm Surface Antigen gene (MPSA gene)/ *annulata* Merozoite Surface Antigen 1 gene (Tams1 gene)} and *T. orientalis* {Major Piroplasm Surface Protein gene (MPSP gene)} were designed by using National Center for Biotechnology Information (NCBI) Primer Designing Tool (NCBI Primer-BLAST). All the oligonucleotide primers were custom synthesized (Eurofins Genomics India Pvt. Ltd., Bengaluru, India). The PCR reaction mixture of 25-µl was prepared containing 12.5-µl master mix (2X) (GoTaq® Green Master Mix, Promega, USA), 1-µl of template DNA, 1-µl (10 pmol) of each primer and nuclease free water (9.50 µl). PCR reaction was performed with 25-µl reaction volume using Mastercycler Nexus Gradient 96-Well Thermal Cycler (Eppendorf, Hamburg, Germany). The primers sets and PCR cycling conditions used for detection of *T. annulata* and *T. orientalis* are shown in table 1,2 and 3.

The PCR products were subjected to agarose gel electrophoresis (2%) with 0.5 µg of ethidium bromide/ml of agarose and visualized by using BIO-PRINT CX4 EDGE Gel Documentation System (Vilber Lourmat, France).

Table 1. Primer Sets used for PCR detection of *T. annulata* and *T. orientalis.*

Sr.	Organism	Gene	Primer Sequence	Amplicon Size	Reference
1.	*T. annulata* (Forward)	MPSA (Tams1) Gene	CCTTTGATACTCGCGACCCT	674 bp	Designed
2.	*T. annulata* (Reverse)	MPSA (Tams1) Gene	GACGATGAGTACTGAGGCGA		
3.	*T. orientalis* (Forward)	MPSP Gene	TCCTCATCGTCTCTGCAACT	826 bp	Designed
4.	*T. orientalis* (Reverse)	MPSP Gene	TGTGAGACTCAATGCGCCTA		

Table 2. PCR Cycling conditions for amplification of MPSA(Tams1) gene of *T. annulata.*

Sr. No.	Condition	Temperature	Time	No. of cycles
1	Initial Denaturation	95 °C	5 min	--
2	Denaturation	95 °C	30 sec	
3	Annealing	57.2 °C	30 sec	30
4	Extension	72 °C	30 sec	
5	Final Extension	72 °C	10 min	--
6	Hold	4 °C	∞	--

Table 3. PCR Cycling conditions for amplification of MPSP gene of *T. orientalis.*

Sr. No.	Condition	Temperature	Time	No. of cycles
1	Initial denaturation	95 °C	5 min	--
2	Denaturation	94 °C	45 sec	
3	Annealing	55 °C	30 sec	35
4	Extension	72 °C	1 min	
5	Final extension	72 °C	5 min	--
6	Hold	4 °C	∞	--

Statistical Analysis

The data generated from different parameters was subjected to independent samples t-test (at the level of P≤0.01 or P≤0.05) by using IBM SPSS software (version 20) for windows.

RESULTS

Out of two hundred and ninety (n=290) suspected cases, forty (n=40) cattle were found positive for theileriosis by blood smear examination indicating an overall 13.79% prevalence. Among forty (n=40) cases, twenty-eight (n=28, 70%) cases infected with *annulata*, eight (n=8, 20%) with *orientalis* and four (n=4, 10%) had mixed infection (*annulata* + *orientalis*).

Blood smear examination of cattle infected with *orientalis* revealed intra-erythrocytic rod- or bayonet-shaped piroplasms (Fig. 1). In contrast, cattle positive for *annulata* showed numerous intra-erythrocytic piroplasms, predominantly signet ring, pyriform, and dot-shaped forms. Moreover, in cases of mixed infection, intra-erythrocytic piroplasms characteristic of both *orientalis* and *annulata* were observed (Fig. 2).

Out of forty blood samples (n=40) positive for theileriosis by smear examination, randomly seven representative samples (n=7) were subjected for molecular diagnosis at species level by PCR. PCR detected two samples (n=2) as positive for *annulata*, one as *orientalis*, and four samples (n=4) for mixed infection with *annulata* and *orientalis* (Fig. 3, 4).

The age wise prevalence of theileriosis in cattle was higher in animals aged above 12 months (n=26, 65%) followed by age group of 3-12 months (n=8, 20%) and lowest prevalence was recorded in age group below 3 months (n=6, 15%). The sex-wise prevalence of theileriosis was higher in females (n=24, 60%) as compared to males (n=16, 40%).

