

Theileriosis in crossbred cows and its therapeutic management: first report from Lushai hill district of Mizoram

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Abstract To know the prevalence of theileriosis infection, haemato-biochemical alteration and efficacy of Oxytetracycline in combination with Diaminazene aceturate injection against theileriosis in Lushai hill district of Mizoram; fifty five cross bred cattle were screened during July, 2013 to November, 2013. The prevalence of tropical theileriosis was 9.09 % based on blood smear examination. Clinical features of the disease were manifested by high rise of body temperature 104.8 ± 1.2 °F, lymphadenopathy, inability to walk, anorexia, listlessness, moderate anaemia, nasal discharge, edema of eye and corneal opacity. There were marked decline ($P < 0.01$) in the haemoglobin 5.28 ± 1.81 gm/dl, total erythrocytic count $2.14 \pm 0.7 \times 10^6$ /ul, packed cell volume 18.10 ± 0.8 % and total leukocyte count $6.95 \pm 1.05 \times 10^3$ /ul, respectively. Erythrocyte sedimentation rate was significantly increased ($P < 0.01$) up to 104.5 ± 12.4 mm/1st hour. The percentage of neutrophils increased 62.75 ± 2.77 and lymphocytes decreased drastically 28.69 ± 1.45 as compared with un-infected cross bred cattle. Clinically infected cattle with *Theileria annulata* had significantly low levels of total proteins and albumin, ($P < 0.05$) but AST, total, direct and indirect bilirubin, BUN, creatinine levels and Ck-MB activity were significantly high ($P < 0.05$).

Oxytetracycline in combination with Diaminazene acetate was found effective against *T. annulata* and could control fever and temperature returns to normalcy by 7th day post treatment along with some haemato-biochemical profiles. In brief, the study revealed that tropical theileriosis was prevalent in Lushai hill district of Mizoram especially among cross breed cattle and had effect on hepatic, renal and cardiac function which can be effectively controlled by Oxytetracycline and Diaminazene acetate injection.

Keywords Crossbred cow · Haemato-biochemical · Mizoram · Theileriosis · Therapeutic

Introduction

Lushai hill district of Mizoram located between 21.85 and 24.35 N latitude and 92.15–93.29E longitude covering 404 km on East with Myanmar and 306 km west with Bangladesh. The livelihood of the peoples of Mizoram is mainly based on agriculture, but the mountainous topography has brought limitations to extensive agricultural operations. Animal husbandry plays an important role in the economy in supplementing the income of the rural house hold. But many haemoprotozoan diseases are constraint to livestock production in many developing countries of the world including India (Khan et al. 2004). They are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock by-products and the loss of draught power. Among the haemoprotozoan diseases *Theileria annulata* a tick borne protozoan parasite of cattle causes tropical Theileriosis with lymphoproliferation followed by leukopenia and high mortality and morbidity in cattle. Bovine tropical

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theileriasis is a serious challenge to the livestock improvement programme in India, but its epidemiology still has not been adequately studied in North east India especially in Mizoram. So, the aim of this study was directed to investigate tropical theileriosis for the first time in Mizoram and clarifying its effects on some blood biochemical constituents as well as treatment of diseased cattle and evaluate the effect of drug on the health of treated animals.

Materials and methods

Field study area and animals

The present investigation was carried out during July, 2013 to November, 2013 in Lushai hill district of Mizoram. Blood samples were analyzed from 55 cross bred cattle suspected for theileriosis. Ages of all animals used in this study ranged from 3 to 5 years. A total of 6 clinically healthy cross bred cattle from tick-free farms were used as a control group for comparison.

Clinical and parasitological monitoring of cattle

The conjunctival, nasal, and oral mucous membranes, lymph nodes, and rectal temperature of the cattle deemed to be undergoing *T. annulata* infection were examined and lymphnode aspiration were taken from enlarged nodes. Clinical and parasitological observations were recorded for all the animals showing the clinical signs of *T. annulata* infection. Blood smears were prepared from the ear tip of animals for identification of *Theileria* organism.

Collection of blood

Blood samples were collected at day 0 of before treatment and at 7th day post treatment from those animals showed positive result by jugular vein puncture. 2 ml blood was collected to a vial containing 10 % EDTA for haematological estimation and 8 ml of blood in sterile test tube was allowed to stand in inclined position at room temperature for serum harvesting. Serum was stored in refrigerator at -4°C for biochemical analysis.

