Indian Journal of Animal Sciences **92** (4): 417–421, April 2022/Article
https://doi.org/10.56093/ijans.v92i4.124017

Prevalence and characteristics of haemoprotozoan infections of cattle in Mizoram

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Received: 1 July 2020; Accepted: 27 December 2021

ABSTRACT

Present study aimed at finding the prevalence and characterestics of different haemoprotozoan infections in cattle of Mizoram. Study consisted of 150 samples collected from 64 cattle farms. The epidemiological parametres were collected along with blood samples. Blood smears were examined and PCR done for all samples. Results showed that 18% of cattle were infected with haemoprotozoan diseases, viz. babesiosis (44.44%), theileriosis (37.03%) and anaplasmosis (11.11%) and concomitant infection (7.40%). Haematobiochemical parametres were also examined and found hypoproteinaemia, hypoalbunminaemia, hypoglobulinaemia, hypoglycemia, increased AST, total bilirubin and creatinine. Therapeutic efficiency of Dimenazine and Buparvaquone along with other drugs were also studied. It is observed that Dimenazine along with Azithromycine and Buparvaquone along with Oxytetracycline showed more effectivenes.

Keywords: Anaplasmosis, Babesiosis, Cattle, Haemoprotozoan, Mizoram, Theileriosis

The state of Mizoram owns 21,000 dairy cattle out of which 11,000 are crossbreds and 10,000 are indigenous cattle breeds (Quinquennial Livestock Census 2019). Haemoprotozoan diseases have considerably increased among the susceptible exotic and crossbreds in the recent years (Sarma *et al*. 2016, Ghosh *et al*. 2020). *Bos indicus* breeds are generally resistant to these diseases because of their long and harmonious association with tick vectors and haemoparasites. But *Bos taurus* breed of cattle imported from cooler climates to the hot and humid regions and their crosses show susceptibility to these haemoparasites (Jabbar *et al*. 2015). The economic loss results from high morbidity and mortality, decreased production of meat and milk and the loss of draught power (Narladkar 2018). The important haemoprotozoan diseases prevailing in India are babesiosis, theileriosis and trypanosomiasis (Maharana *et al*. 2016). The diagnosis of these infections is done with help of examination of prepared blood films stained with Giemsa or other blood stains (Jayalakshmi *et al*. 2019).

But the sensitivity depends on the expertise of the examiner especially at the low level of parasitaemia. Polymerase Chain Reaction (PCR) provides an additional diagnostic option that can detect parasites at low level of parasitaemia and identification at species level with high specificity and sensitivity (Afifi *et al*. 2014). The present study was conducted with the objectives of finding the prevalence of haemoprotozoan diseases in Mizoram along with the clinico-haematological changes and to know the therapeutic efficacy of different drugs against specific haemoprotozoa.

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MATERIALS AND METHODS

The study consisted of 64 unorganized and organized cattle farms in and around Aizawl with different breeds of cattle in different age groups. After subjecting to detailed clinical examination, preliminary epidemiological parameters and history of animals were recorded. The animals were screened on the basis of clinical symptoms, viz. fever, diarrhoea, staggering gait, anaemia, and debilitated condition, enlargement of prescapular lymph node, haematuria and presence of ticks on the animals.

Blood samples were collected from 150 animals (10 mL each) from 64 farms and blood smears were prepared as per standard protocol. From the blood sample, aliquot of 2 mL blood in EDTA (1 mg/mL blood) for haematological, 1 mL whole blood for PCR evaluation and 5 mL blood without anticoagulant for serum separation and 2 mL was kept as reserve. Serum was separated and stored in deep freeze at (–20°C) for biochemical and enzymatic estimation. The smears were subjected to Giemsa staining as per the method described by Nair *et al.* (2011).

Molecular detection of haemoprotozoa: All blood samples were screened for different haemoprotozoan DNA using standard primers with PCR as per standard method (Table 1) (Lorenz 2012).

