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Case Report

The Molecular Detection and Therapeutic Management of Pathogenic *Theileria luwenshuni* Infection in a Goat: A Case Report

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Abstract

A 2-year-old female Assam Hill goat was presented with a clinical history of anorexia, fever, mild anemia, rough body coat, dehydration, tachycardia, dyspnea and swelling of palpable lymph nodes. Hematology revealed low hemoglobin, packed cell volume, red blood cell and thrombocyte count. Biochemical analysis showed increased serum concentration of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea in comparison to the normal reference range. Microscopic examination showed intra-erythrocytic forms of Theileria species. Molecular and phylogenetic analysis of partial 18S rRNA gene sequence confirmed Theileria luwenshuni infection. The goat was treated with buparvaquone and oxytetracycline and recovered uneventfully. A three-month follow-up showed no recurrence. This study reveals the presence of T. luwenshuni in Assam, India and it should be considered in differential diagnosis and as one of the important pathogens of clinically sick goats. The present case report provides a rational approach to diagnosis and treatment for a goat infected with pathogenic T. luwenshuni in Assam, India. To our knowledge, the present communication describes about the first successful therapeutic management of pathogenic T. luwenshuni infection in a goat supported with molecular evidence from Assam, a north-eastern state of India.

Introduction

Small ruminant theileriosis is a tick-borne hemoprotozoan disease transmitted by the ixodid ticks of the genus *Rhipicephalus* and *Hyalomma* species. The disease is caused by various spp. of *Theileria* i.e., *T.lestoquardi, T. uilenbergi* and *T. luwenshuni* which are highly pathogenic whereas *T.ovis, T. seperata* and *T. recondite* are less pathogenic (1). The morbidity rate of pathogenic *T. luwenshuni* infections in small ruminants varies between 18.8% and 65%, the mortality between 17.8% and 75.4% (2).

The disease is economically important and considered one of the major threats to the animal production sector (3), especially in a developing nation like India, where it is acknowledged as "poor man's cow" for serving as source of income to landless or marginal farmers. The hot and humid climatic conditions of India serve as an excellent conducive environment for the propagation of its vector. The infection can be acute, subacute or chronic.

Classic predominate signs include anemia, fever, anorexia, lymphadenopathy, dyspnea and death (3,4). Diagnosis can be achieved by direct microscopy of the blood smear, serology and molecular assay (4). In 2019, only a single study on caprine theileriosis was reported from Assam, India (5). However, till now, there is no reliable report about successful therapeutic management for caprine malignant theileriosis supported by various diagnostics at field level from this part of the country.

The present case report provides a rational approach to achieve diagnosis and treatment of pathogenic *T. luwenshuni* infection in a goat from Assam, India.

Case Description

A 2-year-old female Assam Hill goat weighing about 18 kg was attended at the farmer's door step from Hajo (26°14'55"N 91°31'32"E), a rural village located in the lower Brahmaputra valley region of Assam, India. The complaints of the farmer include anorexia, prostration and lethargy. On anamnesis, it was also communicated that two other goats that died last week also showed similar symptoms and were being treated by local veterinarians. Routine clinical examination revealed depression, pyrexia (105.2 °F), rough body coat, slightly pale mucus membrane, dehydration, tachycardia, dyspnea, along with mild swelling of palpable lymph nodes. No evidence of ticks or flea was visible on gross clinical inspection. A spot blood smear was prepared by pricking the marginal ear vein to obtain a drop of blood. Additionally, around 2ml of blood in EDTA and 3ml in clot activator tubes were collected by the venipuncture of the jugular vein for hemato-biochemical and molecular tests. Prepared blood smear was stained by Giemsa's stain and examined under an oil immersion objective (100X) of the compound microscope (Advanced Binocular Labomed®) for the detection of parasites within the red blood cells and lymphocytes. The parasites were identified on the basis of their characteristic morphology (6).

The PCR assay was conducted as per the previously described method for identification of *Theileria* sp. by a genus specific primer (7). DNA extraction was carried out from collected blood sample using DNeasy Blood (Quiagen® Kit). Extracted DNA's were subjected to PCR reaction in order to get a product size of 1098 bp using nucleotide sequence 989 5'-AGTTTCTGACCTATCAG-3' as a forward primer and 990 5'-TTGCCTTAAACTTCCTTG-3' as a reverse primer. The PCR was performed in reaction volume of 25µl PCR mixture comprising of 5 µl DNA template, 12.5 µl DyNAzyme II PCR mixture, 5.5µl Nuclease free water and 1 µl (10 pmol) of each *Theileria* specific primer. The reaction was performed with 30 cycles each at 94ºC for 1 minute for denaturation, 56

ºC for 1 minute for annealing and 72 ºC for 1 min for final extension and holding at 4 ºC in a semi-automated thermal cycler (TC-5000; Bibby Scientific, Burlington, USA).

