

## PREVALENCE, CLINICOTHERAPEUTIC AND PROPHYLACTIC STUDIES ON THEILERIOSIS IN DISTRICT SAHIWAL (PAKISTAN)

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### ABSTRACT

A total of 300 exotic dairy cattle from various private livestock farms of district Sahiwal, Pakistan were selected to assess the incidence, effects on blood parameters, disease progression, therapeutic, and prophylactic aspects of tropical theileriosis. Animals were divided into two groups *viz* A and B. Group A (n=185) was kept as non-infected control whereas the group B (n=115) was diagnosed for theileriosis on clinical basis and further subdivided into two subgroups B1 (n=75): adult animals, and B2 (n=40): calves up to 1 year of age. The animals of group B (n=115 /38.33%) were treated with buparvaquone and long acting oxytetracycline at the dose rate of 2.5 and 20 mg/kg body weight intramuscularly, respectively and repeated at 48 hours interval. The same regimen was administered as a single dose to the healthy animals, prophylactically. The overall bi-monthly incidence rate recorded in April-May, June-July and August-September was 31.0, 59.0 and 25.0%, respectively. Group B1 accounted for 61.54% of the disease with the highest frequency during June-July. The overall case fatality rate was 18.26% being significantly higher during months of June-July (22.03%). Hematological parameters showed a significant decrease in the mean values of hemoglobin concentration, Packed Cellular Volume (PCV), Total Erythrocyte Count (TEC), and Total Leukocyte Count (TLC) in comparison to those of healthy animals. Therapeutic and prophylactic efficacy of Buparvaquone and oxytetracycline was 81.73 and 100%, respectively. The recovery rates for group B1 and B2 were 81.33 and 82.50%, respectively. It may be concluded that buparvaquone and long acting oxytetracycline in combination are quite effective both for treatment and prevention of disease.

**Key words:** Theileriosis, buparvaquone, hematology, cross-breeding, case fatality.

### INTRODUCTION

Tropical theileriosis is a disease of global economic importance in cattle, caused by the tick borne protozoan parasite *Theileria annulata*, and transmitted by ticks of the genus *Hyalomma* (Brown, 1997; Preston, 2001). The parasite is a serious constraint to cattle production in endemic areas, causing lethal infections in exotic cattle and considerable mortality in indigenous as well as crossbred stocks (Forsyth *et al.*, 1997). The economic losses to animals in terms of morbidity and mortality are due to their heavy incidence (Fadrago *et al.*, 1991). Although the sporadic cases are seen throughout the year, however, the outbreak in exotic and crossbred cattle is mostly reported during the hot and humid months (July-September) of the year (Ashfaq *et al.*, 1983; Zahid *et al.*, 2005).

Theileria parasites enter the bovine host as sporozoites during tick infestation, and rapidly invade mononuclear leukocytes. They are transformed into macroschizonts and induce proliferation of the host cell. Macroschizonts develop further into microschantons and ultimately into merozoites, which are released from the leukocyte. The merozoites invade erythrocytes and develop into piroplasms (Radostits *et al.*, 2000). Cattle with subclinical infection in endemic regions become

carrier of piroplasms and act as a source of infection for the vectors (Brown, 1990).

Climatic conditions of the country are conducive to the multiplication and growth of ticks which are most important ectoparasites of livestock both in tropical and sub-tropical countries. Ticks not only cause direct losses by sucking blood of the host animal, but also are responsible for various blood-borne diseases such as tropical theileriosis, anaplasmosis and babesiosis (Durrani *et al.*, 2008). The ultimate consequence is a decline in production of the livestock (Sajid *et al.*, 2007).

Weight loss, weakness, anorexia, pyrexia, conjunctival petechia, swollen lymph nodes, anemia, and cough are the most common symptoms. However, the later stages of theileriosis are associated with diarrhea, dysentery and lateral recumbency (Radostits *et al.*, 2000; Stockham *et al.*, 2000). Tentative diagnosis of theileriosis in the field is mainly based on clinical signs and tick infestation on the infected animals. However, confirmation of the diagnosis depends on microscopic examination of Giemsa stained thin blood smears (Aktas *et al.*, 2006).

