International Journal of Livestock Research, Vol. 10 (9) Sep' 2020
http://www.ijlr.org
eISSN: 2277-1964
NAAS Rating 2020 – 5.36

Original Research

Prevalence and Clinico-Therapeutic Management of Bubaline Theileriosis in Marathwada Region of Maharashtra

A. A. Bhosale[1], A. U. Bhikane[2], S. G. Chavhan[3], R. K. Jadhav[4*], Anand Mohan[5] and Neelam Kushwaha[6]

[1]M.V.Sc. Scholar, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary & Animal Sciences, Udgir, Maharashtra, INDIA
[2]Professor and University Head, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary & Animal Sciences, Udgir, Maharashtra, INDIA
[3]Assistant Professor, Department of Veterinary Pathology, College of Veterinary & Animal Sciences, Udgir, Maharashtra, INDIA
[4]Assistant Professor, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Udgir, Maharashtra, INDIA
[5]Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Udgir, Maharashtra, INDIA
[6]Hospital Registrar, Teaching Veterinary Clinical Complex, College of Veterinary & Animal Sciences, Udgir, Maharashtra, INDIA

*Corresponding Author: jadhavrk11@gmail.com

How to cite this paper: Bhosale, A., Bhikane, A., Chavhan, S., Jadhav, R., Mohan, A., & Kushwaha, N. (2020). Prevalence and Clinico-Therapeutic Management of Bubaline Theileriosis in Marathwada Region of Maharashtra. *International Journal of Livestock Research*, 10(9), 155-165. doi: http://dx.doi.org/10.5455/ijlr.20200608041459

Received : Jun 08, 2020
Accepted : Sep 18, 2020
Published : Sep 30, 2020

Abstract

Theileriosis has widely been reported in cattle particularly in crossbred cattle in India with scarce reports on bubaline theileriosis. Hence, the present study was designed with to study the prevalence and clinico-therapeutic management of bubaline theileriosis in Marathwada. Buffaloes admitted to College clinics were screened based on history, clinical signs, and hematology and blood smear examination for theileriosis. Epidemiological aspects, clinical signs, haemato-biochemistry was studied and confirmed cases were treated. Out of 877 clinical cases of buffaloes examined at clinics for of one year, 21 buffaloes were found positive for Theileria spp. with an overall hospital prevalence of 1.6 %. The highest prevalence was observed in Murrah buffaloes < 5 years of age yielding > 4 liters/day during post-monsoon. The typical clinical signs were fever, tachycardia, anorexia, weakness, pale mucosae, swollen lymphnodes, loss of body weight, epistaxis, cutaneous skin lesions and corneal opacity. The haemato-biochemical analysis showed marked microcytic normochromic anemia, leukocytosis, lymphocytopenia and increased levels of bilirubin, SGPT, blood urea nitrogen and creatinine. A therapeutic trial was conducted on 14 clinical cases using oxytetracycline and buparvaquone along with supportive treatment. In this study, the overall efficacy of buparvaquone was found to be 83.33 % in theileriosis affected buffaloes. Bubaline theileriosis is prevalent in the Marathwada region and buparvaquone was found highly effective in early stage of bubaline theileriosis but it failed to improve the clinical condition of the animal in the later stages of the disease.

Keywords: Bubaline, Theileriosis, Prevalence, Haemato-biochemistry, Treatment

Introduction

Buffalo is the incredible Asian dairy animal, popularly known as the "Black Diamond" which plays a versatile role in the socio-economic upliftment of the rural agricultural communities. It is the largest milk and lean meat producer in India (Gupta and Singh, 2002). In the Indian subcontinent, the prevailing epizootiological determinants offer the most favored and optimum environment for faster propagation of the acarine intermediate host (*H. anatolicum anatolicum*) and *in situ* development of *Theileria annulata* causing bovine tropical theileriosis (Sudan *et al.,* 2014). The apicomplexan parasite has been restricting cross-border movement of cattle and buffalo and export of high-yielding buffalo breeds to foreign countries. The disease accounts for global losses to the tune of US$ 800 million per annum and nearly 70 % of bovine mortality (Brown, 1997). The projected annual loss due to tropical theileriosis in India is could be to the tune of INR 8092 crore (Narladkar, 2018).

Theileriosis has widely been reported in cattle particularly in crossbred cattle by several workers from all over the world (Preston *et al.,* 1992; Omer *et al.,* 2002; Col and Uslu, 2006; Kohli *et al.,* 2014; Jagtap *et al.,* 2015; Devadevi *et al.,* 2018). However, there are few published reports of theileriosis in buffaloes (Singh *et al.,* 2012; Vahora *et al.,* 2012; Chaudhari *et al.,* 2013). Hence, the present study was designed to study the prevalence and clinico-therapeutic management of bubaline theileriosis in the Marathwada region of Maharashtra.

Materials and Methods

Study Area and Selection of Animals

Total of 877 clinical cases of buffaloes admitted to Teaching Veterinary Clinical Complex and Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, College of Veterinary and Animal Sciences, Udgir from Latur and Nanded districts of Marathwada during June 2016 to May 2017 were included in the study. Out of 877 clinical cases, 112 buffaloes exhibiting clinical signs such as fever, enlargement of superficial lymph node, pale mucosae or no response to usual antibiotics and suspected for theileriosis were screened by using blood smear examination. Positive samples were selected based on the presence of intra-erythrocytic the ilerial piroplasms and Koch's Blue Bodies in blood smears.