Haematological parameters: The haematological parameters, viz. haemoglobin (Hb), packed cell volume (PCV), total leukocyte count (TLC), total erythrocyte count (TEC), mean corpuscular volume (MCV), mean

Table 1. Primers used for haemoprotozoan screening with PCR

Organism	Primer	Primer sequence pair	Product size	Reference
Babesia bigemina	Forward	5′ TGG CGG CGT TTA TTA GTT CG 3′	1124 bp	Laha *et al*. 2012
	Reverse	5′ CCA CGC TTG AAG CAC AGG A 3′		
Babesia bovis	Internal Forward	5′ GGG TTT ATA TAG TCG GTT TTG T 3′	711 bp	Patarapadungkit *et al*. 2004
	Internal Reverse	5′ ACC ATT CTG GTA CTA TAT GC 3′		
Theileria annulata	Forward	5′ GTA ACC TTT AAA AAC GT 3′	721 bp	d'Oliveira *et al*.1995
	Reverse	5′ GTT ACG AAC ATG GGT TT 3′		
Anaplasma marginale	Forward	5′ CAC ATT TCT TGG AGC TGG 3′	160 bp	Figueroa *et al*.1993
	Reverse	5′ TCT CTG GCA CTT TGA ACC 3′		
Theileria orientalis	Forward	5′ CTT TGC CTA GGA TAC TTC CT 3′	776 bp	Kamau *et al*. 2011
	Reverse	5′ ACG GCA AGT GGT GAG AAC T 3′		

corpuscular heamoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and thrombocyte count were done with semi automated blood cell counter (MS4e, Netherlands).

Biochemical parameters: The total protein and albumin was estimated by the biuret method as described by Bertholf (2014) whereas globulin was estimated by calculation. The blood glucose was determined by using glucose oxidase method (Kumar and Gill 2018). The concentration of aspartate aminotransferase (AST) was determined spectrophotometrically using the method of Wang *et al.* (2016). The level of alkaline phosphatase (ALP) in the serum was determined as described by Omotainse *et al*. (1994) using spectrophotometry method. Creatinine (Syal *et al*. 2013), Blood Urea Nitrogen (BUN) (Patke and Kansara 2018) and total and direct bilirubin (Choosongsang *et al.* 2011) was also estimated by using spectrophotometry method.

Serum globulin in g/dL = Total proteins (in g/dL) – Albumin (in g/dL)

Therapeutic potential of Azithromycin-Dimenazine aceturate against babesiosis: Cows with babesia positive were randomly divided into two groups, viz. T2 and T3 and each group had six animals. Six healthy cows were taken as healthy control group (T1) for comparison of therapeutic efficacy. The following therapeutic model was followed: T1 (Healthy): No treatment; T2 (Standard therapy): Diminazine Aceturate @ 12 mg/kg Body Weight (BW) as a single dose + other supportive therapy (Injection (Inj.) Meloxicam @ 0.02 mg/kg BW for three days, Inj. Iron (III) hydroxide polymaltose complex and folic acid @ 1 mL/20 kg BW for 10 days every alternate day, normal saline @ 500 mL IV for three days, mineral mixture @ 30 g orally daily for 15 days); T3 (Experimental therapy): Azithromycin @ 15 mg/kg BW for three days + Diminazine Aceturate @ 12 mg/kg BW as a single dose + other supportive therapy (Inj. Meloxicam @ 0.02 mg/kg BW for three days, Inj. Iron (III) hydroxide polymaltose complex and folic acid @ 1 mL/20 kg BW for 10 days every alternate day, normal saline @ 500 mL IV for three days, mineral mixture @ 30 g orally daily for 15 days).

