



Clinico-Physiological and Haematological studies in cattle-calves naturally infected with *Theileria annulata* by nested PCR

Pavan Goyal¹, Anju Chahar², R.K. Tanwar³, Fakhruddin³

1. M.V.Sc. Scholar, Department of Epidemiology and Preventive Veterinary Medicine, Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334 001, Rajasthan, India

2. Professor and Head

3. Professors

Email: drpawangoyal880@gmail.com

ABSTRACT

Bovine tropical theileriosis is a protozoan disease caused by blood protozoa Theileria annulata and it is transmitted by tick Hyalomma anatolicum anatolicum. A total of 100 cattle-calves were examined irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner for treatment. Most common clinical findings in nested PCR positive cattle-calves (n=41) were anorexia, presence of ticks, Icteric/anaemic/congested mucous membrane, swollen prescapular, parotid and prefemoral lymph node (s), melena/bloody diarrhoea, mucous coated diarrhoea, salivation, nasal discharge, lacrimation, ecchymotic haemorrhages on sclera, circling movement, lateral recumbency, dehydration, dyspnoea, pica, swelling at knee joint (s) and rough hair coat. Physiological examination revealed highly significant increase (P<0.01) in temperature and pulse rate while significant (P<0.05) increase in respiration rate in nPCR positive cattle-calves as compared to nPCR negative cattle-calves. Haematological examination revealed statistically highly significant (P<0.01) decrease in Mean \pm SE values of haemoglobin (Hb gm/dl), packed cell volume (PCV %) and lymphocyte count while non-significant (P>0.05) decrease in total erythrocyte count (TEC), and highly significant (P<0.01) increase in total leucocyte count (TLC), neutrophils and eosinophils while non-significant (P>0.05) increase in monocytes and basophils, non-significant (P>0.05) increase in mean corpuscular volume (MCV), significant (P<0.05) increase in mean corpuscular haemoglobin (MCH) and highly significant (P<0.01) increase in mean corpuscular haemoglobin concentration (MCHC) values in nPCR positive cattle-calves as compared to nPCR negative cattle-calves.

Keywords: Theileria annulata, Hyalomma anatolicum, melena, cattle-calves, ecchymotic haemorrhages, pica

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INTRODUCTION

Theileria annulata was described in Transcaucasian cattle in 1904 and was first named *Piroplasma annulatum*. It was reclassified as *T. annulata* after identification of schizont stage in its life-cycle [32]. Bovine tropical theileriosis is a protozoan disease caused by blood protozoa *Theileria annulata* and it is transmitted by tick *Hyalomma anatolicum anatolicum*. It causes significant economic losses in large parts of Asia [17]. It is mainly seen in cattle, sheep and goat as well as in wild and captive ungulates [23]. This intracellular infection inflicts economic burden on cattle breeders in terms of mortality and morbidity as well as expenses spent on prophylactic measures against disease and treatment [10].

Theileriosis is a burning veterinary problem of the rural livestock oriented communities. It has a profound effect on haematological values and causes huge morbidity and mortality in cattle population, which reflects economic losses and elevates the poverty level [21].

T. annulata sporozoites infect the host mononuclear cells (macrophages/ monocytes and B-lymphocytes) in the lymph nodes draining the site of inoculation of the sporozoites by ticks. The sporozoites transform into schizonts in the mononuclear cells. Host cells become transformed and proliferate in synchrony with the parasite during this process, named the macroschizont stage [15]. The schizonts undergo further differentiation to merozoites, which are released by the lysis of the infected cells. Later, the merozoites invade red blood cells. It is followed by the development of piroplasms in erythrocytes, and these intra-erythrocytic piroplasms become available to the vector [7, 12].

Theileria spp. infection can cause acute, subacute or chronic disease pathology [14]. In *T. annulata* infection, the most common clinical symptoms are weakness, weight loss, anorexia, high body temperature, petechia on the conjunctival mucosa, swollen lymph nodes, anaemia and cough. On later stages of theileriosis, infected animals can not stand up, their body temperatures are under normal values (< 38.5°C), and icterus, dehydration and blood in faeces are the occasional clinical symptoms [4, 23].

Calves (≤ 4 months of age) infected with *Theileria annulata* suffered from emaciation, anaemia, unilateral or bilateral exophthalmia, petechiae in conjunctiva, oral and nasal mucosa, and occasionally in the pinnae. Widespread subcutaneous nodules with 0.5 to 3.0 cm diameter are also detected, as well as enlarged superficial lymph nodes, particularly the submandibular, the retropharyngeal and sometimes the prescapular [5].