Antitheilerial drugs such as buparvaquone have been used effectively in the treatment of tropical theileriosis in the field, stabilizing it as a drug of choice (Unsuren *et al.*, 1988; Muhammad *et al.*, 1999). However, the precise data regarding prophylactic

measures of this disease are still scanty. The present study was, hence, designed not only to monitor the prevalence, effects on hematological parameters, and therapeutic aspects but also the prophylactic measures of theileriosis in exotic/crossbred dairy cattle kept at private livestock farms in Sahiwal district.

## MATERIALS AND METHODS

**Study area and animals:** The study was conducted in private livestock farms of two Tehsils *i.e.* Sahiwal and Cheechawatni, district Sahiwal, Pakistan. A total of 300 animals (80 Holstein Friesian, 100 Jersey and 120 cross bred dairy cattle), of all ages and sexes were selected for the study and were divided into two groups A and B. Group A (n=185) was kept as non-infected control whereas the group B (n=115) was diagnosed for theileriosis on clinical basis and further subdivided into two subgroups B1 (n=75) which included adult animals, and B2 (n=40) which comprised of calves up to 1 year of age.

**Frequency of Disease:** Month and age wise frequency distribution of the disease was determined bi-monthly during 6 months period *i.e.* April-May, June-July and August-September.

**Clinical observations and blood sampling:** Diagnosis of theileriosis was made on the basis of clinical signs, infestation of the ticks and blood smear examination. Blood smears were prepared from the marginal ear vein of all animals and labeled accordingly. The smears were air dried, fixed with methanol, stained with Giemsa stain and examined under the oil immersion lens of a light microscope (Zafar *et al.*, 2006). The parasites were identified according to the characters described by Soulsby (1982). For haematological analysis, approximately 5ml blood was taken from the jugular vein of all animals with a syringe containing EDTA. The blood samples were subjected to haematological parameters *viz.* hemoglobin (Hb) concentration, Total Erythrocyte Count (TEC), Packed Cell Volume (PCV), and Total Leukocyte Count (TLC) as described by Coles, (1986).

**Collection and study of ticks:** Ticks were collected from the external body surface of the animals and were identified by their morphological characteristics as described by Urquhart *et al.* (1996).

**Therapeutic and prophylactic studies:** Based on the clinical and blood smear analysis, the animals found positive for theileriosis (Group 'B') were treated with buparvaquone at the dose rate of 2.5 mg/kg b.wt. and long acting oxytetracycline at the dose rate of 20 mg/kg b.wt., intramuscularly. This treatment was repeated after 48 hours (Radostits *et al.*, 2000; Muhammad *et al.*,

1999). In order to control the infection in the healthy animals (Group A), long acting oxytetracycline (20 mg/kg b.wt.) and concurrently buparvaquone (2.5 mg/kg b.wt.) were administered once intramuscularly in a period of two months. Ticks were controlled by spraying all infected cattle and the surrounding environment with cypermethrin (ecofleece<sup>®</sup>) at the dose rate of 1ml/litre and 10ml/litre of water, respectively twice, 2 weeks apart (Radostits *et al.*, 2000).

**Statistical analysis:** The data were analyzed through one way analysis of variance (ANOVA), technique followed by pair wise means comparisons, using Duncan multiple range tests. Differences were considered significant at  $p < 0.05$  (Duncan, 1955).

## RESULTS AND DISCUSSION

In this study, there were no clinical or parasitological findings in the control group (n=185). Rectal temperatures of all the cattle were within the normal ranges and neither schizonts nor piroplasms were found in any of the animals. Hematological parameters were also within the normal ranges. However, Group B animals (n=115) (38.33%) were diagnosed clinically for theileriosis and found to be positive with *T. annulata*, through the blood smear examinations. Hematological parameters revealed a significant ( $p < 0.05$ ) decrease in hemoglobin concentration, PCV, TEC, and TLC in infected cattle compared to normal ranges (Table 1).