Prevalence

The hospital prevalence of the ileriosis in buffaloes concerning age, sex, breed, physiological status, stage of lactation/pregnancy, milk yield, month, season and degree of tick infestation were analyzed from generated data. The age groups were divided as <5 years, 5 to 10 years and >10 years. The stage of lactation was categorized as early (0-3 months post-partum), mid (4-6 months) and late (> 7 months). The stage of pregnancy was categorized as early (0-3 months), mid (4-6 months) and late (> 7 months). The seasons were classified as monsoon (June-September), post-monsoon (October-November), winter (December-February) and summer (March-May).

Haematology

Blood samples of 112 buffaloes suspected for haemoprotozoan infections were collected in EDTA vials by jugular venipuncture. Complete blood count analysis was done on automated veterinary specific haematology analyzer (Model: Abacus Junior Vet, Diatron GMBH, Austria).

Blood Smear Examination

Thin blood smears prepared from fresh anti-coagulated blood obtained from jugular vein of 112 buffaloes were air dried, fixed in methanol and subjected for Giemsa staining (Soulsby, 1982). Positive samples were selected based on presence of intra-erythrocytic the ilerial piroplasms in different forms (pleomorphic) such as signet ring/crescent, pyriform/pear, dot/round and rod/bayonet shaped indicating *Theileria spp.* and in few cases intra-lymphocytic schizonts (Koch's Blue Bodies) in blood smears. For every individual positive case, about 20-30 high power oil immersion microscopic fields at different locations of Giemsa stained thin blood smears were scanned to determine the average number of intra-erythrocytic the ilerial piroplasms and further categorization was performed. The blood smears with average 1-2, 3-6 and more than 7 intra-erythrocytic the ilerial piroplasms per high power oil immersion microscopic field were categorized as mild positive, moderately positive and highly positive respectively.

Biochemistry

About 4 ml of blood was collected by jugular venipuncture from study buffaloes (n=14) in heparinized vials and subjected to centrifugation at 3000 rpm for 5 minutes. Harvested plasma samples were analyzed for total, direct and indirect bilirubin, SGOT, blood urea nitrogen and creatinine using standard diagnostic kits manufactured by Span Diagnostics, Surat, Gujarat on semi-automated biochemical analyzer (Chemistry Analyzer-CA 2005 B4B Diagnostic division, China, Model no. CA 2005).

Treatment

Among 112 buffaloes screened, 21 buffaloes were found positive for *Theileria spp*. on blood smear examination. Out of 21 buffaloes, 14 buffaloes with variable degree of parasitaemia and showing typical clinical signs were included while seven buffaloes with atypical signs and very low degree of parasitemia were excluded from the study. The mild positive cases in early stage (n=3) of theileriosis were treated with oxytetracycline @ 20 mg/kg IV once daily for five days. Eight (n=8) moderate to highly positive cases of theileriosis with moderate duration of illness and one mild case early stage (n=1) which did not respond to oxytetracycline was treated with single dose of buparvaquone @ 2.5 mg/kg IM. The chronic cases (n=3) which were sick for one month were treated with two doses of buparvaquone at 48 hours interval. Supportive treatment included Inj. Dextrose 20% @ 1-lit intravenously for 5 days, Inj. Vitamin B complex @ 10 ml intramuscularly for 10 days, and haematinic bolus (Ferrous fumerate) @ 1 po bid for 20 days. Efficacy of treatment was evaluated based on improvement in clinical parameters and restoration of haemato-biochemical parameters in the study animals after treatment.

Statistical Analysis

Statistical analysis was carried out as per Snedecor and Cochran (1994). The student 't' test for the equal number of observations was used for comparison of haemato-biochemical values in theileriosis affected cases (n=14) with a healthy control group (n = 14) and for comparison of values in theileriosis affected buffaloes before and after treatment.

The Chi-square (X^2) value was calculated to determine the significance of an association between the disease and hypothesized causal factor. The following formula was used as-

$$X^2 = O\text{-}E = (O\text{-}E)^2 = (O\text{-}E)^2/ E$$

Where,
X^2 - Chi-square
O- Observed animals
E- Expected animals

Result and Discussion

Prevalence

Out of 877 clinical cases of buffaloes, 14 animals were found clinically positive for theileriosis, indicating an overall prevalence of 1.6%. Similar to present observations, Bansal *et al.* (1977), Khattak *et al.* (2007) and Singh *et al.* (2012) have also reported 0.07%, 2% and 2.32% prevalence of theileriosis in buffaloes, respectively. Age-wise prevalence of theileriosis was found to be highest in buffaloes below the 5-years age group (2.30%) followed by 6-10 years (1.20%) age group. Singh *et al.* (2012) and Maharana *et al.* (2016) also reported theileriosis in buffaloes above 1 year of age. The sex-wise prevalence of theileriosis in buffaloes revealed an occurrence in females (1.6%) only. The present findings are in agreement with Tuli *et al.* (2015) who reported a higher incidence of theileriosis in females and attributed it to higher hormonal stress in milch animals. Breed-wise higher prevalence of theileriosis was observed in Murrah (3.70%) buffaloes, followed by non-descript (1.70%) while least prevalence was noticed in Marathwadi (0.84%) buffaloes. The effect of breed on the prevalence of theileriosis in buffaloes was found to be highly significant ($P<0.01$) which might be attributed to the stress of high milk yield in Murrah buffaloes.