MATERIAL AND METHODS

In the present study, one hundred cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal Science, Bikaner for treatment were screened for bovine tropical theileriosis. After clinical examination of cattle-calves, 2.0 ml of blood sample was collected from Jugular vein of each cattle-calf suspected for bovine tropical theileriosis aseptically in ethylene diamine tetra acetic acid (EDTA @ 1 mg/ml) vacutainer for haematological analysis such as haemoglobin, packed cell volume, total erythrocyte count, total leucocyte count, differential leucocyte count, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC). Forty one cattle-calves were found positive for *Theileria annulata* by nPCR. The statistical analyses were carried out by standard methods as described by [29].

RESULTS

Most common clinical signs recorded in bovine tropical theileriosis positive (by nPCR) cattle-calves (n=41) are presented in Table 1 and Fig.1 to 10.

Table 1: Clinical signs recorded in bovine tropical theileriosis positive cattle-calves (n = 41)

S.No.	Clinical signs	Total observation	Frequency (%)
1	Pyrexia	31	75.61
2	Presence of ticks	30	73.17
3	Icteric mucous membrane	3	7.32
4	Anaemic mucous membrane	28	68.29
5	Congested mucous membrane	6	14.63
6	Swollen prescapular lymph node(s)	41	100.00
7	Swollen parotid lymph node (s)	8	19.51
8	Swollen prefemoral lymph node(s)	11	26.83
9	Melena/Bloody diarrhoea	22	53.66
10	Mucous coated diarrhoea	6	14.63
11	Salivation	7	17.07
12	Nasal discharge	8	19.51
13	Lacrimation	4	9.76
14	Anorexia	38	92.68
15	ecchymotic haemorrhages on sclera	2	4.88
16	Circling movement	1	2.44
17	Lateral recumbency	12	29.27
18	Dehydration	5	12.20
19	Dyspnoea	1	2.44
20	Pica	3	7.32
21	Swelling at knee joint (s)	1	2.44
22	Rough hair coat	2	4.88



Fig.1: Heavy tick infestation

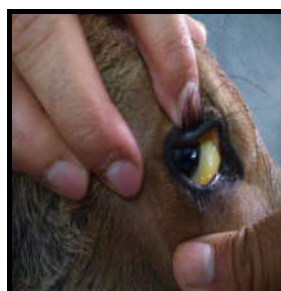


Fig.2: Icteric mucous membrane



Fig.3: Swollen pre-scapular lymph node



Fig.4: Swollen parotid lymph node

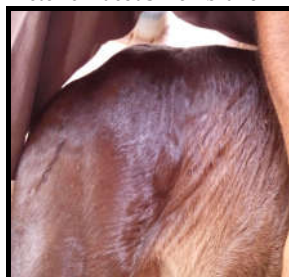


Fig.5: Swollen pre-femoral lymph node

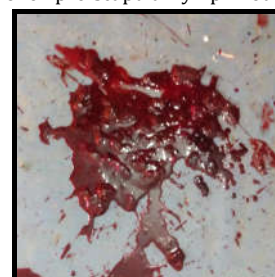


Fig.6: Melena



Fig.7: Blood in faeces

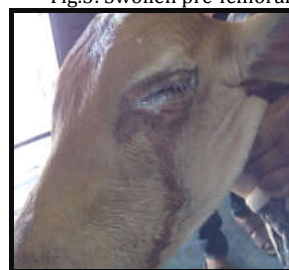


Fig.8: ocular discharge

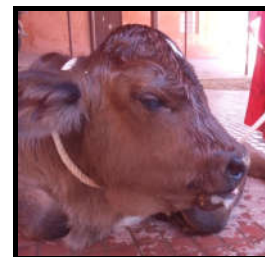


Fig.9: Frothing from mouth



Fig.10: Swelling at knee joint

Physiological parameters:

In the present investigation, statistically highly significant increase ($P < 0.01$) in temperature and pulse rate while significant ($P < 0.05$) increase in respiration rate was observed in nPCR positive cattle-calves as compared to nPCR negative cattle-calves.

