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CASE REPORT ON PCR-BASED DIAGNOSIS OF CAPRINE THEILERIOSIS CAUSED BY *THEILERIA LUWENSHUNI* IN THE VETERINARY SURGEON'S DIVISION IN AMPARA, AMPARA DISTRICT, SRI LANKA

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SUMMARY: Theileriosis is a tick-borne disease of small ruminants that causes substantial economic losses in tropical and subtropical regions of Asia, Europe, Africa, and the Middle East. This case report describes the diagnosis and treatment of theileriosis in a goat herd in Sri Lanka. The outbreak occurred in a herd of 42 goats located in Ambagahawella Grama Niladhari Division within the Ampara Veterinary Surgeon's Range, Ampara District. The herd experienced seven deaths over a period of 2–3 weeks, preceded by anorexia, lethargy, and diarrhoea. Clinical examination of affected animals revealed pale conjunctivae, pyrexia, superficial lymphadenopathy, a dull hair coat, and severe tick infestation. Differential diagnoses included gastrointestinal parasitism and haemoparasitism. Faecal examinations of four clinically affected goats and six apparently healthy animals were negative for gastrointestinal parasites. However, microscopic evaluation of Leishman-stained thin blood smears revealed intra-erythrocytic stages consistent with *Theileria* spp. Whole blood samples were submitted to the Division of Parasitology, Veterinary Research Institute, Peradeniya, for molecular confirmation. PCR testing confirmed the presence of the pathogenic species *Theileria luwenshuni*, while *T. uilenbergi* and *T. lestoquardi* were not detected. Infected goats were treated with an anti-protozoal and an antibiotic with supportive therapy. Three of the four clinically affected goats responded to treatment and recovered, whereas one severely affected animal succumbed to the infection. This report highlights the emergence of *T. luwenshuni* infection in goats in Sri Lanka and emphasises the importance of early diagnosis, prompt treatment, and tick control for effective disease management.

BACKGROUND

Goat and sheep farming is a widely practiced livelihood activity among rural communities in Sri Lanka, which has an untapped potential to contribute significantly to income generation and nutritional security (Anuththara and Weerathilake, 2021). The national goat and sheep population has shown a gradual increase over the past few years, reaching approximately 0.37 million in 2022, followed by a slight decline in the following year (Department of Census and Statistics, 2024). Apparently, nearly 65% of the country's small ruminant population is concentrated in the dry and intermediate zones (Department of Census and Statistics, 2024), where the availability of nutritionally rich fodder resources and grazing land is relatively high. In Sri Lanka, goat production is primarily aimed at meat production while milk, skins, and manure serve as valuable by-products that enhance the economic sustainability of the sector (Anuththara and Weerathilake, 2021).

Theileriosis is an important tick-borne disease of small ruminants caused by protozoan parasites belonging to the family *Theileriidae*, transmitted by ixodid ticks (Metwally *et al*., 2021). The disease is endemic in tropical and subtropical regions of Asia, Africa, the Middle East, and parts of Europe (Shruthi *et al*., 2017). To date, six *Theileria* species have been identified in goats and sheep, of which *T. lestoquardi*, *T. luwenshuni*, and *T. uilenbergi* are regarded as pathogenic. Among these, *T. lestoquardi* is the etiological agent of malignant theileriosis in small ruminants, a condition associated with high morbidity and mortality rates (Bijan Esmaeilnejad *et al*., 2025). The clinical manifestations typically include pyrexia, lymphadenopathy, anaemia, respiratory distress, progressive emaciation, and in severe cases, jaundice. *T. luwenshuni* and *T. uilenbergi* are also recognised as major causative agents of small ruminant theileriosis in various parts of the world, with affected animals exhibiting fever, anaemia, jaundice, growth retardation, and reduced productivity (Lu *et al.*, 2015). In Sri Lanka, *T. luwenshuni* has recently been reported in clinically asymptomatic sheep from the Jaffna District (Sandamali *et al.*, 2025).

Accurate and early diagnosis of theileriosis is crucial for initiating timely treatment and reducing associated economic losses. Diagnosis is often based

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on clinical findings and supported by laboratory investigations. The conventional diagnostic approach involves microscopic examination of Giemsa or Leishman stained thin blood smears to identify intra-erythrocytic piroplasms of *Theileria* spp. (Criado-Fornelio, 2004). Although microscopy is a simple and cost-effective method, its sensitivity is limited, particularly in chronic and subclinical infections where parasitemia is low (Nagaraj *et al*., 2019). Furthermore, differentiation between *Theileria* species is not possible using microscopy alone (Lu *et al*., 2015).

Molecular diagnostic techniques, particularly polymerase chain reaction (PCR), have gained prominence for the detection and identification of *Theileria* at the species level in small ruminants. These methods are highly sensitive and specific, allowing detection of infections with low parasitemia and identification of emerging pathogenic species (Abdelsalam *et al*., 2023).