Physiological status-wise the highest number of clinical cases of theileriosis was observed in lactating buffaloes

(42.85%) followed by pregnant buffaloes (35.71%) and least in buffalo heifers (21.42%). The present observations were supported the views of Radostits *et al.* (2010) who stated that several clinical cases of theileriosis occurring in cattle and buffaloes are usually caused by exposure to some stress such as parturition, lactation and starvation. The effect of physiological status on the prevalence of theileriosis was found to be significant ($P < 0.01$). The highest occurrence of theileriosis was observed in buffaloes in early lactation (66.66%) followed by mid-lactation (33.33%) and no case during late lactation. Further higher occurrence of theileriosis was observed in buffaloes having average milk yield > 4 lits/day (77.77%) as compared to buffaloes yielding < 4 lits/day (22.23%). The present findings were might be due to hormonal imbalance culminating in stress during early and mid-lactation.

The clinical cases of theileriosis in buffaloes were recorded more or less throughout the year but the highest prevalence was recorded during post monsoon (2.56%) followed by winter (2.52%), monsoon (1.11%) and summer (0.58%). However, earlier workers have recorded a higher incidence of theileriosis during summer and monsoon (Vahora *et al.,* 2012; Maharana *et al.,* 2016; Durrani *et al.,* 2008) and attributed it to higher activities of ticks during summer and the rainy season (Jithendran, 1997). The higher prevalence recorded during post-monsoon in this study can be attributed to stress of early lactation as it is the calving season of buffaloes in the study area.

Out of 14 clinical cases of theileriosis, tick infestation was observed in six buffaloes; however, tick infestation was negligible in the remaining eight buffaloes. No correlation was found between the severity of tick infestation and the occurrence of disease. The occurrence of disease in 57.14 % buffaloes without tick infestation may be attributed to breakdown of immunity due to stress factors resulting in precipitation of the disease or flaring up of latent infection. All six buffaloes were found to be infested with *Hylomma ananatolicum anatolicumn* ticks. Many species of ticks have been incriminated as the natural vectors, but *H. anatolicum anatolicum* was reported to be a major vector of bovine theileriosis all over the world (Radostits *et al.,* 2010).

Clinical Signs

The duration of illness in affected buffaloes ranged from 2 to 45days (<5 days in 42.85% cases, 5-10 days and > 10 days in 28.57% cases each) signifying acute to chronic nature of theileriosis. The characteristic clinical signs observed were the variable rise in body temperature, tachycardia, anorexia, weakness, pale mucosae, enlargement of superficial lymph nodes and loss of body weight. Several workers (Aulakh and Singla, 2006; Vahora *et al.,* 2012; Radostits *et al.,* 2010) earlier reported more or less similar clinical signs in theileriosis affected cattle and buffaloes. The visible mucus membranes appeared slightly pale (71.42%) in initial stage and pale (28.57%) in later stages of disease (Fig. 1). The lymph nodes were swollen in 50% cases (Fig. 2) and normal in the remaining 50% cases. The color of urine was found to be normal in four (28.57%) and yellow in ten (71.42%) buffaloes.

Figure 1: Pale conjunctival mucous membrane in buffalo ailing from theileriosis

Figure 2: Swollen prescapular lymph node in buffalo ailing from theileriosis

Epistaxis observed in two (14.28%) cases (Fig. 3) was attributed to thrombocytopenia (75 & 129 $x10^3$/ µl) noticed in these buffaloes. Cutaneous skin lesions (Fig. 4) were observed in one (7.14%) buffalo moderately positive for theileriosis, which might be attributed to the presence of schizonts below dermis (Muhammad *et al.,* 1999; Soulsby, 1982). Corneal opacity (Fig. 5) was also noticed in one buffalo heifer, which might be attributed to white blood cells infiltration (Osman and Al-Gaabary, 2007; Mahmmod *et al.,* 2011; Ali and Radwan, 2011). Mean clinical values in theileriosis affected and healthy buffaloes (Table 1) showed a highly significant ($P<0.01$) increase in rectal

temperature, heart rate and respiration rate and highly significant (P<0.01) decrease in ruminal motility compared to normal healthy buffaloes. Tachycardia was directly proportional to the severity of anaemia. The rectal temperature ranged from 101.2-104.4 ^{0}F.

Figure 3: Thrombocytopenia induced bilateral epistaxis in buffalo suffering from theileriosis

Figure 4: Generalized cutaneous lesions in buffalo ailing from theileriosis

Figure 5: Corneal opacity in the left eye of buffalo heifer ailing form theileriosis

Effect of duration of illness on clinical parameters in theileriosis affected buffaloes revealed rectal temperature ranging from 100.5-104 ^{0}F (102.35±0.65 ^{0}F) in six buffaloes with < 5 days duration of illness, 101.3-104.4 ^{0}F (102.48±0.67 ^{0}F) in four buffaloes with 5-10 days duration of illness while 104.4-105 ^{0}F (103.53±1.05 ^{0}F) in four buffaloes with > 10 days duration of illness. From present findings, it was concluded that as the duration of illness increases the body temperature also increases. However, Deore *et al.* (1979) reported the highest body temperature during first 12 days of illness, which subsequently decreased as the duration of illness increased. On analysis of data on body temperature it is interesting to note that among affected buffaloes, 42.85% animals were having normal body temperature (<102 ^{0}F) and 58.15% animals exhibited elevated body temperature (> 102 ^{0}F). Negligible changes were observed in heart rate, respiration rate and ruminal motility concerning the duration of illness.