Breed-wise prevalence of theileriosis was found highest in Holstein Friesian breed (n=18, 45%) followed by Deoni (n=10, 25%), Red Kandhari (n=5, 12.5%), non-descript (n=3, 7.5%), Gir (n=2, 5%), Khillar and Jersy (n=1, 2.5%) in present study.

The theileriosis affected cattle showed important

clinical signs *viz;* fever, mucous membrane as pale (n=21, 52.5%), icteric (n=9, 22.5%), congested (n=8, 20%) or papery white (n=2, 5%), enlargement of superficial lymph node (n=25, 62.50%) and presence of tick infestation. However, in a few cases, clinical signs such as diarrhoea (n=7,12.50%), melena (n=2,5%), haemoglobinuria (n=2, 5%) and brisket oedema (n=4,10%) were also noticed.

Theileriosis affected cattle showed highly significant increase (P≤0.01) in body temperature (103.44±0.12 *vs* 100.90±0.15°F), heart rate (82.32±3.97 *vs* 45.76±0.38 beats/min), respiration rate (42.57±4.88 *vs* 25.76±0.34 breaths/min) and pulse rate (79.83±3.35 *vs* 46.47±0.44 pulse/min) as compared to healthy control animals.

The haematological analysis of blood samples of theileriosis affected cattle showed highly significant decrease (P ≤ 0.01) in total erythrocyte count, hemoglobin concentration, packed cell volume and MCHC, while the significant increase (P ≤ 0.05) in total leucocyte count was recorded in affected cattle as compared to control animals. Platelet counts of theileriosis affected cattle revealed highly significant decrease (P ≤ 0.01) as compared to healthy control animals. Moreover, the non-significant increased values of MCV, MCH and absolute counts of neutrophils, lymphocytes, monocytes and eosinophils were observed in affected cattle as compared to healthy control animals (Table 4).

Serum biochemical level of ALP, AST, total bilirubin, direct bilirubin, indirect bilirubin, BUN, creatinine, CK-MB, CK-NAC and adenosine deaminase (ADA) were significantly elevated in theileriosis affected cattle while the serum levels of total protein, albumin, calcium and phosphorus showed significant decrease in affected animals. Biochemical parameters such as ALT showed non-significant increase while magnesium showed non-

Table 4. Haematological changes in theileriosis affected in cattle (Mean ± SE).

Sr.No.	Parameter	Infected (n=40)	Healthy Control (n=17)	't' value
1.	TEC (10^{12}/L)	4.92 ± 0.31	7.19 ± 0.24	3.94**
2.	Hb (g/dl)	6.84 ± 0.39	10 ± 0.41	4.34**
3.	PCV (%)	22.28 ± 1.34	31.96 ± 1.31	3.89**
4.	MCV (fL)	48.16 ± 1.48	44.61 ± 1.31	1.30NS
5.	MCH (pg)	14.42 ± 0.37	13.97 ± 0.42	0.64NS
6.	MCHC (%)	29.49 ± 0.26	31.31 ± 0.19	3.79**
7.	TLC (x10^9/L)	13.33 ± 1.20	9.28 ± 0.62	2.09*
8.	Lymphocytes (x10^9/L)	5.29 ± 0.65	5.02 ± 0.42	0.22 NS
9.	Monocytes (x10^9/L)	1.28 ± 0.32	0.36 ± 0.10	1.73 NS
10.	Eosinophils (x10^9/L)	0.83 ± 0.16	0.48 ± 0.06	1.53 NS
11.	Neutrophil (x10^9/L)	5.05 ± 0.67	3.55 ± 0.34	1.24 NS
12.	Platelets (x10^9/L)	163.78 ± 13.78	213.18 ± 10.69	2.93**

NS- Non-significant *Significant (P≤0.05) **Highly Significant (P≤0.01)

Table 5. Biochemical changes in theileriosis affected cattle (Mean ±SE).