This case report describes the PCR-based diagnosis of caprine theileriosis in a goat herd within the Ampara Veterinary Surgeon's Division, Sri Lanka. The report further outlines the treatment protocol applied, and discusses the recommended farm-level management and preventive measures for the effective control of theileriosis in goats.

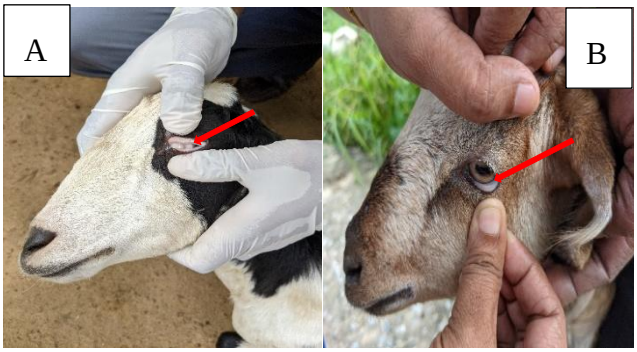
CASE PRESENTATION

A goat farmer from Ambagahawella Grama Niladhari Division in Ampara Veterinary Surgeon's Division of Ampara District, Sri Lanka, reported recent deaths of seven animals in his herd. The affected animals showed loss of appetite and inactivity for a period of two to three weeks prior to death, and another four animals are currently exhibiting similar signs. The farm was promptly visited to assess the condition of the farm and the status of the affected and apparently healthy goats.

All the animals were semi-intensively managed in stilted houses. A variety of clinical signs were observed upon thorough clinical examination of the four reported sick goats, as listed in Table 1. Pale conjunctiva (Figure 1A and Figure 1 B 2), pyrexia, tachycardia, tachypnea, dull coat and lethargy (Figure 2) were observed in sick animals with varying severity. Moreover, the history of deworming and ectoparasite control was obtained through questioning the farmer. The herd had been treated with an anthelmintic for gastroenteric parasite control within three months prior, as reported by the goat farmer. Differential diagnoses were considered including haemoparasitism and gastrointestinal parasitism, with particular attention to *Haemonchus contortus* infection based on the available information and observed clinical signs.

Table 1. Signalment and clinical signs of the affected goats

Animal No	Age	Sex	Breed	Clinical signs
01	6 months	F	Cross bred	Pale conjunctiva (Figure 1A), tachycardia (89-92 bpm) and fever (104.2 ºF)
02	18 months	F	Cross bred	Slightly increased respiratory rate (38 bpm), pale conjunctiva (Figure 1B) and tachycardia (90-95 bpm)
03	6 months	M	Cross bred	Pale conjunctiva, tachycardia (85-90 bpm), fever (104.7 ºF) and lethargy (Figure 2)
04	24 months	F	Cross bred	Severe pale conjunctiva, tachycardia (90-93 bpm), fever (104.0 ºF) and enlarged lymph nodes



Figures 1A and 1B: Pale conjunctivae in affected goats

Figure 2: A lethargic goat

Samples were collected from both symptomatic and asymptomatic goats to ensure diagnostic confirmation. A total of ten animals were sampled, which included four symptomatic and six asymptomatic goats. Three milliliters of whole blood was aseptically collected from the jugular vein into 5 ml EDTA tubes from each goat for laboratory examination of haemoparasites. In addition, dung

samples were collected directly from the rectum to assess gastrointestinal parasitic infections. All samples were carefully labelled with the respective animal identification number and date of collection. The additional information, such as age, breed, sex and clinical signs was recorded on a supplementary data sheet at the time of sample collection. All samples were placed in an ice box at 4 °C and immediately dispatched to the Veterinary Investigation Centre, Ampara. Dung samples were examined for the detection of parasitic eggs using the simple salt floatation method. However, parasitological examination of dung samples revealed no evidence of gastrointestinal parasitic infections. Then thin blood smears were prepared and stained using Leishman's stain and examined under ×1000 magnification to detect haemoparasites. Microscopic examination of thin blood smears revealed an anaemic blood picture in all animals with varying degrees of severity. The blood pictures of clinically affected goats were anaemic and positive for the presence of intracellular parasitic stages (Figure 3), suggestive of *Theileria* species.