Hematological Changes

Highly significant (P<0.01) decrease in mean haemoglobin, packed cell volume and total erythrocyte count with significant (P<0.05) reduction in MCV and non-significant changes in MCH and MCHC values were observed in affected buffaloes as compared to normal healthy buffaloes (Table 1), indicative of microcytic-normochromic anemia. Anaemia observed in theileriosis affected buffaloes in the present study might be attributed to a) Increased phagocytosis of parasitized erythrocytes (Omer *et al.,* 2002), b) Presence of intra-erythrocytic piroplasms or parasite- induced lysis (Boulter and Hall, 2000), c) Alteration in the antigenicity of erythrocytes due to the entry of parasites, leading to autoimmune reaction in the body triggering the removal of infected erythrocytes from circulation (Muraleedharan *et al.,* 2005) due to autoimmune reaction (Hooshmand-Rad, 1976), d) Release of TNF α by infected cells due to *T. annulata* causing suppression of haematopoetic progenitors (Tizard, 1992), and e) Oxidative damage to RBCs (Ali and Radwan, 2011).

Table 1: Mean (± S.E.) clinical and haemato-biochemical values in theileriosis affected and healthy (Control) buffaloes

S. No.	Parameters	Affected (n=14)	Healthy (n=14)	't' value
1.	Body temperature (^{0}F)	102.72 ± 0.44	100.11 ± 0.053	6.169**
2.	Heart rate (Per minute)	70.78 ± 1.27	49.42 ± 0.64	14.757**
3.	Respiration rate (Per minute)	28.64 ± 0.70	23.14 ± 0.45	6.788**
4.	Ruminal motility (Per 5 minute)	3.14 ± 0.17	4.71 ± 0.32	-6.904**
5.	Hb (g/dl)	7.52±0.40	11.82±0.28	-8.223**
6.	PCV (%)	22.00±1.24	37.38±0.85	-9.844**
7.	TEC (× 10^6/µl)	3.71±0.28	4.88±0.19	-3.870**
8.	MCV (fl)	61.07±2.59	68.92±1.66	-2.297*
9.	MCH (pg)	21.10±1.00	21.78±0.44	-0.615 ^{NS}
10.	MCHC (g/dl)	34.58±0.62	34.12±0.81	0.434 ^{NS}
11.	TLC (× 10^3/µl)	10.51±1.02	6.68±0.39	3.269**
12.	Lymphocyte(x10^3/µl)	4.45±0.48	4.59±0.25	-0.276^{NS}
13.	Monocyte (×10^3/µl)	0.37±0.06	0.91±0.01	3.974**
14.	Neutrophil (×10^3/µl)	5.66±0.76	1.99±0.20	4.552**
15.	PLT (× 10^3/µl)	193.50±16.65	215.92±10.52	-1.092 ^{NS}
16.	Total bilirubin(mg/dl)	1.76±0.20	0.66±0.032	5.362**
17.	Direct bilirubin(mg/dl)	1.07± 0.16	0.36±0.02	4.387**
18.	Indirect bilirubin(mg/dl)	0.69±0.08	0.30±0.02	4.820**
19.	BUN (mg/dl)	28.72±2.28	17.48±1.52	5.027**
20.	Creatinine (mg/dl)	2.02±0.22	0.35±0.22	6.879**
21.	SGPT(U/L)	70.57±4.13	42.35±0.97	26.290**

*NS – Non significant; * - Significant (P<0.05); ** - Highly significant (P < 0.01)*

Theileriosis affected buffaloes with a mild, moderate and severe degree of disease revealed decrease in Hb (8.63±0.52 *vs* 7.68±0.40 *vs* 5.15±0.75 g/dl), PCV (25.07±0.53 *vs* 22.63±1.32 *vs* 14.15±2.15 %) and TEC (3.94±0.21 *vs*, 3.95±0.35 *vs* 2.28±0.66 x10^6/µl) values respectively, suggestive of decreasing trend with an increase in the severity of the infection. Theileriosis affected buffaloes with a duration of illness < 5 days, 5-10 days and > 10 days revealed Hb, PCV and TEC (7.93 ± 0.52 *vs* 7.77±0.91 *vs* 6.67±0.79 g/dl; 23.80±1.72 *vs* 21.63 ±2.45 *vs* 19.66±2.53% and 3.89±0.38 *vs* 3.47±0.44 *vs* 3.67±0.80 x10^6/µl), respectively, indicative of a decrease in erythrogram with an increase in the duration of illness. These findings may be attributed to continued hemolysis and chronic loss of appetite owing to persistence of infection.

The leukogram revealed a highly significant (P<0.01) increase in total leukocyte and neutrophil counts along with non-significant decrease in lymphocyte counts in theileriosis affected buffaloes as compared to healthy buffaloes (Table 1). The observations are in agreement with reports of earlier workers (Aulakh and Singla 2006; Ariyaratne *et al.*, 2014). On the contrary, some workers have reported a significant decrease in total leukocyte count in *Theileria annulata* infected buffaloes (Tuli *et al.*, 2015; Memon *et al.*, 2016). In the present study, lymphopenia might have resulted from large-scale destruction of lymphocytes during schizogony in lymphoid organs and infiltration of these cells in to various organs resulting in variable peripheral blood mononuclear leukocyte count and ratio in the peripheral circulation (Sandhu *et al.*, 1998). Non-significant changes in platelet count were noticed in affected buffaloes as compared to healthy buffaloes.

