



Assessment of haemato-biochemical alterations in neurological form of *Theileria luwenshuni* infection in goats

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Abstract

The cerebral form of theileriosis with nystagmus, opisthotonus and circling as the predominant clinical signs, is rarely reported in small ruminants. Fifteen goats with many or all of the following neurological signs like circling, head pressing, ataxia, incoordination, staggering, tremors, muscle twitching, seizures, blindness, opisthotonus, paralysis, bruxism, abnormal posture, reduced or absence of reflexes were selected for the study. Presence of merozoites of T. luwenshuni was confirmed in the blood of six (40%) of the animals by blood smear examination and PCR targeting 18SrRNA gene. Statistically significant alterations were not detected in any of the biochemical parameters assessed, but moderate reduction in serum levels of magnesium, copper and zinc were noticed.

Keywords: *Theileria luwenshuni*, goats, haemato-biochemical alterations

Neurological diseases are an important part of small ruminant medicine, but studies dealing with their aetiopathology are limited. Probable aetiologies for neurological disorders in goats are manifold, such as viral, bacterial, helminth and protozoan infections, as well as nutritional deficiency. Additionally, many neurological signs could be secondary manifestations of diseases other than of neurological origin. A better understanding of the exact aetiology of neurological diseases of the goats in our locality is essential for developing an effective therapeutic protocol, but is yet to be elucidated.

Sheep and goats are susceptible to infection by six species of *Theileria*. Among these, *Theileria lestoquardi*, *Theileria luwenshuni*, and *Theileria uilenbergi* are considered highly pathogenic (Taha et al., 2013). The cerebral form of theileriosis is very rare in small ruminants. Razmi et al. (2019) reported neurological signs like nystagmus, opisthotonus and circling in sheep which was experimentally infected with *Theileria lestoquardi*. Abimanyu et al. (2024) documented a rare case of cerebral theileriosis in a two-year-old non-descript goat with concurrent gastrointestinal parasitism, which exhibited atypical clinical signs including anorexia, labored breathing, an arched and extended neck, unidirectional head turning, severe respiratory distress, and blindness.

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This study was designed to investigate the role of *T. luwenshuni* as an aetiology for caprine neurological signs, and determine the hematobiochemical variations among the affected animals.

Materials and methods

Study samples

Fifteen goats presented to the Teaching Veterinary Clinical Complex (TVCC), Mannuthy and University Veterinary Hospital (UVH), Kokkalai with neurological signs formed the subjects of the study. Goats with many or all of the clinical signs such as depression, dullness, circling, head pressing, ataxia, incoordination, staggering, tremors, muscle twitching, seizures, blindness, opisthotonus, paralysis, bruxism, abnormal posture, reduced or absence of reflexes were selected and included in the study. Signalment, anamnesis, management practices, preliminary general examination as well as detailed clinical examinations were carried out in all the animals.

Thin peripheral blood smears were prepared from clinically suspected animals, air-dried, fixed in methanol and stained with Fields stain. Eight mL of blood was collected under aseptic conditions from the jugular vein of goats using a 21 G scalp vein and 10mL disposable syringe. Subsequently, two mL of blood was transferred to a sterile EDTA coated vial and three mL was transferred to each of two sterile clot activator vials. A complete blood count, including the following parameters such as haemoglobin (Hb), volume of packed red cells (VPRC), total leukocyte count (TLC), differential leucocyte count (DLC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelets was done using ORPHEE Mythic 18 Vet and ORPHEE Mythic 5 Vet PRO, Switzerland, automated haematology analysers within 2 hours of collecting the samples. Genomic DNA was extracted from blood samples using a commercially available genomic DNA kit (kit No.112124DGK, Origin Diagnostics & Research, Kerala). which contained the following reagents: proteinase K, absolute ethanol (96-100 percent), Buffer GA, Buffer GB, Buffer GD, Buffer PW and Buffer TE

Molecular identification of *Theileria* species

All the samples were subjected to PCR assay by

using species specific primers for identification of *Theileria luwenshuni*, (Yin et al., 2008), *Theileria lestoquardi/ovis* (Jalali et al., 2014) and *Theileria uilenbergi* (Yin et al., 2008).

The PCR amplification of *Theileria lestoquardi/ovis* was done using BIORAD thermal cycler, USA. PCR tubes were incubated at 94°C for 5 minutes to denature genomic DNA, followed by 30 cycles at 94°C for 30 seconds for DNA denaturation, 59°C for 1 minute for primer annealing, and 72°C for 1 minute for elongation. The PCR reaction was ended with a final extension step at 72°C for 5 minute. PCR amplification targeting *Theileria luwenshuni* was performed with initial denaturation at 94 °C for 3 minutes to ensure complete denaturation of the genomic DNA. This was followed by 35 amplification cycles consisting of denaturation at 94 °C for 30 seconds, primer annealing at 57 °C for 1 minute, and extension at 72 °C for 1 minute. The protocol was completed with a final extension step at 72 °C for 7 minutes to allow full elongation of all amplified products. To initiate the PCR amplification of *Theileria uilenbergi*, the reaction mix was subjected to an initial denaturation at 94 °C for 5 minutes. This step was followed by 30 cycles consisting of denaturation at 94 °C for 30 seconds, primer annealing at 59 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension at 72 °C for 5 minutes was included to ensure complete synthesis of the amplified products.