Sr.No.	Parameter	Infected (n=40)	Healthy Control (n=17)	't' value
1	ALP (U/L)	273.68 ± 15.38	135.10 ± 13.57	2.98**
2	SGPT (ALT) (U/L)	49.32 ± 1.73	42.22 ± 1.94	1.37^{NS}
3	SGOT (AST) (U/L)	171.76 ± 8.02	72.04 ± 3.60	4.13**
4	Total Bilirubin (mg/dl)	3.25 ± 0.38	0.39 ± 0.02	2.53**
5	Direct Bilirubin (mg/dl)	1.57 ± 0.18	0.12 ± 0.02	2.62**
6	Indirect Bilirubin (mg/dl)	1.68 ± 0.20	0.27 ± 0.01	2.34*
7	Total Protein (g/dl)	5.09 ± 0.12	6.54 ± 0.10	4.13**
8	Albumin (g/dl)	2.40 ± 0.07	3.07 ± 0.05	3.11**
9	BUN (mg/dl)	48.49 ± 3.80	21.14 ± 0.70	2.43*
10	Creatinine (mg/dl)	2.86 ± 0.25	1.56 ± 0.07	2.06*
11	Calcium (mg/dl)	7.41 ± 0.27	10.21 ± 0.40	5.99**
12	Phosphorus (mg/dl)	4.72 ± 0.30	5.77 ± 0.11	2.50*
13	Magnesium (mg/dl)	1.94 ± 0.08	1.99 ± 0.07	0.43^{NS}
14	CK-MB (IU/L)	224.68 ± 41.19	65.16 ± 4.22	3.97**
15	CK-NAC (IU/L)	152.34 ± 33.89	43.12± 2.75	3.42**
16	ADA (IU/L)	159.85 ± 82.30	13.70 ± 0.95	2.49*

NS- Non-significant *Significant (P≤0.05) **Highly Significant (P≤0.01)

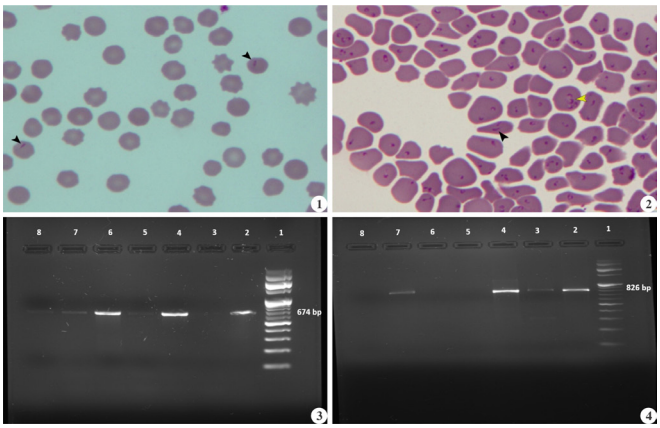

Fig.1. Blood Smear: *orientalis*: Presence of intra-erythrocytic rod or bayonet shaped *orientalis* piroplasm's (black arrowheads) (1000X, Giemsa Stain);**Fig.2.** Blood Smear: Mixed Infection: Note the presence of numerous intra-erythrocytic signet ring, pyriform and dot shaped *annulata* (yellow arrowhead) and rod or bayonet shaped *orientalis* (black arrowhead) piroplasm's (1000X, Giemsa Stain); **Fig. 3.** PCR amplification of MPSA (Tams1) gene of *annulata* from genomic DNA extracted from the blood samples of affected cattle. Lane 1: 100 bp DNA marker, Lanes 2-8: 674 bp amplicons of MPSP gene of *annulata*; **Fig.4.** PCR amplification of MPSP gene of *orientalis* from genomic DNA extracted from the blood samples of affected cattle. Lane 1: 100 bp DNA marker, Lanes 2-8: 826 bp amplicons of MPSP gene of *orientalis*.

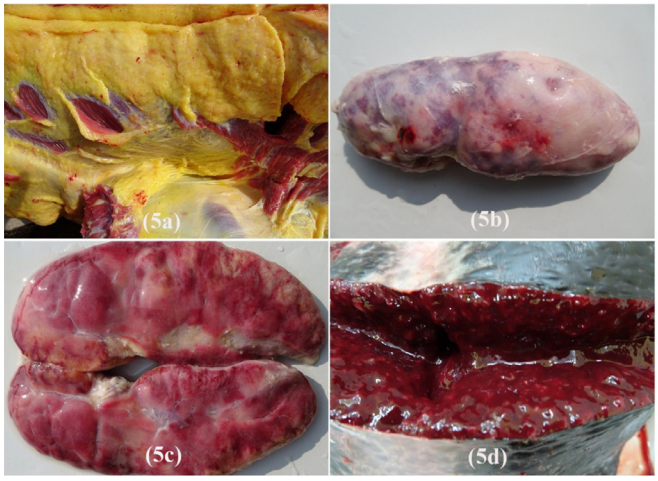

Fig. 5. Postmortem Lesions: Cattle: Theileriosis: **(5a)** Icteric discolouration of subcutaneous tissue; **(5b-5c)** Lymph node: Marked enlargement and haemorrhages in lymph nodes; **(5d)** Spleen: Semisolid or mushy appearance of splenic parenchyma.

significant decrease as compared to control animals (Table 5).