Biochemistry

Highly significant (P < 0.01) increase in plasma total, direct and indirect bilirubin (Table 1) noticed in theileriosis affected buffaloes could be attributed to increased destruction of parasitized erythrocytes by erytrophagocytosis in the spleen, lymph nodes and other organs of reticuloendothelial system (Sandhu *et al.*, 1998). Highly significant (P

< 0.01) increase in SGPT values observed in affected buffaloes was attributed to hepatic injury resulting from anemic anoxia (Hasanpour *et al.*, 2008) in buffaloes. The elevated level of BUN and creatinine observed in the present study was in agreement with Tuli *et al.* (2015) which might be attributed to increased turnover of proteins.

Diagnosis

No single test is the gold standard test for definite diagnosis of theileria and none of the tests is 100% sensitive or specific. Three basic types of tests are routinely used for diagnosis of theileriosis *viz.* light microscopy, serology and molecular testing (Chauhan *et al.*, 2015). Microscopy remains the simplest and most accessible diagnostic test for most veterinarians. During acute infections, microscopy is a reasonably sensitive tool for the detection of small round or oval shaped piroplasms in erythrocytes and schizonts in the lymphocytes in Giemsa stained blood smears. The diagnosis of theileriosis in chronically infected and carrier animals however remains a significant challenge due to very low, often intermittent parasitemia (Syed *et al.*, 2014). Among 21 positive samples, 10 (47.61%), 9 (42.86%) and 2 (9.52%) cases showed a mild, moderate and high degree of infection, respectively. Positive samples were selected based on the presence of intra-erythrocytic theilerial piroplasms in different forms (pleomorphic) such as signet ring/crescent, pyriform/pear, dot/round and rod/bayonet shaped indicating *Theileria spp.* and in few cases intra-lymphocytic schizonts (Koch's Blue Bodies) in blood smears.

The majority of positive cases showed the presence of ring/crescent and pyriform as predominant morphology of theilerial piroplasms (Fig. 6), while in remaining cases either rod/bayonet, dot/round or comma shaped morphology (Fig. 7) of theilerial piroplasms was observed. The number of piroplasms varied from 1-4 in a single erythrocyte. The incidence of single piroplasms in an erythrocyte was high. The highly positive and few moderately positive cases frequently showed presence of more than one piroplasms in a single erythrocyte. Very frequently in all degree of theilerial infections, the intra-cytoplasmic dark blue spherical inclusions of varying size were evident in lymphocytes indicating schizonts stages (Koch's Blue Bodies) (Fig. 8) of *Theileria spp.*

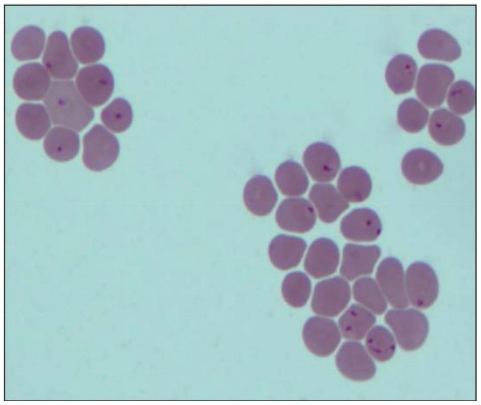

Figure 6: Ring or crescent and pyriform Theilerial piroplasms in erythrocytes (1000X, Giemsa Stain)

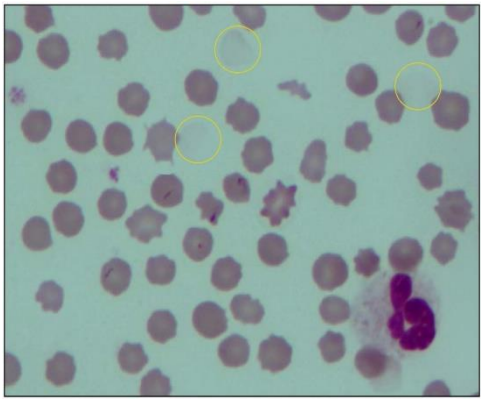

Figure 7: Note the presence of rod, dot, pyriform and bayonet shaped Theilerial piroplasms in erythrocytes and ghost cells (yellow circles) (1000X, Giemsa Stain)

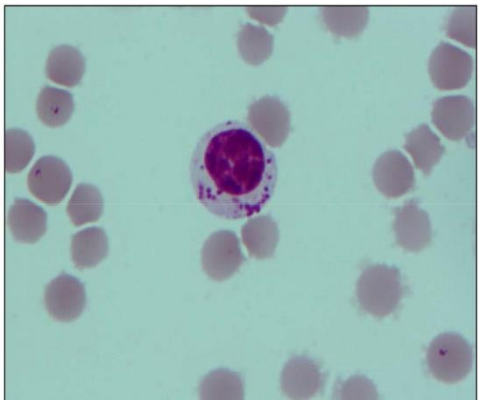

Figure 8: Note three pyriform or signet ring shaped piroplasms in erythrocytes and intracytoplasmic Koch's Blue Bodies in lymphocyte (1000X, Giemsa Stain)

The other findings observed in few mild and moderately positive and in all highly positive cases included the presence of ghost cells indicating intravascular hemolysis leading to haemolytic anaemia.