Parameters of study

Hematological parameters viz. hemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocytic count (DLC), red blood cell indices were studied with the help of automatic blood cell counter (MS4, Netherland).

Biochemical study was conducted for estimation of total protein and albumin (Biuret method), BUN (Alkaline picrate method) and direct and total bilirubin (modified Jendrasik and Grof method) with the help of commercial kit (Span diagnostic kit, Span Diagnostic Limited, Surat, India).

Serum enzyme profile viz. serum asparate aminotransaminase (AST/SGOT) and Creatine kinase MB (CK MB) (IFCC 1989) was measured by standard diagnostic kit.

Therapeutic remedy

All the positive cases were treated with oxytetracycline @ 22 mg/kg body weight intravenously for 5 days with Diaminazene aceturate @3.5 mg/kg body weight as a single dose intramuscularly. Dextrose normal saline @400 ml intravenously for 5 days, Vitamin B complex @ 10 ml IM for 7 days every alternate day and Iron dextran @ 10 ml intramuscularly every alternate days for 10 days were given as supportive therapy. Criterias for evaluating the efficacy of treatment were return from clinical illness after clinical remission viz. normal temperature, loss of parasitemia and improvement of haematobiochemical parameters on day 7 post treatments.

Statistical analysis

The results obtained are expressed as mean \pm SD. Anova test was used to compare the means of the groups. Statements of statistical significance are based on $P < 0.05$.

Results

The present study showed the overall prevalence of *Theileria spp.* was 9.09 % (5/55) in crossbred cattle of Lushai hill district of Mizoram. Clinical features of the disease



Fig. 1 Enlarged superficial lymph node

were manifested by high rise of body temperature 104.8 ± 1.2 °F, enlarged superficial lymph nodes (Fig. 1), inability to walk, anorexia, listlessness, moderate anaemia, nasal discharge (Fig. 2), weight loss and emaciation (Fig. 3) edema of eye, corneal opacity and ticks were found on various parts of the body. Blood sucking ticks were collected from different parts of the body and were identified as *Hyalomma* spp.

Examination of Giemsa- stained blood films from animals with clinical theileriosis revealed the presence of macroschizonts (Fig. 4) or koch blue bodies (Fig. 5) in lymph node aspiration smear.

Significantly decreased ($P < 0.05$) haemoglobin concentration, packed cell volume, and total erythrocyte counts, were observed in infected cattle as compared to healthy (Table 1). There was significant decrease ($P < 0.05$) in total leucocytes count in infected animals compared to healthy. In differential leukocytic count, neutrophils count was significantly reduce ($P < 0.05$) in infected animal while lymphocyte count was significantly higher ($P < 0.05$) in theileriosis infected cattle as compared to healthy. There was, however, no significant difference in absolute basophile, eosinophil and monocyte counts between healthy and infected cattle. Regarding ESR value, it was significantly higher ($P < 0.05$) in infected animals. The level was significantly reduce after treatment as compared with infected animal (before treatment) but significantly increase ($P < 0.05$) as compared with healthy. There was no significant difference of MCV and MCH in infected group and healthy animals while MCHC was significantly decreased ($P < 0.01$) in infected animals. In infected cattle, serum total proteins, albumins and globulins concentrations were significantly decreased ($P < 0.05$) when compared with healthy. Serum direct and total bilirubin concentrations, BUN, SGOT and CK MB levels were



