MOLECULAR DETECTION, HAEMATOLOGICAL AND THERAPEUTIC STUDIES ON THEILERIOSIS IN BUFFALOES

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ABSTRACT

Tropical theileriosis poses major threat for buffaloes causing significant economical loss to livestock farmers. Early detection and prompt treatment helps to minimise mortality and economical loss. This study was conducted on 79 female buffaloes presented with the signs suggestive of theileriosis. Evaluation of PCR for detection and buparvaquone for efficacy was undertaken. Overall prevalence of *T. annulata* infection recorded was 22.78 % by PCR. Adult buffaloes showed higher prevalence (13.92 %) compared to young buffaloes (8.86 %). Blood smear examination revealed 38.89 % sensitivity in detection of *Theileria* piroplasms. Haematological observations showed significant decreased values of Hb, TEC, PCV and TLC. Neutropenia, monocytopenia, eosinopenia and lymphocytosis were recorded. Buparvaquone was 100 % effective in complete elimination of *T. annulata* in infected buffaloes.

Keywords: buffaloes, *Theileria annulata*, PCR, Buparvaquone

INTRODUCTION

Tropical theileriosis caused by *Theileria annulata* poses major threat for buffaloes causing significant economic losses to the livestock farmers.

Early diagnosis and prompt treatment is essential to prevent the production loss. Diagnosis of *T. annulata* infection is usually based on microscopic examination of blood smears stained with Giemsa. However apart from difficulty in species differentiation blood smear technique is not suitable for detection of infection where parasitaemia is low (Nayel et al. 2012). The methods of detection of Theileria species involving serological tests are not sensitive due to cross-reactions and disappearance of antibodies in long term carriers (Passos et al. 1998). Therefore a sensitive and highly specific method for the diagnosis of piroplasms is required. Recently species specific polymerase chain reaction (PCR) methods have been developed for the detection of *Theileria* species (D' Oliveira et al. 1995; Roy et al. 2000; Ica et al. 2007; Altay et al. 2008; Ali and Radwan 2011; Kundave et al. 2015; and Ganguly et al. 2015). Hence the present study was conducted to know the haematological alterations, use of PCR for detection and suitable treatment for *Theileria annulata* infection in buffaloes.

MATERIALS AND METHODS

The study was conducted on 79 female buffaloes (26 young and 53 adult) presented to Veterinary College Hospital, Bidar, Karnataka, India with the signs of pyrexia, lymphadenopathy,

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pale mucus membrane, anorexia and tick infestation.

Haematological examination

Thin blood smear were prepared from ear vein and stained using Giemsa stain. The parasites were identified according to the characters described by Soulsby (1982). The blood samples were collected from jugular vein in EDTA coated vacutainers for PCR analysis. The blood samples found positive for *T. annulata* on blood smear examination were subjected to haematological examination as described by Schalm *et al*., (1975). Similarly blood samples collected from apparently healthy buffaloes were subjected to haematological examination and served as control.

Isolation of DNA from blood samples

Genomic DNA was extracted from 200 µl of the whole blood using DNA extraction kit (Bio basic) according to the manufacturer's instructions. The isolated DNA was quantified spectrophotometrically and run on 0.8 % agarose gel. Aliquots of extracted DNA were stored at -20°C for PCR analysis.

Polymerase chain reaction

The primer was designed on the coding sequence of the 30 kDa major merozoite surface antigen (Tams 1 gene) of *Theileria annulata.* The forward primer sequence is 5'-CCA GTA ACC TTT AAA AAC GT – 3' and reverse primer sequence is 5'- GTT ACG AAC ATG GGT TT -3'. The PCR reaction on total volume of 15 µl containing 30 ng of template DNA, 7.5 µl of 2x master mix (Fermentus), 0.5 µl of each forward and reverse primer (10 pmol/µl), 5.5 µl of nuclease free water. Reaction condition were initial denaturation at 98°C for 5 minutes, followed by 37 cycles of

95°C for 30 seconds, 45°C for 30 seconds, 72°C for 30 seconds, with a final extension step of 72°C for 1 minute and holds at 4°C in a thermal cycler (Eppendorf). A negative control (Sterile water), a positive control DNA from *Theileria annulata* were included in each amplification run. PCR products were analysed by electrophoresis on 1.5 % agarose gel and documented with documentation system (Gel Doc, Syngene).

Treatment

Buffaloes infected with *T. annulata* confirmed by blood smear examination were treated with dose of Buparvaquone 2.5 mg/kg body weight. Therapeutic efficacy was evaluated based on changes in clinical parameters and parasitological status on 7th day post treatment.

Statistical analysis

The difference of means of haematological values between *T. annulata* infected and healthy control group were compared using student t-test (Snedecor and Cochran, 1994)

RESULTS AND DISCUSSION

Out of 79 blood samples 18 (11 adult and 7 young) were positive for *T. annulata* by PCR indicated overall prevalence of 22.78 %. Higher prevalence (13.92 %) of *T. annulata* was recorded in buffaloes more than 2 year age group when compared to less than two year age group (8.86 %) Similarly higher prevalence of *T. annulata* in adult bovines was recorded by Anand et al (2009) and Kundave *et al*. (2015). Higher rate of disease occurrence in adult buffaloes could be attributed to stress of pregnancy and lactation (Durrani 2003). The age-related resistance in young cattle to most

tick-borne protozoan diseases has been reported by Dumanli *et al*. (2005).