The positive control for *Theileria* sp was provided by the Department of Veterinary Parasitology, College of Veterinary Science, Khanapara, Guwahati, India. Distilled water was used as a negative control. The PCR product was subjected to agarose gel electrophoresis followed by visualization in the gel documentation system. *Theileria* spp. positive PCR product was gel purified by NucleoSpin® Gel and PCR Clean-up Kit as per the manufacturer's instruction and was outsourced for sequencing (1st Base, Malaysia). The consensus partial 18S rRNA gene sequence was submitted in the GenBank database of the National Center for Biotechnology Information.

Hematological and biochemical assessments were performed by an automated animal blood cell counter (ProCyte Dx Analyser; IDEXX Laboratories, Shanghai 200336, China) and a semi-automated biochemical analyzer (C-61; Benesphera®, Avantor, Mumbai, India) respectively.

The study was approved by the Institutional Animal Ethics Committee, College of Veterinary Science, Assam, India (770/ac/CPCSEA/FVSc/AAU/IAEC/16-17/402).

Results

Microscopic examination revealed the presence of intra-erythrocytic pleomorohic forms of *Theileria* species, occurring as a single entity measuring 4.00–5.00 µm, under different microscopic fields as shown in Fig. 1. Therefore, the case was diagnosed as caprine theileriosis. Hematology revealed low hemoglobin, PCV, RBC and thrombocyte counts. Additionally, increased nucleated RBCs (four per 100 white blood cells) and immature neutrophils were also noticed. Further, biochemical analyses indicated an increase in the concentration of hepatic enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Moreover, kidney function tests revealed azotemia along with elevated creatinine and blood urea nitrogen (BUN) values (Table 1).

Table 1: The hemato-biochemical parameters measured in the study

Parameters	*Patient values*	*Reference interval (15,16)*
WBC (× 10^3 µL-1)	21.71	4.00-14.00
PCV (%)	18.29	22.00-39.00
RBC (× 10^6 µL-1)	6.71	8.00-18.00
Hemoglobin (g dL-1)	6.43	8.00-12.00
Thrombocyte (× 10^3 µL-1)	110.00	200-600
AST (U/L)	43.07	164.00-174.
ALT (U/L)	214.51	18.00-22.00
BUN (mg dL-1)	20.62	25.00-60.00
Creatinine (mg dL-1)	2.32	1.02-1.90