Therapeutic potential of Buparvaquone-oxytetracycline: T1 (Healthy): No treatment; T2 (Standard therapy): Buparvaquone @ 12 mg/kg BW as a single dose + other supportive therapy (Inj. Meloxicam @ 0.02 mg/kg BW for three days, Inj. Iron (III) hydroxide polymaltose complex and folic acid @ 1 mL/20 kg BW for 10 days every alternate day, normal saline @ 500 mL IV for three days, mineral mixture @ 30 g orally daily for 15 days); T3 (Experimental therapy): Buparvaquone @ 12 mg/kg BW as a single dose + oxytetracycline @ 10 mg/kg BW for three days + other supportive therapy (Inj. Meloxicam @ 0.02 mg/kg BW for three days, Inj. Iron (III) hydroxide polymaltose complex and folic acid @ 1 mL/20 kg BW for 10 days every alternate day, normal saline @ 500 mL IV for three days, mineral mixture @ 30 g orally daily for 15 days).

Therapeutic evaluation was done with the help of improvement of clinical signs, and blood haemato-biochemical parameters and absence of haemoprotozoan organism. All the data were analyzed by using independent t-test and ANOVA by Statistical Package SPSS 16 (SPSS, Science, Chicago, USA).

RESULTS AND DISCUSSION

Diagnosis of haemoprotozoan infections: The blood smears revealed abnormal erythrocytes morphology along with presence of pyriform organism in red blood cells (RBCs) in case of babesiosis (Fig. 1), Koch's blue body in infected lymphoctes in case of theileriosis (Fig. 2) and blue-purple inclusions in RBCs in case of anaplasmosis (Fig. 3). The variations in erythrocytic morphology are mainly due to haemoprotozoans, erythrocytic oxidation, intravascular thrombi formation and immune mediated process (Stockham *et al*. 2000). PCR method showed 10 positive samples in addition to the 17 positively stained samples (Fig. 4). Parasites are often missed on blood smear examination in patients with haemoprotozoan infection when they seek medical care, because usually less RBCs are parasitized early in the course of the illness (Mosqueda *et al*. 2012). Phylogenetic analyses inferred from *Ct* amplification of parasite DNA is far more sensitive than parasite detection by conventional Giemsa stain. The

concentration of the parasite may be extremely low but PCR sensitivity is extremely high which will amplify even a minute amount of haemoparasite (Parthiban *et al*. 2010).

Prevalence of haemoparasites in cattle of Mizoram: The present study revealed that 18% of cattle were found infected with haemoprotozoan diseases, viz. babesiosis (44.44%), theileriosis (37.03%) and anaplasmosis (11.11%) and concomitant infection (7.40%). This study reported less prevalence of haemoprotozoan than the previous study in Mizoram (33.3%) (Ghosh *et al*. 2020). The less prevalence might be due to variation of study areas or availability of tick vectors. Variations in geo-climatic conditions, breed, age of the animals and exposure of vectors might contribute to variable prevalence of haemoprotozoan diseases in the study area (Muhanguzi *et al*. 2010).

Season wise prevalence (%) of various haemoprotozoan diseases: The results of the present study showed that monsoon season (62.90%) and summer (29.62%) are important risk factors for the prevalence of babesiosis, theileriosis and anaplasmosis. The results were in agreement with Jayalakshmi *et al*. (2019). Constable *et al*. (2016) observed that higher incidence of haemoprotozoan diseases were found soon after peak of tick population depending on temperature, humidity and rainfall which might be accounted for higher prevalence of haemoprotozoan infections in rainy season of the study.

Breed wise prevalence of haemoparasites: In the present study, crossbred animals (70.37%) were mostly affected than Holstein Friesian (18.51%) and Jersey (11.11%) and this observation was in agreement with previous workers. Velusamy *et al.* (2014) reported high prevalence of haemoprotozoan diseases in Jersey cross breeds and Holstein Friesian. This might be due to the high percentage of crossbred cattle in the study area.