Fig. 2 Nasal discharge



Fig. 3 Emaciation of the cattle

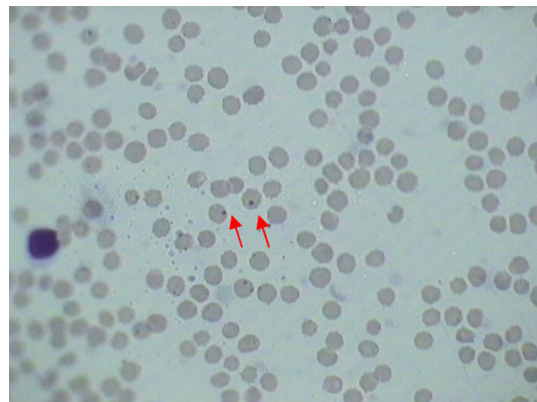


Fig. 4 Blood smear showed *T. annulata* organism

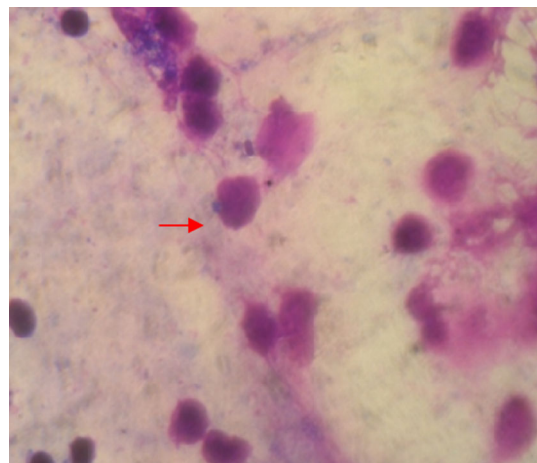


Fig. 5 Lymph node smear slides showing the macroschizont (also known as Koch's blue body) stage of *Theileria annulata*

significantly increase ($P < 0.05$) in theileriasis infected animals as compared with healthy.

The animals responded to treatment within 7 days. Improvement in attitude and appetite was noted and rectal temperature returned to within the normal range. Recovery continued, and on day 7, the cattle became bright, more

Table 1 Hematological value of theileriosis affected cattle (before and after therapy)

S. no.	Parameters	0 day (BT)	7 days (AT)	Healthy
1	Hb (g/dl)	5.28 ± 1.81 ^a	8.67 ± 1.04 ^b	9.78 ± 0.98 ^b
2	PCV (%)	18.10 ± 0.8 ^a	27.12 ± 0.98 ^b	29.45 ± 0.07 ^b
3	RBC (×10 ⁶ /ul)	3.04 ± 0.7 ^a	4.08 ± 0.91 ^b	4.67 ± 0.96 ^b
4	TLC (×10 ³ /ul)	4.95 ± 1.05 ^a	7.56 ± 1.08 ^b	8.67 ± 0.05 ^b
5	N (%)	50.44 ± 2.77 ^a	57.34 ± 1.34 ^b	65.00 ± 0.06 ^b
6	L (%)	41.00 ± 0.04 ^a	35.56 ± 1.34 ^b	28.69 ± 1.45 ^b
7	M (%)	6.00 ± 0.07	5.89 ± 0.67	4.75 ± 0.08
8	E (%)	2.56 ± 0.06	1.21 ± 0.56	1.56 ± 0.07
9	MCH (pg)	24.00 ± 1.06	22.00 ± 1.05	15.48 ± 0.06
10	MCV (fl)	59.53 ± 1.11	66.47 ± 1.34	63.06 ± 1.01
11	MCHC (g/dl)	29.17.00 ± 1.02 ^a	31.96 ± 0.87 ^b	33.20 ± 0.07 ^b
12	ESR (mm/1st hr)	104.5 ± 12.4 ^a	53.09 ± 10.32 ^b	18.00 ± 8.96 ^c

Values are mean ± SE. Values in the different column with the different superscripts are significantly different at P < 0.05

Table 2 Serum biochemical value of theileriosis affected cattle (before and after therapy)

S. no.	Parameters	0 day	7 days	Healthy
1	Albumin (gm/dl)	2.48 ± 0.09 ^a	3.12 ± 0.08 ^{ab}	4.09 ± 0.98 ^b
2	T. Protein (gm/dl)	4.57 ± 1.02 ^a	6.23 ± 1.11 ^b	7.08 ± 0.67 ^b
3	Globulin (gm/dl)	2.09 ± 0.98	3.11 ± 0.93	2.99 ± 0.77
4	Ck-MB (u/l)	6579.34 ± 33.78 ^a	678.00 ± 23.10 ^b	78 ± 3.12 ^c
5	SGOT (I U/l)	58.00 ± 0.97 ^a	46.34 ± 0.07 ^a	29.78 ± 0.69 ^b
6	BUN (mg/dl)	34.30 ± 0.99 ^a	22.12 ± 1.23 ^{ab}	18.34 ± 1.11 ^b
7	Direct bilirubin (mg/dl)	0.234 ± 1.04 ^a	0.188 ± 1.07 ^b	0.175 ± 0.08 ^b
8	Total bilirubin (mg/dl)	0.396 ± 1.23 ^a	0.304 ± 0.98 ^a	0.187 ± 0.66 ^b

Values are mean ± SE. Values in the different column with the different superscripts are significantly different at P < 0.05

alert and eating. Blood samples showed no gametocytes and hematological and serum biochemical analysis indicated the improvement (Table 1) on 7th day after post treatment though some parameters were significantly increase viz ESR, SGOT, CKMB and total bilirubin in comparisons to healthy animal but non significantly decrease from day 0 (Tables 1 and 2).