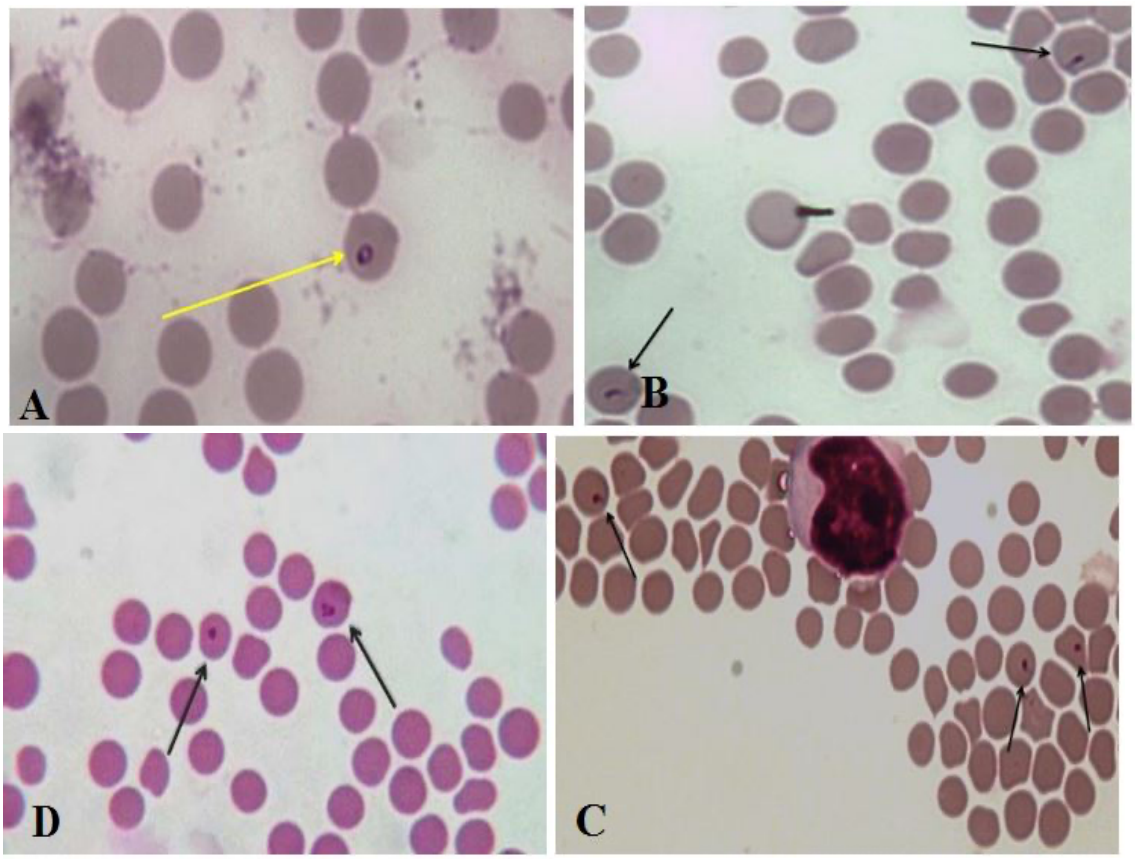

Fig. 1: Giemsa-stained blood smear from a 2-year-old female Assam hill goat showing intra-erythrocytic pleomorphic piroplasm of *Theileria* spp. A) Ring form; B) Nail Form; C) Comma-shaped; D) Dot shaped. (100X, arrows)

The molecular testing of the sample confirmed infection with *Theileria* spp. Meanwhile, no PCR amplification products were seen in a negative control (Fig. 2). By analyzing the sequence data, a partial 18S *r*RNA gene sequence of *Theileria* sp. was obtained, which was submitted to GenBank under the accession number MG694565. The BLASTn result indicated that the submitted gene sequence has 99.90% identity with *T. luwenshuni* (NCBI GenBank Accession No. MH20863.1) reported from Yunnan province of China. Further the phylogenetic tree constructed with MEGA X also revealed similar result as the *T. luwenshuni* of Assam was nearest to Chinese strain (MH20863) within the clad (Fig. 3). Treatment was initiated with a single intramuscular injection of buparvaquone (Intervet Inc. MSD Animal Health Co., India; 2.50 mg kg-1) in combination with oxytetracycline injection (Zydus Cadila Vet. Co., India; 5.00 mg kg-1,q12) intravenously for 5 days. Furthermore, Normal Saline Solution (NSS) (Infutec Healthcare Co., India; 30 mg kg-1, q12) was administered for the next three days, along with a single dose of meloxicam (Excellar Healthcare Pvt. Ltd., India; 0.30 mg kg-1) which was injected subcutaneously for pyrexia. An oral multivitamin preparation containing haematinics was also advised for 1 month (Flanca Lifesiences Co., India; 5.00 mL, od). The clinical signs improved after four days and a 3 month follow-up study showed no recurrence. Three months after the first diagnosis, blood smear and PCR were also negative for the parasite.

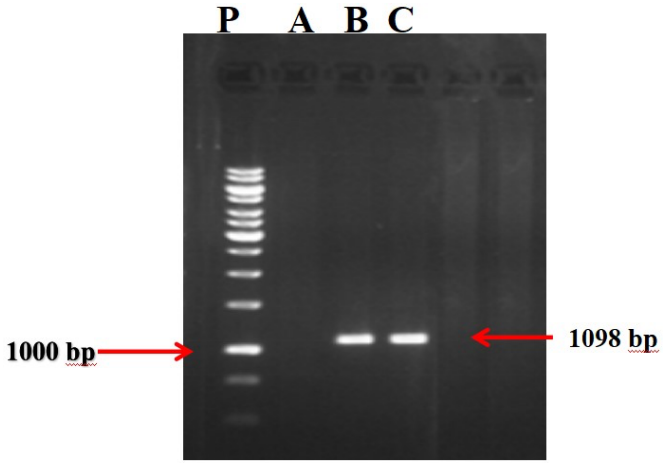

Fig. 2: Agarose gel electrophoresis of 1098 bp fragments of *Theileria spp* DNA
Lane P: 1 Kb DNA ladder; Lane A: Negative control; Lane B: Positive control;
Lane C: PCR product of *Theileria spp* sample