Age wise prevalence (%) of haemoprotozoan infection: In this study, higher susceptibility of adult cattle (above five years) to haemoprotozoan diseases was found consistent with the findings of Velusamy *et al*. (2014) who reported higher prevalence in animals aged more than two years. Zintl *et al.* (2005) opined that in calves, inflammatory response occurs early and is concentrated in the spleen while a delayed and systemic inflammatory response occurs in adult animals which is incompetent and contributes to the pathogenesis.

Haematological profile of cattle with various haemoprotozoan infections: Significant decrease in Hb, PCV, TEC, TLC, platelets and granulocyte count in cattle ($P<0.05$) in present study were in agreement with Bock *et al*. (1999). Anaemia and decreased TEC observed could be attributed to blood sucking by ticks as well as intravascular haemolysis of RBCs (Kaur *et al*. 2017). Leucogram showed decrease in TLC, related to destruction of leucocytes in lymphoid organs and infiltration of these cells in other organs and can be attributed to persistent harmful effect of haemoprotozoan on the haemopiotic organs especially bone marrow and their interference with the process of leucogenesis. (Sarma *et al.* 2016) (Table 2).

Table 2. Haematological profile of cattle with mixed infection

Parameter	Mean ±SE		t
	Healthy	Mixed infection	
Hb (g/dl)	10.56±0.30	3.50±0.10	20.287*
PCV (%)	31.90±0.47	10.5±0.29	36.312*
TEC (M/mm^3)	7.03±0.36	3.23±0.04	9.391*
TLC (m/mm^3)	11.71±0.52	6.65±0.89	5.079*
Platelet (10^3/ul)	7.35±0.59	4.03±0.46	4.281*
MCV (fl)	46.05±2.77	32.60±1.34	4.072*
MCH (pg)	15.26±1.01	10.85±0.45	3.703*
MCHC (g/dl)	33.09±0.49	33.33±0.50	-.435 NS
Lymphocytes (%)	39.08±1.88	48.66±0.30	-4.567*
Monocytes (%)	7.23±0.43	10.56±0.27	-6.200*
Granulocytes (%)	53.68±2.00	40.76±.03	5.833*

* Significant; NS, Non significant.

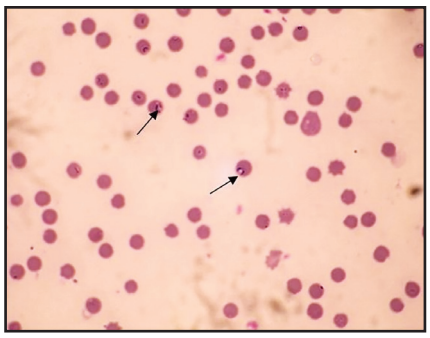

Fig. 1. Giemsa stain blood smear showed *Babesia bigemina* (100x) as pyriform.

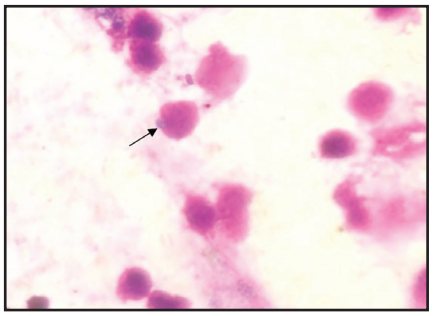

Fig. 2. Giemsa stain blood smear showed *Theileria spp.* (100x) as Koch blue body in lymphnode.

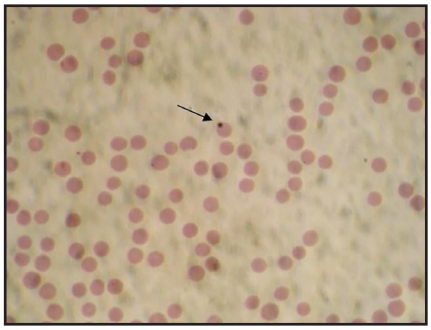

Fig. 3. Giemsa stain blood smear showed *Anaplasma marginale* (100x) as dense, homogeously staining blue-purple inclusions.