The external examination of carcasses of theileriosis affected cattle revealed presence of pale or papery white and icteric conjunctival mucous membranes, swollen lymph nodes and tick infestation. On incision, the subcutaneous tissue and fat showed marked dark yellow or icteric discolouration indicating jaundice (Fig. 5a). The affected lymph nodes appeared swollen, oedematous and

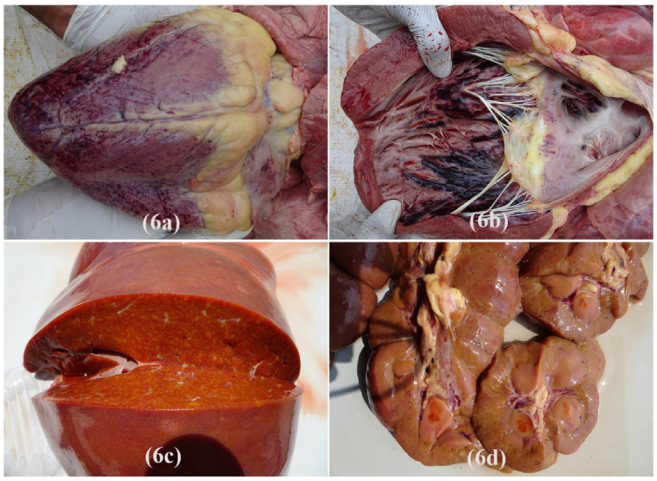

Fig. 6. Postmortem Lesions: Cattle: Theileriosis: **(6a)** Heart: Epicardial haemorrhages; **(6b)** Heart: Endocardial haemorrhages; **(6c)** Liver: Note the presence of marked icteric discolouration and hepatomegaly; **(6d)** Kidney: Note the presence of greenish yellow discoloration and presence of bile pigment aggregates in renal cortex.

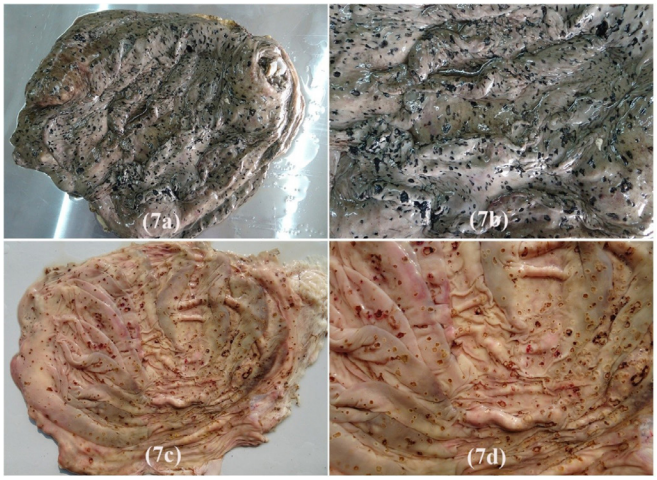

Fig. 7. Postmortem Lesions: Cattle: Theileriosis: **(a-d)** Abomasum: Note the presence of numerous punched out ulcers over the abomasal mucosa of affected cattle.

showed cortical haemorrhages intermixed with necrotic foci in few cases (Fig. 5b-5c). Marked splenomegaly was noticed in theileriosis affected cattle along with frequent presence of soft semisolid splenic pulp or parenchyma (Fig. 5d).

The gross lesions such as epicardial and endocardial haemorrhages and icteric discoloration pericardial fat were frequently noticed in affected cattle (Fig. 6a-6b). Liver of affected cattle frequently showed marked hepatomegaly along with dark icteric parenchymal discoloration indicating jaundice (Fig. 6c). The gross lesions such as yellowish green discoloration and presence of bile pigment aggregates in renal cortex were evident in kidneys of affected cattle (Fig. 6d). The gross examination of abomasum of affected cattle showed

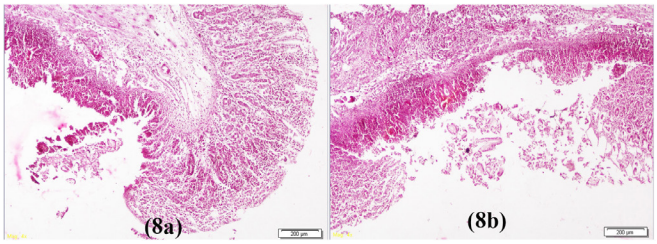

Fig. 8. Histopathological Lesions: Cattle: Theileriosis:(a-b) Abomasum: Mucosal ulcerations and presence of inflammatory response. (H & E Stain, a-b: bar=200 µm).

widespread mucosal punched out ulcers with necrotic centres and haemorrhagic borders as well as marked oedema of rugal folds (Fig. 7a-d).