Nucleotide sequencing

Nucleotide sequence of the PCR amplicons was detected by Gene Spec, Ernakulam, India with both genus specific and species primers. Sequences were edited and aligned by using Emboss Merger Website and global sequence comparisons were performed by using Basic Local Alignment Search Tool (BLAST) hosted by the National Centre for Biotechnology Information (NCBI). New sequences were deposited in the Gen Bank database for procuring accession number

Haemato-biochemical analysis

Diagnostic kits to estimate serum levels of alanine amino transferase (IU/L), alkaline phosphatase (IU/L), aspartate amino transferase (IU/L, gamma glutamyl transferase (IU/L) , calcium (mg/dL), phosphorous (mg/dL), magnesium (mg/dL), were procured from Erba

Table 1. Primers and references used for molecular detection of different species of *Theileria* in goats

Sl.No.	Name of Gene	Primer	Amplicon size	References
1	<i>Theileria lestoquardi/ovis</i> 18S rRNA	For:GCATTCGTATTTAACTGTCAGAGG Rev:GATAAGGTTCCACAAAACCTCCCTAG	861	Jalali et al. (2014)
2	<i>Theileria luwenshuni</i> 18S rRNA	For: GGTAGGGTATTGGCCTACTGA Rev: TCATCCGGATAATACAAGT	389	Yin et al. (2008)
3	<i>Theileria uilenbergi</i> 18S rRNA	For: GGTAGGGTATTGGCCTACCGG Rev: ACACTCGGAAAATGCAAGCA	388	Yin et al. (2008)

technologies, Mumbai. These biochemical parameters were estimated in an advanced semi-automatic analyser (ERBA Chem-5 Plus v2 Mannheim, Germany).

Stored serum was thawed at 4°C and 500 µL serum was mixed with 4.5 ml acidified water (1% nitric acid) in a test tube and mixed well using a vortex. Minerals, copper and zinc of the mixture was obtained by flame atomic absorption spectrometry in PerkinElmer Atomic Absorption Spectrometer PinAAcle 900H.

Results and discussion

Theileria luwenshuni is considered to be a rare pathogen of goats, being reported sporadically from Karnataka (Mamatha et al., 2017), Assam (Begam et al., 2018) and Maharashtra (Dhaygude et al., 2020) and Kerala (Nagaraj et al., 2019). Out of the 15 goats screened, four (26.67%) revealed the presence of *Theileria* piroplasms and eleven animals had no evidence of hemoparasites in blood smears. All the 15 blood samples were subjected to species specific PCRs targeting *Theileria luwenshuni*. Among these six (40%) yielded amplicons of 389 bp, specific for *Theileria* genus. One representative sample was sent for sequencing to confirm identity. Among these, six (40%) yielded positive result, producing a specific amplicon at 389 bp. Out of the six samples positive by species-specific PCR for *Theileria luwenshuni*, one was selected for sequencing. The obtained sequence was analyzed using the Basic Local Alignment Search Tool (BLAST) under the NCBI database to assess sequence homology and was subsequently submitted to GenBank with the accession number. BLAST analysis revealed 100 per cent identity with existing *Theileria luwenshuni* sequences in GenBank, with accession number PX937222.

Paresis/recumbency, hyperaesthesia, nystagmus, tremors and head tilt were the most common clinical

manifestations. The common clinical signs reported have been pyrexia, inappetence, and progressive weight loss to enlargement of lymph nodes, respiratory distress (such as coughing, nasal discharge, and dyspnea), anemia, jaundice, and diarrhea. Razmi et al. (2019) reported neurological signs like nystagmus, opisthotonus and circling in sheep which was experimentally infected with *Theileria lestoquardi*. Labored breathing, an arched and extended neck, severe respiratory distress, and blindness, as reported by Abimanyu et al., (2024) was not seen in any of the animals. The major neurological signs observed in the affected goats were depression (80%), dullness (80%), paresis or recumbency (66.7%), hyperaesthesia (46.7%), bruxism (46.7%), nystagmus (40%), tremors (33.33%), and head tilt (33.33%), along with varying occurrences of head pressing, behavioural changes, blindness, circling, convulsions, and other neurologic abnormalities. The organism exerts its pathogenic mechanisms by transforming the affected lymphocytes into lymphoblasts, which migrate to various tissues, including the nervous tissue.

Haematological and serum biochemical parameters of *Theileria luwenshuni* infected goats were done by one sample t test. Lymphopaenia and monocytosis were observed in goats infected with *Theileria luwenshuni* and neurological manifestations (Table 2). Mahmoud et al. (2019) observed lymphopaenia in goats infected with *Theileria* spp and there was no significance difference noticed for monocytes. Manohar et al (2021) identified monocytosis in goats infected with *Theileria* spp similar to our findings.