The bi-monthly monitoring revealed that the incidence of the disease was highest during June-July (59%) followed by April-May (31%) and August-September (25%) Calves up to 1 year of age (Group B1) accounted for 61.54% of the disease with the highest frequency during June-July (Table 2).

Blood-sucking ticks were found on many body parts of the cattle and were identified to be *Hyalomma* species.

Out of 115 animals treated with the therapeutic doses of buparvaquone and long acting oxytetracycline, 94 (81.73%) responded positively and recovered. The recovery rates for group B1 and B2 were 81.33 and 82.50%, respectively (Table 3). The body temperature dropped from 105°F-106°F to 100°-102°F after 1-2 days following initiation of the treatment. Mortality and case fatality rates were 7 and 18.26%, respectively, in the treatment group (Table 4). Group A animals (n=185) which were administered prophylactic doses of the adjunct therapy showed no signs till last monitoring.

The results of this study showed that Sahiwal, Pakistan is an endemic area for tropical theileriosis which is in consistence with the work reported by Muhammad *et al.*, (1999) and Zahid *et al.*, (2005) in other districts of Punjab, Pakistan. The climatic conditions of Pakistan being conducive to the growth and maturation of the ticks

make it susceptible to tick borne infections and this may be the explanation why Pakistan is an endemic area for tropical theileriosis.

The positive animals of Group B showed high levels of parasitemia and exhibited clinical signs typical to tropical theileriosis. These results are in line with those reported by Osman and Al-Gaabary, (2007), and Omer *et al.* (2003) who reported that clinical signs in the infected animals were pyrexia (105-106 °F), enlargement of superficial lymph nodes, nasal and ocular discharges, salivation, anemia, respiratory distress and eye lesions. However, the slight variation in clinical signs showed by the animals in the present study may be attributed to various housing and management practices along with the degree of infection.

Haematological profile indicated significantly reduced hemoglobin concentration, TEC, PCV, and TLC as compared to normal values. The decrease in hemoglobin concentration (anemia) and TEC could be attributed to the toxic effects of metabolites of *Theileria* species, persistent loss of blood caused by permanent blood sucking ticks, (Geerts *et al.* 2001), and Tumor necrosis factor- $\alpha$  (TNF-  $\alpha$ ) on erythropoiesis (Boulter and Hall, 2000). The decrease in TLC has been reported by Goddeeris *et al.* (2004) and Graham *et al.* (2001). This decrease is related to a destruction of leucocytes in lymphoid organs and infiltration of these cells in other organs (Clark *et al.*, 1986).

The findings of the present study are in accordance with those of Sayin *et al.* (2003) who reported tropical theileriosis to appear soon after the appearance of tick infestation, and reaches the highest prevalence in July. Similarly, Ashfaq *et al.* (1983), Muhammad *et al.*, (1999), and Habela *et al.*, (1999) have reported maximum outbreaks of the disease from June to September. High

ambient temperatures are conducive for the growth and multiplication of the ticks, making the *Hyalomma* more active and hence increasing the transmission of theileriosis.

In the present study, the ticks collected from the infested cattle were identified to be *Hyalomma* species. Durrani and Shakoori (2009) have also reported highest prevalence of *Hyalomma* ticks (12%), followed by *Boophilus* (8.1%), *Haemaphysalis* (5%), and *Rhipicephalus* (3.1%) in various districts of Punjab, Pakistan. Similar results have also been reported by Sajid *et al.*, (2008) for districts Layyah and Muzaffargarh of Lower Punjab; and Khan *et al.*, (1993) for Faisalabad, Pakistan.