Therapeutic Management

The primary goal of therapy in theileriosis affected animals is to eliminate the parasite and reverse the life threatening anaemia. Numerous drugs have been used in the treatment of theileriosis. Currently, buparvaquone and oxytetracycline are the most widely used drugs for the treatment of theileriosis in cattle around the world (Syed *et al.*, 2014). In the present therapeutic trial, 14 theileriosis affected buffaloes were treated with either oxytetracycline alone or buparvaquone alone or combination of two doses of buparvaquone and five doses of oxytetracycline. Out of three mild and early clinical cases of theileriosis in buffaloes treated with oxytetracycline alone @ 20 mg/kg I/V once daily for five days, two buffaloes completely recovered, while one buffalo did not respond to treatment. All nine clinical cases of theileriosis in buffaloes, which included eight fresh moderate to severe cases and one mild case, which did not respond to oxytetracycline were successfully treated with single dose of buparvaquone @ 2.5 mg/kg body weight IM. However, out of three severely affected chronic cases of buffaloes treated with two doses of buparvaquone at 48-hour interval, only one case completely recovered after treatment, indicated high efficacy in early cases and poor efficacy in chronic longstanding cases. In this study, the overall recovery rate in buffaloes treated for theileriosis with buparvaquone was found to be 83.33%.

In recovered buffaloes, appetite and water intake improved in 3 to 5 days after initiation of treatment and restored to nearly normal on 7^{th} to 8^{th} day. The animals, which were dull and depressed before treatment, became alert and active by 4^{th} to 5^{th} day of treatment. The skin coat regained its normal luster two weeks after treatment while body condition gradually improved. The clinical, haematological and biochemical parameters restored to normal in treated animals (Table 2) owing to the cessation of intravascular haemolysis, supplementation of haematinics and restoration of normal physiological functions of liver and kidneys.

Table 2: Mean (± S.E.) clinical and haemato-biochemical values before and after treatment of theileriosis affected buffaloes with buparvaquone (n=9)

S. No.	Parameter	Before treatment	After treatment	't' value
1	Body temperature (ºF)	102.48 ± 0.57	100.6±0.15	3.006*
2	Heart rate (Per minute)	72.66 ± 1.58	56.44±1.08	8.894**
3	Respiration (Per minute)	29.33 ± 0.86	21.55±0.64	6.549**
4	Ruminal motility (Per 5 minute)	3.22 ± 0.22	4.66±0.37	-4.914**
5	Hb(g/dl)	7.38±0.62	12.31±0.68	-7.349**
6	PCV (%)	21.45±1.85	37.97±2.36	-7.266**
7	TEC ($\times 10^6$ /µl)	3.42±0.39	5.99±0.33	-5.933**
8	MCV (fl)	65.11±2.72	63.00±1.29	0.962 ^{NS}
9	MCH (pg)	22.35±1.10	20.58±0.55	1.737 ^{NS}
10	MCHC(g/dl)	34.35±0.58	32.74±0.79	1.982 ^{NS}
11	TLC ($\times 10^3$ /µl)	10.35±1.44	7.28±0.68	0.611**
12	Lymphocyte (%)	47.36 ±6.37	52.31±2.05	-0.824 ^{NS}
13	Monocyte (%)	4.24±0.63	5.32±1.18	-0.791 ^{NS}
14	Neutrophil (%)	48.30±6.52	41.24±2.65	1.228 ^{NS}
15	PLT ($\times 10^3$ /µl)	189.77±23.31	229.47±20.55	1.520 ^{NS}
16	Total Bilirubin (mg/dl)	1.53±0.24	0.86±0.22	1.807 ^{NS}
17	Direct Bilirubin (mg/dl)	0.90± 0.19	0.35±0.064	2.760*
18	Indirect Bilirubin (mg/dl)	0.63±0.08	0.36±0.05	2.286 ^{NS}
19	BUN (mg/dl)	31.13±3.10	17.83±0.87	8.389**
20	Creatinine (mg/dl)	1.96±0.34	0.76±0.30	4.791**
21	SGPT(U/L)	70.57±4.13	33.07±2.32	2.932*

*^{NS} – Non significant; * - Significant (P<0.05); ** - Highly significant (P < 0.01)*

Oxytetracycline's @ 20mg/kg are less effective in theileriosis while buparvaquone @ 2.5 mg/kg proved effective which is in agreement with Radostits *et al.* (2010). Buparvaquone has been successfully used @ 2.5 mg/kg body weight IM, for the treatment of tropical theileriosis with 86.66-100% efficacy by several workers in cattle and buffaloes (Osman and Al-Gaabary, 2007; Bhojne *et al.*, 2010; Syed *et al.*, 2014). The present finding on the efficacy

of buparvaquone in chronic or advanced cases of theileriosis is in agreement with Osman and Al-Gaabary (2007*)* who concluded that early treatment with buparvaquone is 100% effective in eliminating the protozoan parasites from the blood and lymph nodes whereas in the later stages it is unable to eliminate the parasites from blood and lymph node as well as failed to improve the clinical condition of the animal. Buparvaquone is usually not effective in cerebral and advanced cases of the theileriosis (Dhar *et al.*, 1987). Many authors have also reported the development of resistance to buparvaquone (Mhadhabi *et al.*, 2010; Sharifiyazdi *et al.*, 2012).

In the present study, the theileriosis ailing buffaloes were given supportive treatment with dextrose to correct hypoglycemia resulting from anorexia, B complex to stimulate appetite and metabolism and iron preparations to stimulate erythropoiesis. Similarly, Sarma *et al.* (2008) and Syed *et al.* (2014) have used dextrose, B complex and iron preparations for supportive therapeutic management of theileriosis in cattle.

Conclusion

In conclusion, theileriosis is prevalent in the buffalo population of Marathwada region and buparvaquone is highly effective in early stage of bubaline theileriosis but it failed to improve the clinical condition of the animal in the later stages of the disease.

Conflict of Interests

There is no conflict of interest.