Nithya et al. (2025) have reported hypomagnesaemia among goats affected with *T. luwenshuni* in the study area, which was not evident in our study, even though statistically insignificant reduction in the serum levels of magnesium, copper and zinc were evident (Table 3). This could be the first report of deficiency, albeit statistically insignificant, of multiple minerals being reported in ovine theileriosis. The pathologic mechanism by which *T. luwenshuni* could derange macro and micro mineral metabolism is yet to be elucidated. Denishan et al. (2017) have attributed the decreased serum copper and iron concentrations in *Theileria*-infected cattle to the inability of the damaged liver to synthesize ceruloplasmin and transferrin, respectively. The reasons of lower magnesium concentration are thought as increase in tubular resorption and loss of appetite due to gastrointestinal atony. Cintia et al (2015) has stated that decrease in food intake resulted by anorexia lead to impairment of carbohydrate metabolism is also thought to cause decrease in Cu levels in cattle with theileriosis.

Conclusion

Theileria luwenshuni was identified as a major and frequent pathogen causing neurological signs in



Fig 1. Amplicons of 18S rRNA gene of *Theileria luwenshuni*

Lane L: 100 bp DNA ladder
Lane 2,3 : positive samples
Lane 4: positive control
Lane 5: negative control

Table.2. Haematological parameters of *Theileria luwenshuni* infected with normal reference values

Variable	Minimum	Maximum	Mean±SE	Normal range	t-value (p-value) for comparison with	
					Lower limit	Upper limit
WBC (10 ³ /μL)	6	18.2	10.07± 2.03	4-13	2.99 ^{ns}	1.44 ^{ns}
					-0.98	-0.89
LYM %	19.1	55.8	44.81± 5.29	59-79	2.68**	0.45 ^{ns}
					-0.02	-0.99
MON %	3	34.1	13.26± 4.37	0-4	3.034 ^{ns}	2.12**
					-0.98	-0.04
NEUT %	15.5	69.28	41.68 ± 7.40	10-59	4.28 ^{ns}	2.34 ^{ns}
					-0.99	-0.97
EOS %	0.11	0.3	0.21± 0.03	0-10	6.53 ^{ns}	292.77 ^{ns}
					-0.99	-1
RBC (10 ⁶ /μL)	9.69	17.65	13.16± 1.25	8-18	4.13 ^{ns}	3.86 ^{ns}
					-0.99	-0.99
HGB (g/dL)	6	11.1	8.61± 0.80	8-14	0.77 ^{ns}	6.68 ^{ns}
					-0.76	-0.99
HCT%	15.2	52.2	25.00± 5.59	19-38	1.07 ^{ns}	2.32 ^{ns}
					-0.83	-0.96
PLT (10 ³ /μL)	153	490	315.33± 66.30	247-912	1.03 ^{ns}	8.99 ^{ns}
					-0.83	-0.99

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant

Table.3. Serumbiochemical parameters of *Theileria luwenshuni* infected goats with normal reference values

Variables	Minimum	Maximum	Mean	Normal range	t-value (p-value)	
					Lower limit	Upper limit
ALP (U/L)	14.38	189.6	56.15 ± 26.92	93-387	1.37 ^{ns} (0.11)	12.29 ^{ns} (1)
ALT (U/L)	9.72	52.16	29.52 ± 5.66	6-19	4.15 ^{ns} (-0.99)	1.86 ^{ns} (0.06)
AST(U/L)	64	470.1	159.39 ± 66.91	167-513	0.11 ^{ns} -0.46	5.28 ^{ns} -0.99
GGT U/L)	15.47	71.16	35.83 ± 8	24-64	1.48 ^{ns} -0.9	3.52 ^{ns} -0.99
Ca (mg/dL)	4.55	9.38	7.76 ± 0.74	8.9-11.7	0.300 ^{ns} -0.767	6.616 ^{ns} (<0.001)
P (mg/d L)	1.21	11.03	5.1± 1.56	4.2-9.1	1.53 ^{ns} -0.9	2.54 ^{ns} -0.99
Mg (mg/d L)	1.76	3.22	2.50 ± 0.25	2.8-3.6	0.59 ^{ns} (-0.71)	2.54 ^{ns} (-0.97)
Cu (mcg/d L)	30	304	134.66 ± 47.24	101.7- 203.4	1.19 ^{ns} -0.14	4.38 ^{ns} -0.99
Zn (mcg/d L)	11	138	83.83 ± 32.84	69.9 -130.0	0.70 ^{ns} (-0.74)	1.45 ^{ns} (-0.89)

** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant

goats even though reports designate it as a are pathogen. The prominent clinical signs among the affected animals included paresis/recumbency, hyperaesthesia, nystagmus, tremors and head tilt, which were also reported by others.. Deficiency of serum magnesium, copper and zinc, even

though statistically insignificant, was found to play a role in aggravation of clinical signs. The sample size was not large enough for determining whether the differences were significant or not.

Conflict of interest

The authors declare that they have no conflict of interest

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