Histopathological examination of abomasum -revealed multiple haemorrhagic ulcerations and marked necrosis of mucosal epithelium and heavy mononuclear cell infiltration were evident in abomasum of affected cattle. The denuded and necrosed mucosal epithelium frequently found trapped in fibrin and adhered to the native mucosal surface appearing as multiple deep eosinophilic masses. Marked lymphomononuclear cell infiltration was also frequently evident within underlying lamina propria, submucosa and around blood vessels (Fig. 8a-b).

Lymph nodes of affected cattle showed moderate lymphoid depletion, presence of fibrinoid material, cortical oedema and haemorrhages (Fig. 9a-b). In few cases, the marked proliferative changes (diffuse hyperplasia) of lymphoid follicles of cortex were noticed and cortex was replaced by sheets of lymphocytes and macrophages. Histopathological examination of spleen showed marked congestion, haemorrhages and hemosiderin deposition (presence of golden yellow to brown hemosiderin pigment scattered throughout the red and white pulp *i.e.* hemosiderosis) (Fig. 9c-d).

Marked widespread or diffuse hepatocellular vacuolar degeneration and cholestasis were evident in livers from affected cattle along with moderate infiltration of mononuclear cells within sinusoids and periportal areas. Marked tubular epithelial cells necrosis and mononuclear cell infiltration in interstitium was observed in kidneys of affected cattle. Marked mononuclear cell infiltration and myofibers degeneration in heart of affected cattle. Lungs revealed the presence of marked oedema and mononuclear cell infiltration within interstitium or alveolar septa, congestion and haemorrhages.

DISCUSSION

Effective disease control relies on accurate diagnosis. Traditionally, the microscopic examination of Giemsa-

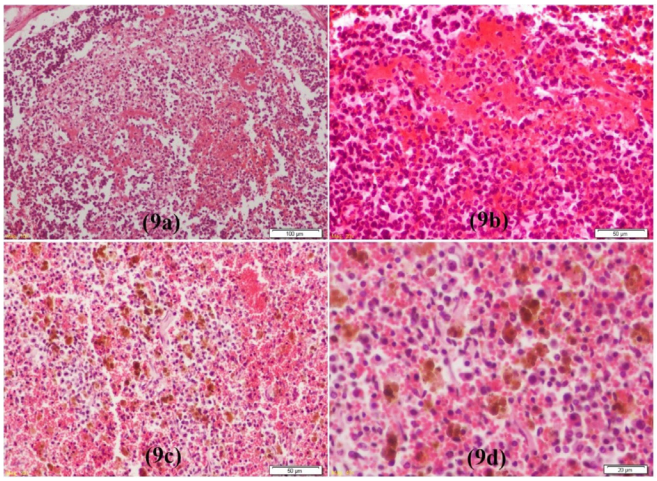

Fig. 9. Histopathological Lesions: Cattle: Theileriosis:**(9a-9b)** Lymph Node: Marked lymphoid depletion and presence of fibrinoid material and oedema; **(9c-9d)** Spleen: Presence of golden yellow to brown hemosiderin pigment scattered throughout the red and white pulp (hemosiderosis). (H & E Stain, a: bar=100 µm, b-c: bar=50 µm, d: bar=20 µm).

stained blood or lymph node smears has been the gold standard for field diagnosis of bovine theileriosis. While cost-effective and accessible, microscopy suffers from significant limitations, particularly low sensitivity in detecting carrier animals with low parasitemia and in many cases inability to reliably differentiate between morphologically similar species[6,35].

The advent of molecular diagnostic techniques, particularly Polymerase Chain Reaction (PCR) and its variants (nested PCR, real-time PCR, Loop-Mediated Isothermal Amplification), has revolutionized the detection of theileriosis. PCR assays targeting specific genes such as the Tams1 gene for T. annulata and the Major Piroplasm Surface Protein (MPSP) gene for T. orientalis offer superior sensitivity and specificity, enabling the detection of cryptic infections and the precise identification of genotypes[6,7,11,35].

Microscopic diagnostic features of *Theileria annulata* and *orientalis* which were recorded in blood smears of theileriosis affected cattle of present study were also found in consonance with earlier literature[7-8]. The findings of present study regarding molecular (PCR) detection of *annulata* and *orientalis* infection in cattle of present study were also found in consonance with studies of earlier authors[7,11-14].