Out of 79 blood samples, Giemsa stained blood smear examination revealed presence of *Theileria piroplasms* in 7 samples (Figure 1) which indicated 38.89% sensitivity compared to PCR as base reference. A desired product size of 721 bp was obtained in *T. annulata* positive samples (Figure 2). Blood smear examination in the present study had shown false negative under light microscope, which shows low sensitivity of the test. It could be attributed to low parasitaemia, destruction of piroplsms in red blood cells due to haemolysis and unsuitable blood smear staining (Hoghooghi *et al*., 2011). Moreover the microscopic detection of piroplasms in blood samples that were negative by PCR was not possible. The samples positive by stained blood smears were found to be positive with PCR assay. This fact confirms the superiority of PCR over blood smear examination. The results of the present study are in agreement with earlier workers (Sanchez *et al*., 1999; Roy *et al*., 2000; Azizi *et al*., 2008; Mohammod *et al*., 2011;

Ganguly *et al*., 2015 and Kundave *et al*., 2015)

The haematological values of *T. annulata* infected and healthy control groups have been presented in Table 1. The infected group showed significant decreased (p≤0.01) values of total erythrocyte count, haemoglobin, packed cell volume than healthy group, indicating normocytic normochromic anaemia. Significant decrease (p≤0.01) in total leukocyte count and neutrophil count and non-significant decrease in monocyte and eosinophil count was observed in infected group. Similar haematological observations in *Theileria annulata* infected buffaloes were made by Sharma *et al*. (1985), Osman and Al-Gaabary (2007) El-Deeb and Younis (2009). Decreased total erythrocyte count, haemoglobin and PCV in *Theileria annulata* infected buffaloes could be attributed to erythrophagocytosis (Yagi *et al*., 2002). Relative increase in lymphocyte count reflects compensatory mechanism in target cells in response to their invasion with *T. annulata* (Lamia, 1997).

Seven buffaloes infected with *T.annulata*

Table 1. Hemogram in Healthy and *Theileria* infected buffaloes (mean ± S.E).

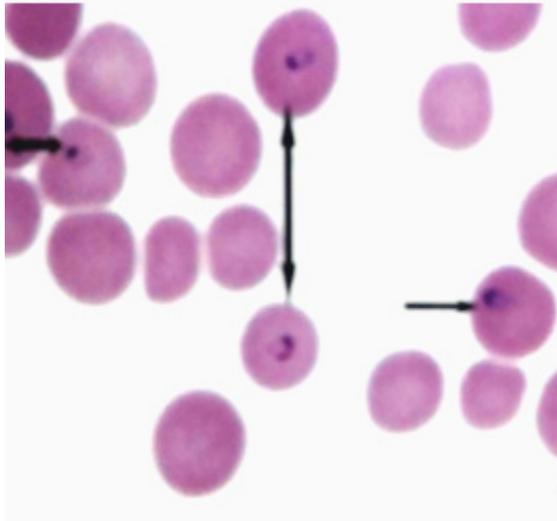| Variable	Healthy buffaloes (*N*=07)	Infected buffaloes (*N*=07)
TEC (10^6/µl)	5.78 ± 0.13	3.70 ±0.18
Hb (g/dl)	10.11 ± 0.22	7.60 ± 0.19
PCV (%)	32.71 ± 0.83	22.99 ± 0.86
MCV (fl)	56.5 ± 0.50	60.41 ± 1.57
MCH (pg)	17.44 ± 0.21	20.81 ± 0.61
MCHC (g/dl)	30.9 ± 0.50	34.50 ± 0.85
TLC (10^3µl^{-1})	6.45 ± 0.19	5.60 ± 0.28
Neutrophils (10^3 µl^{-1})	33.85 ± 0.67	39.86 ± 0.55
Lymphocytes (10^3 µl^{-1})	61.42 ± 1.08	56.00 ±0.61
Monocytes (10^3 µl^{-1})	2.85 ± 0.50	2.71 ± 0.42
Eosinophils (10^3 µl^{-1})	1.85 ± 0.34	1.43 ± 0.20

Figure 1. Piroplasmic forms of *T. annulata* by Giemsa staining method.
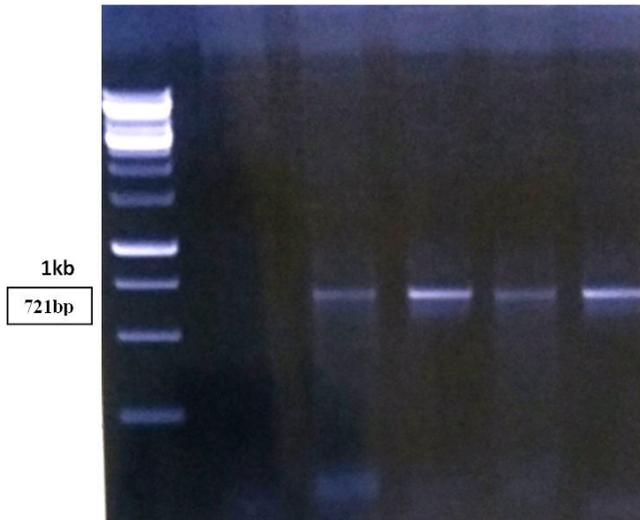

Figure 2. Detection of *T. annulata* in blood by PCR.

confirmed with blood smear examination were treated with single dose of Buparvaquone at the rate of 2.5 mg/kg body weight intramuscularly. All seven buffaloes revealed clinical improvement and absence of intracellular piroplasms by seventh day with the efficacy rate of 100 percent. This is an agreement with the report of Osman and Al-Gaabary (2007).

From the findings of the present study it can be concluded that prevalence of *Theileria annulata* infection was high in adult buffaloes and haematological changes observed are useful in understanding disease pathogenesis and corrective measures for supportive therapy. PCR can reliably be used for accurate detection of *T. annulata*. Buparvaquone treatment is indicated in the treatment of tropical theileriosis in buffaloes.

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