Therapeutic effectiveness for the adjunct therapy of buparvaquone and long acting oxytetracyclin was 81.73% in the present study. These results are in accordance to the work of Sharma and Mishra (1990). Similarly, Muhammad *et al.* (1999) reported 93 % effectiveness of buparvaquone (Butalex) in bovine theileriosis. Malhotra *et al.* (1988), however, found better recovery rate for buparvaquone. The mortality and case fatality rates in the present study were 7 and 18.26%, respectively. The death of the animals under treatment may be attributed to cerebral theileriosis or an advanced stage of the disease. Fadruga *et al.* (1991) has also reported that haemoparasites inflict losses to animals in term of morbidity and mortality due to their heavy incidence. Buparvaquone is usually not effective in cerebral and advanced cases of the theileriosis (Dhar *et al.*, 1987; Hussain *et al.*, 1990). The prophylactic efficacy of buparvaquone and long acting oxytetracyclin was 100%, as the healthy animals showed no signs of theileriosis till last monitored.

**Table 1: Mean ( $\pm$  SEM) values of haemogram in healthy and diseased exotic dairy cattle**

Parameters	Healthy cattle	Range	Theileriosis affected cattle	Range
RBCs (T/l)	7.37 $\pm$ 0.39	5.59-10.60	6.05 $\pm$ 0.17*	5.48-7.40
Hb (g/l)	120.93 $\pm$ 4.07	93.00-148.00	88.60 $\pm$ 7.08*	60.00-128.00
PCV (%)	34.45 $\pm$ 0.69	27.00-39.00	32.40 $\pm$ 2.04	25.00-46.00
WBCs (G/l)	6.90 $\pm$ 0.39	4.45-12.00	4.90 $\pm$ 0.32*	3.80-6.90

Significant at \* = P < 0.05

**Table 2: Bi-monthly incidence of tropical Theileriosis from April to September at private livestock farms in District Sahiwal**

Months of study	No. of samples	Adults	Calves	Total
April-May	100	29/85 (34.11)	2/15 (13.33)	31/100 (31)
June-July	100	39/75 (52)	20/25 (80)	59/100 (59)
August-September	100	7/75 (9.3)	18/25 (72)	25/100 (25)
Total	300	75/235 (31.91)	40/65 (61.54)	115/300 (38.33)

Values in parenthesis are percentages

**Table 3: Bi-monthly Morbidity, Mortality and Case Fatality rates from April to September due to tropical theileriosis at Private Livestock Farms in district, Sahiwal.**

Months of study	No. of samples	Adults	Calves	Total
April-May	100	31 (31)	4 (4)	4/31 (12.90)
June-July	100	59 (59)	13 (13)	13/59 (22.03)
August-September	100	25 (25)	4 (4)	4/25 (16.0)
<b>Total</b>	<b>300</b>	<b>115/300</b> <b>(38.33)</b>	<b>21</b> <b>(7)</b>	<b>21/115</b> <b>(18.26)</b>

Values in parenthesis are percentages

**Table 4: Group wise treatment regimen and recovery rates**

Group	Subgroup	No. of animals	Treatment regimen	No. of animals recovered	No. of animals not recovered
A	-	185	Oxytetracycline LA (20mg/kg b.wt.) and buparvaquone (2.5 mg/kg b.wt.) I/m	185 (100)	0
B	B1	75	Oxytetracycline LA (20mg/kg b.wt.) and buparvaquone (2.5 mg/kg b.wt.) I/m	61 (81.33)	14
	B2	40	repeated after 48 hrs.	33 (82.50)	7

Values in parenthesis are percentages

**Conclusion:** The geographical location of Pakistan in the Warm Climate Zones (WCZS) of the world, along with large scaled cross-breeding programs has made it an endemic area for theileriosis. Exotic/crossbred animals are more susceptible to the disease. Theileriosis has profound effects on haematological values and is responsible for mortality and losses in production with its highest incidence during spring to late summer. Buparvaquone is not only therapeutically effective in the initial stages of the disease but also has remarkable prophylactic efficacy as well. The future needs include latest diagnostic techniques like PCR, appropriate tick eradication programs, controlled crossbreeding, and thoroughly monitored preventive medicine programs in order to reduce both the incidence and prevalence of the disease.

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