The findings of the present study regarding the overall prevalence of theileriosis in cattle were largely consistent with previous reports[15]. Similar to the current study, earlier researchers also reported a higher prevalence of theileriosis in age groups older than 12 months of age[16-17]. The breed wise prevalence of theileriosis was highest in crossbred animals as compared to indigenous animals. This might be due to the stress due to high milk yield

potential, high tick infestation as compared to indigenous animals, breed resistance, genetic makeup and greater susceptibility of crossbred animals to the disease[18].

The higher prevalence of theileriosis in females than males recorded in current study also consistent with the earlier reports[19].The highest sex wise prevalence of theileriosis in female cattle may be associated to hormonal imbalances, which can lead to a compromised immune system and immunosuppression during advanced pregnancy and lactation in high yielding animals[11].

The clinical signs observed in theileriosis affected cattle of present study were also consistent with the clinical findings which were recorded in earlier studies[20-26]. The high fever in theileriosis is postulated due the liberation of endogenous pyrogens as a result of cellular lysis and high parasitaemia, which stimulates the thermoregulatory centre in the hypothalamus[20]. Furthermore, these earlier researchers[21-22] studied the expression of pro-inflammatory cytokines in cell lines experimentally infected with *annulata* and noted that the development of all the major clinical signs and pathology of acute theileriosis such as pyrexia, anaemia, anorexia, cachexia and disseminated haemorrhages, are significantly influenced by the cytokines produced by infected mononuclear cells, such as TNF-α, IL-1α, IL-1β and IL-6. Moreover, the earlier studies also stated that the numerous schizont production in lymphocytes results into the massive and uncontrolled proliferation of both specific and non-specific T lymphocytes, resulting in enlarged lymph nodes in *T. annulata* and *T. parva* infection[23-24].

The clinical signs such as presence of pale or icteric mucous membranes, icterus and haemoglobinuria as well as hematological changes in erythrocytic parameters which were observed in theileriosis affected cattle of present study may be attributed to anaemia due to haemolysis induced by merozoites of *Theileria spp*. The previous hematological investigations[25-26]on bovine theileriosis concluded that the several contributing factors including immune mediated haemolysis, excessive cytokines production and generation of reactive oxygen species as well as the removal of piroplasm infected erythrocyte by macrophages in the organs of the reticuloendothelial system contributes anaemia in theileriosis.

The other hematological alterations recorded in present study such as marked thrombocytopenia in cases of bovine theileriosis were also found in close proximity with hematological findings of previous studies[27-28]. These studies indicated that the thrombocytopenia observed in theileriosis is probably a result of increased destruction, consumption and degranulation of platelets in the peripheral blood and suppression of platelets release from the bone marrow into the blood stream by parasite and its product.

The serum biochemical changes observed in the present study were also found in consonance with findings of previous studies[29-30]. These earlier studies reported that thesignificant increase of CK-MB level in theileriosis affected cross breed cattle might be due to the severity of anaemia and parasitaemia contributing to the pathophysiology of myocardial damage. Moreover, the increased levels of adenosine deaminase (ADA) in tropical theileriosis in cattle are possibly due to involvement of cellular immune responses. Theileriosis is a progressive lymphoproliferative disease and the increase in the levels of ADA can attributed to lymphoproliferation of T-lymphocytes or mononuclear cell proliferation by schizont-infected cells, causing the increase in ADA levels in infected animals[31].

The serum biochemical alterations such as decreased levels of total protein, albumin, calcium and phosphorus in present study can be well correlated with the findings of earlier researchers stating that the decreased levels of calcium and phosphorus in bovine theileriosis can be linked to hypoproteinaemia, decreased dietary intake, gastrointestinal malfunction hepatic and renal damage[32-33].

More or less similar gross and histopathological lesions observed in present study were found in consonance with the findings of theileriosis in cattle and other animal species reported by previous researcher[7,11,21,32,34].

The earlier studies[21,34] on pathogenesis of gross and histopathological lesions in bovine theileriosis. These studies demonstrated that, the rapidly proliferating schizont infected mononuclear cells disseminates through the lymphoid tissues from the prescapular lymph node to distant lymph nodes and to the spleen and thymus. The parasitized mononuclear cells also spread rapidly into non-lymphoid organs e.g., liver, kidney, lung, abomasum, adrenal glands and pituitary gland and heart. These rapidly proliferating parasitized mononuclear phagocytes produces cytokines viz. TNF-α, IFN-γ and IL-2. These cytokines (TNF-alpha) disrupt the physiological integrity of the host; can also harm host by disrupting the regulation of the immune and endocrine systems. Furthermore, they stated that, these cytokines produced by parasitized mononuclear cells plays a major role in the development of clinical disease and lymphoid hyperplasia and tissue damage such as ulcerative lesions in theileriosis. The pathogenesis of gross and histopathological lesions observed in theileriosis affected cattle of present study can be correlated with the mechanism reported by these earlier workers.