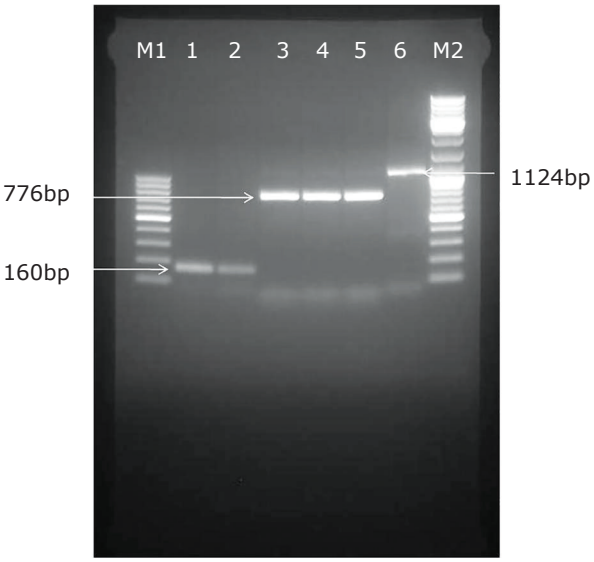

Fig. 4. Amplification of target genes. Lanes 1, 2: *Anaplasma marginalae*; Lanes 3, 4, 5: *Theileria orientalis*; Lane 6: *Babesia bigemina*; Lane M1: 100bp DNA ladder; Lane M2: Gene ruler.

Serum biochemical profile of cattle with various haemoprotozoan infestations: Both babesiosis infected cattle and theleriosis infected cattle showed hypoproteinaemia, hypoalbuminaemia, hypoglobulinaemia, hypoglycemia, increased AST, total bilirubin and creatinine. These observations indicated the harmful effect of toxic metabolites of babesia species on hepatic cell. These results were supported by findings of Yeruham *et al*. (2003) and Hussein *et al*. (2007). Findings in theleriosis infected cattle were supported by Stockham *et al*. (2000).

Anaplasmosis infected cattle showed increased globulin and it signifies that the immune system may be activated leading to elevation of circulating immunoglobulin in the serum to counter the infection in the system (Ashuma *et al*. 2013).

Therapeutic evaluation of Diminazine aceturate and combination of Diminazine aceturate and azithromycin against babesiosis: Diminazine aceturate-azithromycin composition exhibited a higher efficacy than Diminazine aceturate alone in this study. Diminazine aceturate and aromatic diamidine compound can interfere with the synthesis of DNA and aerobic glycolysis and commonly used against infection of babesiosis (Matsuu *et al*. 2008). Unfortunately, babesia developed resistance against Diminazine as it was in use for over half a century (Oguejiofor *et al*. 2010). PCR assay showed that the organisms were still present on day 7 post therapy in Diminazine aceturate alone treated group but negative result was found in Diminazine-azithromycin group and that might be due to synergistic effect of combination therapy.

Therapeutic evaluation of buparvaquone and combination of buparvaquone and oxytetracycline against theileriosis: The combination therapy of oxytetracycline with buparvaquone was effective against theileriosis as compared to buparvaquone alone. Various workers reported that theileriosis can be controlled by long acting oxyteracycline. Saravanan *et al*. (2017) opined that Oxytetracycline helps to ameliorate pneumonic changes in addition to antitheilerial activity. Mbwambo *et al*. (2006) reported that in chronic cases which are usually complicated with pulmonary disorders, using of oxytetracycline with buparvaquone as a combination for treatment of theileriosis was effective.

ACKNOWLEDGEMENTS

This paper is prepared from the MVSc thesis work of first author and the research work is having the approval of Institutional Animal Ethics Committee. The authors are thankful to the Hon'ble Vice Chancellor, Central Agricultural University and Dean, CVSc & AH, Selesih, Aizawl for providing all the facilities for this research work.

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