Table 2: Mean \pm SE values along with range of temperature, pulse and respiration rates of nPCR positive and negative cattle-calves

S. No.	Parameters	Mean \pm SE		Range	
		nPCR positive cattle-calves	nPCR negative cattle-calves	nPCR positive cattle-calves	nPCR negative cattle-calves
1	Temperature ($^{\circ}$ F) **	104.28 \pm 0.19 ^b	103.26 \pm 0.19 ^a	100.7—108	100.4--106.5
2	Pulse rate (Per Min.)**	144.41 \pm 0.68 ^b	116.27 \pm 0.81 ^a	82—183	65—165
3	Respiration rate/ min.*	42.78 \pm 0.50 ^b	38.53 \pm 0.49 ^a	24—68	22—60

*($P < 0.05$) = statistically significant ** ($P < 0.01$) = highly significant
(Means with different superscripted letters in the same row differ significantly)

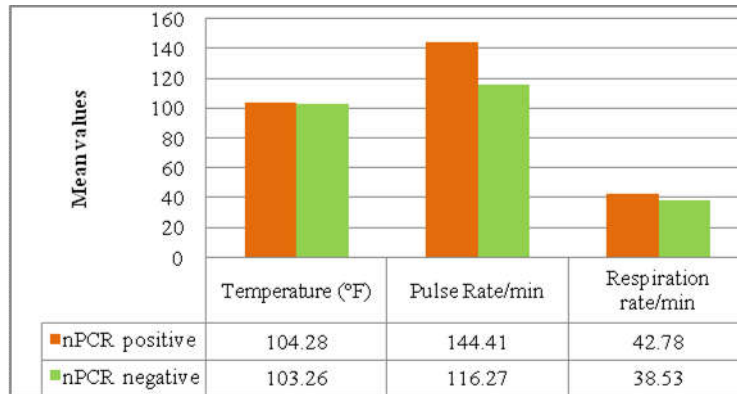


Fig.11: Bar diagram showing mean values of temperature, pulse rate and respiration rate of nPCR positive and negative cattle-calves

Haematological parameters:

In the present study, there was statistically highly significant ($P < 0.01$) decrease in Mean \pm SE values of haemoglobin (Hb gm/dl), packed cell volume (PCV %) and lymphocyte count while non-significant ($P > 0.05$) decrease in total erythrocyte count (TEC), and highly significant ($P < 0.01$) increase in total leucocyte count (TLC), neutrophils and eosinophils while non-significant ($P > 0.05$) increase in monocytes and basophils, non-significant ($P > 0.05$) increase in mean corpuscular volume (MCV), significant ($P < 0.05$) increase in mean corpuscular haemoglobin (MCH) and highly significant ($P < 0.01$) increase in mean corpuscular haemoglobin concentration (MCHC) values in nPCR positive cattle-calves as compared to nPCR negative cattle-calves.

Table 3: Mean \pm SE values along with range of haematological parameters of nPCR positive and negative cattle-calves

S. No.	Parameters	Mean \pm SE		Range	
		nPCR positive cattle-calves	nPCR negative cattle-calves	nPCR positive cattle-calves	nPCR negative cattle-calves
1	Hb (g/dl)**	5.75 \pm 0.18 ^a	6.40 \pm 0.18 ^b	3.0–8.4	3.6–9.2
2	PCV (%)**	18.61 \pm 0.32 ^a	21.64 \pm 0.33 ^b	10–26	11–29
3	TEC (million/ μ l)(ns)	5.30 \pm 0.28	5.87 \pm 0.20	1.6–13.09	2.23–11.09
4	TLC (thousand/ μ l)**	11.29 \pm 0.43 ^b	6.43 \pm 0.21 ^a	1.75–27.15	1.56–10.4
5	DLC (%)				
	● Neutrophils**	47.22 \pm 0.64 ^b	37.10 \pm 0.47 ^a	8–73	20–59
	● Lymphocytes**	49.29 \pm 0.62 ^a	60.49 \pm 0.47 ^b	25–90	40–79
	● Monocytes (ns)	2.39 \pm 0.22	2.00 \pm 0.18	0–7	0–6
	● Eosinophils**	1.05 \pm 0.16 ^b	0.41 \pm 0.13 ^a	0–4	0–3
	● Basophils (ns)	0.05 \pm 0.07	0	0–1	0
6	MCV (fl/cells) (ns)	44.81 \pm 0.69	39.62 \pm 0.58	12.32–97.7	17.18–85.17
7	MCH (pg/cells)*	13.95 \pm 0.40 ^b	11.69 \pm 0.32 ^a	3.7–32.18	2.94–25.87
8	MCHC (%)**	31.11 \pm 0.26 ^b	29.68 \pm 0.22 ^a	23.18–38.57	24.17–33.91

(Means with different superscripted letters in the same row differ significantly)

ns ($P > 0.05$) = non significant * ($P < 0.05$) = statistically significant ** ($P < 0.01$) = highly significant