In conclusion, the present study reports an overall

prevalence of 13.79% of theileriosis in the cattle population in and around Udgir city, Maharashtra state. Molecular techniques, particularly PCR, provide greater accuracy in identifying the specific *Theileria* species involved and are more reliable in detecting mixed infections.

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REFERENCES

1. Sharma A, Kaur P, Bal MS and Singla LD. 2014. Application of multiplex PCR for the simultaneous detection of natural infection of theileriosis, babesiosis and trypanosomosis in cattle. *J Vet Parasitol* **28**: 112-116.
2. Subapriya S, Senthil NR, Gowri B, Chandrasekaran D, Gopalakrishnan A, Arunaman CS and Vairamuthu S. 2021. Prevalence of haemoprotozoal diseases in cattle: a review of 6000 cases. *J Pharm Innov* **10**: 535-541.
3. Maiti SK. 2021. Advances in diagnosis, therapeutic management and control of haemoprotozoan diseases in livestock. Approaches for improving livestock productivity through nutrition and animal health management. National Institute of Agricultural Extension Management (MANAGE) **2**: 50-58.
4. Parthiban M, Saranya R, Magesh M and Raman M. 2010. Detection of theileria parasite in cattle of Tamilnadu using nested PCR. *Tamilnadu J Vet Anim Sci* **6:** 162-163.
5. Gebrekidan H, Perera PK, Ghafar A, Abbas T, Gasser RB and Jabbar A. 2020. An appraisal of oriental theileriosis and the *Theileria orientalis* complex, with an emphasis on diagnosis and genetic characterization. *Parasitol Res* **119:** 11-22.
6. Kundave VR, Patel AK, Patel PV, Hasnani JJ and Joshi CG. 2015. Detection of theileriosis in cattle and buffaloes by polymerase chain reaction. *J Parasit Dis* **39**: 508-513.
7. Jacob SS, Sengupta PP, Kumar HBC, Maharana SM, Goudar A, Chandu AGS, Rakshitha TS, Shivakumar V, Gulati BR and Reddy GBM. 2024. Unveiling genotypic diversity of *Theileria orientalis* in lethal outbreaks among bovines in Karnataka, India. *Parasitol Res* **123**: 202.
8. Soulsby EJL. 1987. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th ed. Bailliere Tindall, London, UK. pp:729-737.
9. Bancroft JD and Gamble M. 2007. Theory and practice of histological techniques. Edinburgh: Churchill-Livingston, Butterworth and Co. (publishers) Ltd, pp: 29-220.
10. Green MR and Sambrook J. 2012. Isolation and quantification of DNA. In: molecular cloning: A laboratory manual. 4th edn. Cold Spring Harbor Laboratory Press, New York. pp: 1-78.
11. Aparna M, Ravindran R, Vimalkumar MB, Lakshmanan B, Rameshkumar P, Kumar KGA Promod K, Ajithkumar S, Ravishankar C, Devada K, Subramanian H, George AJ and Ghosh S. 2011. Molecular characterization of *Theileria orientalis* causing fatal infection in crossbred adult bovines of South India. *Parasitol Int* **60**: 524-529.
12. Gebrekidan H, Gasser RB, Perera PK, McGrath S, Stevenson MA and Jabbar A. 2015. Investigating the first outbreak of oriental theileriosis in cattle in South Australia using multiplexed tandem PCR (MT-PCR). *Ticks Tick Borne Dis* **6**(5): 574-578.
13. Baghel KR, Manisha, Saravanan BC, Sankar M, Ghosh S and Tewari AK. 2021. Incidence of bovine tropical theileriosis in cattle in central and southern regions of Chhattisgarh, India. *Explor Anim Med Res* **11**(2): 237-240.
14. Baghel KR, Saravanan BC, Jeeva K, Chandra D, Singh KP, Ghosh S and Tewari AK. 2023. Oriental theileriosis associated with a new genotype of *Theileria orientalis* in buffalo (*Bubalus bubalis*) calves in Uttar Pradesh, India. *Ticks Tick Borne Dis* **14**(1): 1-6.
15. Naik BS, Maiti SK and Raghuvanshi PDS. 2016. Prevalence of tropical theileriosis in cattle in Chhattisgarh state. *J Anim Res* **6:** 1043-1045.
16. Sharma D, Vatsya S, Harit A, Kumar RR and Kumar S. 2021. Prevalence of theileriosis in large ruminants of Uttarakhand. *Pharma Innov J* **10**: 922-925.
17. Dutta H, Panda SK, Sathapathy S, Mohanty BN, Mishra C and G. R. Jena GR. 2024. Epidemiological and comparative haematological studies of theilerioses at different stages of production and reproduction in cattle. *Vet Pract* **25**: 93.
18. Niranjan AS, Tiwari J, Shanker D, Kumar P and Srivastava MK. 2023. Molecular prevalence and phylogenetic analysis of piroplasmids in cattle under small-scale dairy farming in the western Uttar Pradesh. *Vet Pract* **24**: 94.
19. Atif FA, Khan MS, Iqbal HJ, Arshad GM, Ashraf E and Ullah S. 2012. Prevalence of *Anaplasma marginale*, *Babesia bigemina* and *Theileria annulata* infections among cattle in Sargodha District, Pakistan. *Afr J Agric Res* **7**: 3302-3307.
20. Glass EJ, Craigmile SC, Springbett A, Preston PM, Kirvar E, Wilkie GM, Eckersall PD, Hall FR and Brown CGD. 2003. The protozoan parasite, *Theileria annulata*, induces a distinct acute phase protein response in cattle that is associated with pathology. *Int J Parasitol* **33:** 1409-1418.
21. Brown DJ, Campbell JDM, Russell GC, Hopkins J and Glass EJ. 1995. T cell activation by *Theileria annulata* infected macrophages correlates with cytokine production. *Clin Exp Immunol* **102**: 507-514.
22. Graham SP, Brown DJ, Vatansever Z, Waddington D, Taylor LH, Nichani AK, Campbell JDM, Adamson RE, Glass EJ and Spooner RL. 2001. Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause in vivo. *Vaccine* **19**: 2932-2944.
23. Schneider I, Haller D, Kullmann B, Beyer D, Ahmed JS and Seitzer U. 2007. Identification, molecular characterization and subcellular localization of a *Theileria annulata* parasite protein secreted into the host cell cytoplasm. *Parasitol Res* **101**: 1471-1482.
24. Singla LD and Kaur P. 2022. A comprehensive review on bovine tropical theileriosis under Indian scenario. *Indian J Vet Med* **42**: 1-12.
25. Hasanpour A, Moghaddam GA and Nematollahi A. 2008. Biochemical, hematological, and electrocardiographic changes in buffaloes naturally infected with *Theileria annulata. Korean J Parasitol* **46**: 223-227.
26. Parmar VL, Vagh AA, Patel UD, Bilwal AK, Thakre BJ, Brahmbhatt NN, Patbandha T and Parmar JN. 2024. Study on prevalence, risk factors and clinical phenotypic appraisals of *Theileria annulata* infection in Gir cattle. *Research Square*: 1-26.
27. Kimento BA. 1976. Ultrastructure of blood platelets in cattle with east coast fever. *Am J Vet Res* **37**: 443-447.
28. Col R and Uslu U. 2006. Haematological and coagulation profiles during severe tropical theileriosis in cattle. *Turk J Vet Anim Sci* **30**: 577-582.
29. Tehrani AA, Hosseini E and Bahrami AM. 2013. Biochemical,