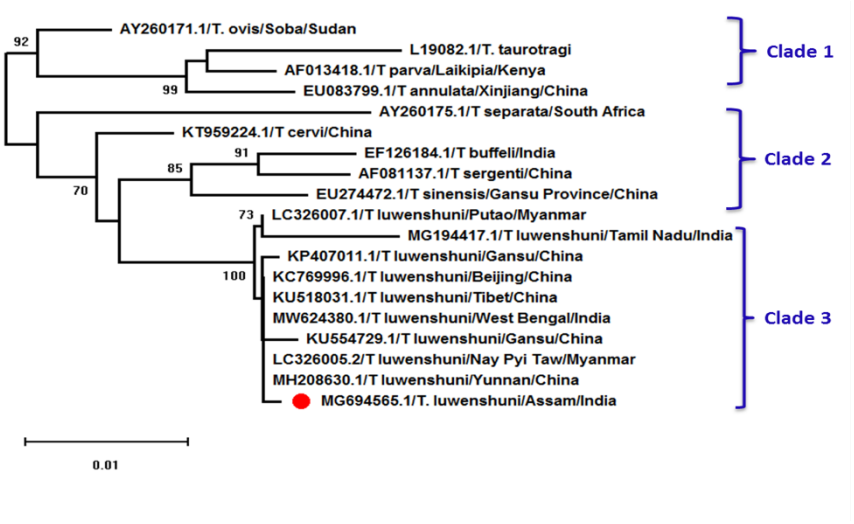

Fig. 3: Phylogenetic tree of *Theileria spp* based on partial 18S rRNA gene sequences. The tree was constructed in MEGA X software by Neighbor-Joining (NJ) method. The tree exhibited three clades based on 18S rRNA gene sequence of different. The 18S rRNA gene sequence obtained from a *Theileria* spp. of Assam formed a clade with *Theileria luwenshuni* (solid red circular marker) which is close to a Chinese strain (MH208630.1) that was reported from Yunnan province of China

Discussion

This study represents the rare case report of *T. luwenshuni* infection in an indigenous goat from Assam, a north-eastern state of India and provides a description of the clinical case at the farmer's doorstep along with morphological and molecular supportive evidence followed by treatment. Previously, only a single study from this region have been conducted (5). The present study, however, offers a more detailed and clinically relevant analysis.

The clinical manifestations and alteration in hemato-biochemical profile of the affected goat were consistent with the previous studies

(8,9). Reduced values of hemoglobin, PCV, RBC and thrombocytes in the present case were suggestive of anemia which is a common feature of this infection (9). The occurrence of anemia in theileria infected animals is due to the pronounced phagocytic activity of macrophages in removing the infected erythrocytes and platelets (8,10). Tachycardia and dyspnea recorded during clinical inspection was due to intravascular hemolysis leading to anemia (11). The increase WBC count in this study might be due to increased proliferation of lymphocytes during the course of disease as a part of the cellular defense mechanism. Similar findings were recorded earlier (8,9,12).

The present case also recorded an elevation of AST and ALT values, which is indicative of hepatic injury caused by T. *luwenshuni*. This elevation of hepatic enzymes is related to the overfunctioning of the liver for compensating the ongoing hemolytic anemia for conjugation of glucoronic acid. The kidney function test showed an increase in BUN and creatinine values greater than reference range, which might be due to the invasion of cells parasitized by the Theileria schizont in the different tissues. The distribution of these schizont-infected cells plays a crucial role in mediating damage to the renal parenchyma, subsequently leading to the leakage of biomarkers into the serum. Also, increased protein catabolism due to anorexia may contribute to the rising BUN value. Similar findings were opined by other authors (8,12).

In field conditions, diagnosing and treating caprine theileriosis is very difficult because of the non-specific clinical signs exhibited by the affected goat. Hence, the examination of stained blood smears can serve as a quick diagnostic test for the start of curative treatment without delay. However, to avoid a false negative result, on account of low parasitemia, the sample must be tested via PCR which is more sensitive (5,13,14). In the present case, a combination of buparvaquone and oxytetracycline was prescribed to treat T. *luwenshuni* infection. Buparvaquone is very effective in clearing *Theileria* parasites from goats. Oxytetracycline used along with buparvaquone is effective against the schizon of stage *Theileria* and showed a synergistic effect (17). In order to correct the dehydration status of the infected goat, judicious fluid therapy was employed. To enhance immunity and to improve the anemic status of the patient, oral multivitamin preparation with haematinic was also advised as supportive medication.

In conclusion, the present study provides the first successful therapeutic management of pathogenic *T. luwenshuni* infection in a goat from Assam, India. This is the first clinical case study on naturally occurring malignant caprine theileriosis supported with clinical presentation, hemato-biochemical and molecular findings from this region. The treatment regimen combining buparvaquone and oxytetracycline, along with supportive care, resulted in the uneventful recovery of the goat with no recurrence observed over a three-month follow-up period.

Conclusion

These findings underscore the importance of considering *T. luwenshuni* in the differential diagnosis of anemic and febrile conditions in goats. The successful management of this case highlights the need for prompt diagnosis, increase awareness, diagnostic capabilities and effective treatment strategies to mitigate the impact of this pathogenic species, subsequently improving animal health and productivity in developing regions. Further studies are warranted to explore the epidemiology, vector dynamics, and preventive measures to control the spread of *T. luwenshuni* in goat populations.

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Conflict of interest

The authors declare no probable conflicts of interest.

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