Discussion

This is the first study in cattle to investigate theileriosis infections in Mizoram. It provides new information on the diversity, distribution, and extent of *Theileria* spp. infecting domestic crossbred cattle in Mizoram. Furthermore, we report infection with *T. annulata* in cattle for the first time in Mizoram with the prevalence of 9.09 % though the incidence of theileriosis in cattle have been earlier reported from various part of India, (Aparna et al. 2011, Sumathi and Veena 2012, Kohli et al. 2014).

The observed clinical findings in crossbred cattle with theileriosis such as anorexia, enlarged superficial lymph

nodes, and corneal opacity were in agreement of El Deeb and Younis (2009) and Sudan et al. (2012). Anorexia could be attributed to persistent fever; moreover the enlargement of superficial lymph nodes could be explained by lymphoid hyperplasia in early stage of the disease. The corneal opacity was explained by Irvin and Mawamachi (1983) as a result of white blood cells infiltration.

In the present study, total erythrocyte counts, packed cell volume percentage and hemoglobin concentrations were significantly lower in infected animals as compared with healthy cattle. Such findings have already been reported by Col and Uslu (2006) and Hasanpour et al. (2008). The decrease in erythrocyte count, packed cell volume and hemoglobin concentration resulted anemia in infected group. Immune-mediated mechanism like erythrophagocytosis might be responsible for the destruction of erythrocytes infected with theileria schizonts (Uilenberg 1981). The normocytic hypochromic anemia observed in cattle with theileriosis (Table 1) could be *Theileria* sp attributed to the toxic metabolites of which has harmful effect on bone marrow as they interfere with the process of erythropoiesis. Leucogram showed significant decrease

($P < 0.05$) in total leucocytic count and neutrophils while the lymphocytes showed significant increase ($P < 0.05$) in comparison with healthy control ones. Such changes in leucogram might be attributed to persistent harmful effects of *Theileria* on the haemopoietic organs especially bone marrow and their interference with the process of leucogenesis and due to proliferation of lymphocytes in the lymphoid organs as defensive response to invading parasite. Relative increase in numbers of lymphocytes and monocytes reflects compensatory mechanism as target cells in response to their invasion with *Theileria*. Similar results were observed in infected cattle by Lamiaa Abo-EL-Hasan (1997). Erythrocytic sedimentation rate (ESR) was significantly increased ($P < 0.05$) in infected animals which is claimed to be due to changes in the plasma protein and insufficient compensatory haemopoietic capacity of the infected animals (Smith and John 1970).

In the current work, it showed that, the mean values of blood serum total and direct bilirubin, SGOT, and BUN revealed significant elevation in *Theileria* infected cross breed (Table 2). In contrary, total serum proteins and albumin showed significant decrease in mean value of infected cross breed. These significant changes probably indicate inflammatory changes in hepatic and glomerular cells which in turn affected their functions. These results are in agreement with Sandhu et al. (1998) and Singh et al. (2001). Significant increase CKMB level of infected animals might be due to severity of anemia and parasitemia which contributed to the pathophysiology of myocardial damage. Similar finding also reported by Fartashvand et al. (2013).

In the present study, It was found that the combination therapy of oxytetracycline with Diaminazene aceturate was effective against theileriosis. Buparvaquone @ 2.5 mg/kg IM as a single dose is a drug of choice for the treatment of theileriosis in cattle (Salama and Gaabarya 2007) but it is very costly for individual treatment of calves and not generally available in Mizoram. Keeping this fact in view, combination therapy of oxytetracycline @22 mg/kg IV daily for five days and Diaminazene aceturate @3.5 mg/kg IM on as a single dose along with supportive therapy was instituted in theileriosis affected crossbred cattle. Combined treatment of oxytetracycline and Diaminazene aceturate has been reported to be effective for the treatment of *Tropical Theileriosis* by earlier workers (Dey 2000; Mehesare S et al. 2012).

Conclusion

Tropical theileriosis an upcoming/emerging issue in *Lushai hill district of Mizoram* especially among cross breed cattle and had effect on hepatic, renal and cardiac function. Based

on the observations, it can be concluded that severe *T. annulata* infection is associated with profound changes in hematological and biochemical profiles and cardiac enzyme parameters and Oxytetracycline with Diaminazene acetate has the potential to manage *T. annulata* infection of cattle.

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