hematological studies in cattle naturally infected with *Theileria annulata*. *Bull Env Pharmacol Life Sci* **2**: 7-10.
30. Col R and Uslu U. 2007. Changes in selected serum components in cattle naturally infected with *Theileria annulata*. *Bull Vet Inst Pulawy* **51**: 15-18.
31. Altug N, Yuksek N, Agaoglu ZT and Keles I. 2008. Determination of adenosine deaminase activity in cattle naturally infected with *Theileria annulata*. *Trop Anim Health Prod* **40**: 449-456.
32. Omer OH, El-Malik KH, Magzoub M, Mahmoud OM, Haroun EM, Hawas A and Omar HM. 2003. Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. *Vet Res Commun* **27**: 15-25.
33. Hosny AM, Aly SA and Ahmed OI. 2010. Oxidative stress and some hematobiochemical changes in blood of cattle during theileriosis. *Assiut Vet Med J* **56**: 1-21.
34. Forsyth LMG, Minns FC, Kirvar E, Adamson RE, Hall FR, McOrist S, Brown CGD and Preston PM. 1999. Tissue damage in cattle infected with *Theileria annulata* accompanied by metastasis of cytokine-producing, schizont-infected mononuclear phagocytes. *J Comp Pathol* **120**: 39-57.
35. Noaman, V. 2014. Comparison of molecular and microscopic technique for detection of *Theileria spp.* in carrier cattle. J Parasit Dis **38**: 64-67