Publisher Disclaimer

IJLR remains neutral concerning jurisdictional claims in published institutional affiliation.

References

1. Ali, A.E.F. and Radwan, M.E.I. (2011). Molecular detection of *Theileria annulata* in Egyptian buffaloes and biochemical changes associated with particular oxidative changes. *Advances in Life Sciences*, 1 (1), 6-10.
2. Ariyaratne, M.E.A.D.S., Gothami, W.S. and Rajapakse, R.V.P.J. (2014). Application of PCR technique on confirming theileria infection in cattle and buffaloes with determining the relationship between animal's PCV and WBC count with the infection. *International Journal of Science and Research*, 4 (7), 2012-2015.
3. Aulakh, G.S. and Singla, L.D. (2006). Clinico-haematobiochemical observations on bovines naturally infected with *Theileria annulata*. *Journal of Veterinary Parasitology*, 20 (1), 49-52.
4. Bansal, G.C., Gaur, S.N.S. and Shah, H.L. (1977). Note on prevalence and cross transmission of *Theileria annulata*. *Pantanagar Journal of Research*, 2 (2), 244-246.
5. Bhojne, G.R., Dakshinkar, N.P., Dhoot, V.M., Kolte, S.W. and Gupta, D. (2010). Herd outbreak and management of bovine theileriosis. *Intas Polivet*, 11 (1), 21-22.
6. Boulter, N. and Hall, R. (2000). Immunity and vaccine development in the bovine theileriosis. *Advances in Parasitology*, 44, 41–97.
7. Brown, C.G.D. (1997). Dynamics and impact of tick-borne diseases of cattle. *Tropical Animal Health and Production*, 29, 15–35.
8. Chauhan, H.C., Patel, B.K., Bhagat, A.G., Patel, M.V., Patel, S.I., Raval, S.H., Panchasara, H.H., Shrimali, M.D., Patel, A.C. and Chandel, B.S. (2015). Comparison of molecular and microscopic techniques for detection of Theileria annulate from the field cases of cattle. Veterinary World, 8 (11), 1370-1374.
9. Chaudhri, S.S. Bisla, R.S., Bhanot, V. and Singh, H. (2013). Prevalence of haemoprotozoan infections in pyretic dairy animals of eastern Haryana. *Indian Journal of Animal Research*, 47 (4), 344-347.
10. Col, R. and Uslu, U. (2006). Haematological and coagulation profiles during severe tropical theileriosis in cattle. *Turkish Journal of Veterinary and Animal Science*, 30, 577-582.
11. Deore, P.A., Sabnis, M.G. and Bendre, V.U. (1979). A case of cutaneous thileriasis in graded cattle. *Indian Veterinary Journal*, 56, 794-795.
12. Devadevi, N., Rajkumar, K., Vijayalakshmi, P. and Perumal, S.V. (2018). Haematobiochemical changes in cattle with *Theileria orientalis* infection. *International Journal of Livestock Research*, 8 (12), 258-263. Doi:10.5455/ijlr.20180321021002.
13. Dhar, S., Malhotra, V., Bushan, C. and Gautam, O.P. (1987). Treatment of clinical cases of bovine tropical