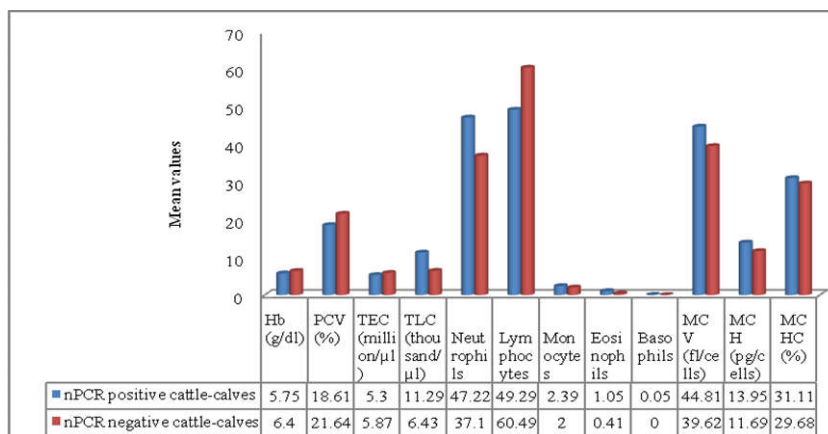


Fig. 12: Bar diagram showing Mean values of haematological parameters of nPCR positive and negative cattle-calves

DISCUSSION

Respiratory signs in theileriosis occur due to accumulation of oedematous fluid inside the lungs and thoracic cavity [20]. Anorexia can be attributed to persistent fever; moreover the enlargement of superficial lymph nodes can be due to lymphoid hyperplasia in early stage of the disease [30]. Cytokines (TNF- α , IL-1, and IL-6) produced by infected mononuclear cells are responsible for the diverse clinical symptoms of tropical theileriosis, such as depression, pyrexia, anorexia, cachexia, and disseminated haemorrhages [15]. Diarrhoea in young animals due to intercurrent infection with intestinal pathogens as the immune response is compromised by lymphocytopenia [26]. However, exophthalmia and corneal opacity noted by [25, 19, 9, 8, 1, 11, 30, 28, 3] were not observed in this investigation.

Increasing body temperature in theileriosis occurs due to the liberation of endogenous pyrogens because of white blood cells lysis especially of neutrophils and macrophages during infection and high level of parasitemia. This leads to the stimulation of thermoregulatory centre in the hypothalamus [24]. Increase in pulse rate occurs as a result of dehydration and anaemia due to decrease of RBCs, Hb and PCV levels. So strong heart beats are heard in the infected animals. It may be attributed that anaemia leads to increased heart beats and cardiac output and decreased circulation time (rapid circulation), which results in cardiac hypertrophy or failure [24]. Respiratory signs in theileriosis occur due to accumulation of oedematous fluid inside the lungs and thoracic cavity [20].

Significant decrease in mean Hb concentration, PCV and RBC count from day 16 onwards may be resulted from destruction of the parasitized erythrocytes by macrophages in the spleen, lymph nodes and other organs of the reticulo-endothelial system [25] or due to toxic effects of metabolites of *Theileria* species, persistent loss of blood caused by permanent blood sucking ticks [13] and Tumor necrosis factor- α (TNF- α) on erythropoiesis [6] and due to high level of activated complement products [22]. Leucocytosis resulted from proliferation of lymphocytes in the lymphoid organs as a defensive response to invading protozoans [25]. The increase in eosinophil count may be due to tick infestation, relating to hypersensitivity [31]. Lymphocytopenia due to the destruction of lymphocytes in lymphoid organs and an infiltration of these cells into various organs [22]. Relative increase in number of monocytes reflected compensatory mechanism as target cells in response to their invasion with *Theileria* protozoan [19].

Non-significant increase in MCV, significant increase in MCH and highly significant increase in MCHC value in nPCR positive cattle-calves as compared to nPCR negative cattle-calves may be due to autoimmune hemolytic anemia, bone marrow damage or chronic inflammatory disorders (inflammatory bowel disease) because in this study there was lymphocytopenia which indicates presence of autoimmune disorders and bone marrow damage while leucocytosis, neutrophilia and eosinophilia indicative of inflammatory condition. This is supported by the fact that toxic metabolites of *Theileria* spp. have harmful effect on bone marrow as they interfere with the process of erythropoiesis [16]. The other important cause to make the anemia in ovine theileriosis is destruction of RBCs by parasite inside RBCs which causes hemolytic anemia [2]. Hemolytic anaemia also reported by [11, 27]. [18] also reported anaemia as a result of autoimmune reaction.

CONCLUSION

Theileriosis is a burning veterinary problem of the rural livestock oriented communities. It has a profound effect on haematological values and causes huge morbidity and mortality in cattle population, which reflects economic losses and elevates the poverty level.

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