theileriosis with buparvaquone. *Indian Veterinary Journal*, 64, 331-334.
14. Durrani, A.Z., Kamal, N. and Khan, M.S. (2008). Epidemiology, Serodiagnosis and Chemoprophylaxis of Theileriosis in Cattle. Doctoral dissertation, University of Veterinary and Animal Sciences, Lahore, Pakistan.
15. Gupta, S.C. and Singh, B.P. (2002). Fasciolosis in cattle and buffaloes in India. *Journal of Veterinary Parasitology*, 16, 139-145.
16. Hasanpour, A., Moghaddam, G.A. and Nematollahi, A. (2008). Biochemical, haematological, and electrocardiographic changes in buffaloes naturally infected with *Theileria annulata*. *The Korean Journal of Parasitology*, 46 (4), 223.
17. Hooshmand-Rad, P. (1976). The pathogenesis of anaemia in *Theileria annulata* infection. *Research in Veterinary Science*, 20, 324–329.
18. Jagtap, R.B., Gupta, A. and Chaphalkar, S.R. (2015). Flow cytometry-based profiling of leukocytes: A new method for diagnosis of tropical theileriosis in crossbred cattle. *Veterinary World*, 8 (12), 1379.
19. Jithendran, K.P. (1997). Blood protista of cattle and buffaloes in Kangra Valley, Himachal Pradesh. *Indian Journal of Animal Science*, 67 (3), 207-208.
20. Khattak, T., Ali, A., Rehman, N., Khan, K., Shoaib, M. and Shah, M.Y. (2007). Prevalence of haemoparasites in cattle and buffaloes in DI Khan, NWFP (Pakistan). *International Journal of Life Science*, 1 (1), 1-4.
21. Kohli, S., Atheya, U.K., Srivastava, S.K., Banerjee, P.S. and Garg, R. (2014). Outbreak of theileriosis and anaplasmosis in herd of Holstein crossbred cows of Dehradun district of Uttaranchal, India: A Himalayan region. *International Journal of Livestock Production*, 5 (1), 6-9.
22. Maharana, B.R., Kumar, B., Prasad, A., Patbandha, T.K., Sudhakar, N.R., Joseph, J.P. and Patel, B.R. (2016). Prevalence and asssessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India. *Indian Journal of Animal Research*, 50 (5), 733–739.
23. Mahmmod, Y.S., Elbalkemy, F.A., Klaas, I.C., Elmekkawy, M.F. and Monazie, A.M. (2011). Clinical and haematological study on water buffaloes (*Bubalus bubalis*) and crossbred cattle naturally infected with *Theileria annulata* in Sharkia province, Egypt. *Ticks and Tick-Borne Diseases*, 2 (3), 168–171.
24. Memon, M.I., Memon, N., Kachiwal, A.B., Memon, M.R. and Bhutto, B. (2016). Prevalence of theileriosis and its impact on haemotological values in naturally infected buffaloes at Hyderabad. *Pakistan Jouarnal of Agriculture Agricultural Engineering and Veterinary Science*, 32 (1), 85–94.
25. Mhadhabi, M., Naouach, A., Boumiza, A., Darghouth, M.A., Benabderrazak, S. and Darghouth, M.A. (2010). *In vivo* evidence for the resistance of *Theileria annulata* to buparvaquone. *Veterinary Parasitology*, 169 (3-4), 241-247.
26. Muhammad, G., Saqib, M., Athar, M., Khan, M.Z. and Asi, M.N. (1999). Clinico-and therapeutic aspects of bovine theileriosis. *Pakistan Veterinary Journal*, 19 (2), 64-71.
27. Muraleedharan, K., Ziauddin, K.S., Hussain, P.M., Puttabyattappa, R. and Seshadri, S. J. (2005). Haematological observations on *Theileria annulata* infection in cattle and buffaloes. *Journal of Veterinary Parasitology*, 19, 71-72.
28. Narladkar, B.W. (2018). Projected economic losses due to vector and vector-borne parasitic diseases in livestock of India and its significance in implementing the concept of integrated practices for vector management. *Veterinary World*, 11 (2), 151.
29. Omer, O.H., El-Malik, K.H., Mahmoud, O.M., Haroun, E.M., Hawas, A., Sweeney, D. and Magzoub, M. (2002). Haematological profiles in purebred cattle naturally infected with *Theileria annulata* in Saudi Arabia. *Veterinary Parasitology*, 107 (1-2), 161-168.
30. Osman, S.A. and Al-Gaabary, M.H. (2007). Clinical, haematological and therapeutic studies on tropical theileriosis in water buffaloes (*Bubalus bubalis*) in Egypt. *Veterinary Parasitology*, 146 (3), 337-340.
31. Preston, P.M., Bell-Sakyi, G.I.W. and Sanderson, A. (1992). Tropical theileriosis in *Bos taurus* and Bos *indicus* calves: response to infection with graded doses of sporozoites. *Research in Veterinary Science*, 53, 230–243.
32. Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2010). Theileriosis: In Veterinary Medicine.10th Edn. Saunders, Philadelphia, pp. 1526-1531.
33. Sandhu, G.S., Grewal, A.S., Singh, A., Kondal, J.K., Singh, J. and Brar, R.S. (1998). Haematological and biochemical studies on experimental *Theileria annulata* infection in crossbred calves. *Veterinary Research Communications*, 22 (5), 347-354.
34. Sarma, G.V.K., Reddy, S.U.M., Subrahmanyam, P., Murthy, S.S., Rao, T.M. and Krishna, P.G. (2008). Clinical efficacy of iron sorbitol citric acid complex+ folic acid+ hydroxycobalamin acetate (FERITAS) injection in treatment of the haemoprotozoan diseases and post parturient haemorrhagic complications. *Intas Polivet*, 9 (1), 64-66.

35. Sharifiyazdi, H., Namazi, F., Oryan, A., Shahariari, R. and Razavi, M. (2012). Point mutations in the *Theileria annulata* cytochrome b gene is associated with buparvaquone treatment failure. *Veterinary Parasitology*, 187 (3-4), 431-435.
36. Singh, N.K., Singh, H., Haque, M. and Rath, S.S. (2012). Prevalence of parasitic infections in buffaloes in and around district Ludhiana, Punjab, India. *Journal of Buffalo Science*, 1 (1), 113-115.
37. Snedecor, G.M. and Cochran, W.C. (1994). Statistical Methods, 8th edition, Iowa State University Press, Ames, Iowa.
38. Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals.7thedn. Bailliere Tindall and Cassel Ltd. London.
39. Sudan, V., Sharma, R.L., Yadav, R. and Borah, M.K. (2014). Turning sickness in a riverine
a. buffalo naturally infected with *Theileria annulata* and its successful therapeutic management. *Comparative Clinical Pathology*, 23, 39-42.
40. Syed, A.M., Bhikane, A.U., Masare, P.S., Ghoke, S.S. and Moregaonkar, S.D. (2014). Epidemio-clinical studies and chemotherapeutic evaluation against theileriosis in cattle. *Veterinary Practitioner*, 15 (2), 314-315.
41. Vahora, S.P., Patel, J.V., Patel, B.B., Patel, S.B. and Umale, R.H. (2012). Seasonal incidence of Haemoprotozoal diseases in crossbred cattle and buffalo in Kaira and Anand districts of Gujarat, India. *Veterinary World*, 5 (4), 223-225.
42. Tizard, I.R. (1992). Immunology: An introduction. 3rd Edn. Saunders College Publishing. Philadelphia, pp 1–13.
43. Tuli, A., Singla, L.D., Sharma, A., Bal, M.S., Filia, G. and Kaur, P. (2015). Molecular epidemiology, risk factors and hematochemical alterations induced by *Theileria annulata* in bovines of Punjab (India). *Acta Parasitologica*, 60 (3), 378–390.

****