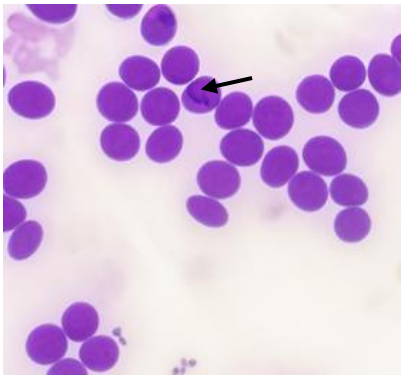


Figure 3: A blood smear stained with Leishman's stain illustrating intra-erythrocytic stages (arrow)
suggestive of *Theileria* species (x1000)

Ten whole blood Samples were transported in an ice box at 4°C to the Division of Parasitology, Veterinary Research Institute, Peradeniya, for further confirmation of *Theileria* species by Polymerase Chain Reaction (PCR). Parasitic DNA was extracted from blood samples using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Extracted DNA was quantified and stored at -20°C until further use. PCR amplification was performed using species-specific primers for *Theileria lestoquardi*, *Theileria luwenshuni* and *Theileria uilenbergi.* Conventional PCR was performed in a 25 µl reaction mixture containing 12.5µl of 2×PCR Taq Mixture (HIMEDIA, India), 1 µl of each forward and reverse primer (10 µM), 4 µl of template DNA and 6.5 µl of molecular grade water following the manufacturer's instructions. The PCR assay for *Theileria lestoquardi* targeted 30 kDa MSP gene and was performed as described by (Kirvar *et al*., 1998), while PCR assays for *Theileria luwenshuni* and *Theileria uilenbergi* targeted 18S rRNA gene as described by (Yin *et al*., 2008). The primer sequences, amplicon size and the amplification profile for each PCR assay are summarised in Table 2. The PCR products (8 µL) were loaded into 1% agarose gel containing ethidium bromide, and the bands were visualised under UV light after electrophoresis for 50 minutes at 100 V.

PCR analysis revealed that all the samples were negative for *Theileria lestoquardi* and *Theileria uilenbergi.* In contrast, six out of ten samples tested were positive for *Theileria luwenshuni,* demonstrating amplified bands of approximately 389 bp as illustrated in Figure 4. All four clinically affected goats were positive for *Theileria luwenshuni* by PCR along with two asymptomatic goats.

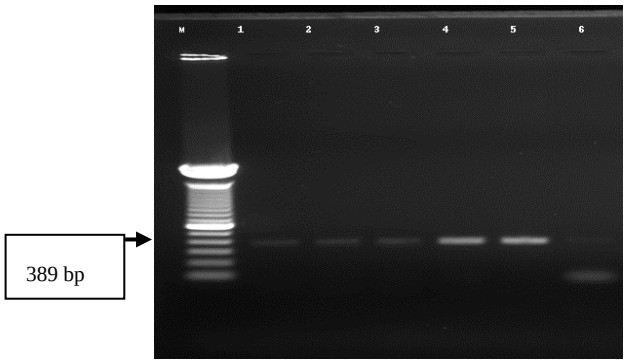


Figure 4: Gel electrophoresis of amplified 18S rRNA gene of *Theileria luwenshuni* (approximately at 389 bp, arrow) (1% Agarose gel stained with ethidium bromide). Lane M-100bp DNA Ladder, Lanes 1 – 4: samples from clinically affected goats, Lanes 5 – 6: samples from asymptomatic goats

Table 2: Primer sequences, amplicon size and the amplification profile for each PCR assay (Kirvar *et al*., 1998 and Yin *et al*., 2008)

Theileria **Species**	**Primer Sequence**	**Amplicon Size (bp)**	**Amplification profile**
Theileria lestoquardi	F 5'-GTGCCGCAAGTGAGTCA-3'	785	3 min at 94°C, and then 40 cycles with each cycle consisting of 30 sec at 94°C, 30 sec at 65°C, and 2 min at 72°C. Final extension for 5 min at 72°C.
	R 5'-GGACTGATGAGAAGACGATGAG-3'		
Theileria luwenshuni	F 5'-GGTAGGGTATTGGCCTACTGA-3'	389	3 min at 94°C, and then 35 cycles with each cycle consisting of 30sec at 94°C, 1 min at 57°C, and 1 minute at 72°C. Final extension for 7 min at 72°C.
	R 5'-TCATCCGGATAATACAAGT-3'		
Theileria uilenbergi	F 5'-GGTAGGGTATTGGCCTACCGG-3'	388	3 min at 94°C, and then 35 cycles with cycle consisting of 30 sec at 94°C, 1 min at 55°C, and 1 min at 72°C. Final extension for 7 min at 72°C.
	R 5'-ACACTCGGAAAATGCAAGCA-3'		

As immediate intervention was highly indicated, the condition was tentatively diagnosed as infection with *Theileria* species based on the clinical signs and examination of thin blood smears before the laboratory confirmation by PCR was available. Affected animals were treated with 12% imidocarb dipropionate, a drug with anti-protozoal effect, and 20% oxytetracycline. These injections were administered as a single dose in subcutaneous and intramuscular routes, at a rate of 0.5 mg/kg and 1 ml/10 kg of body weight, respectively. Moreover, a steroid (dexamethasone, 3 mg/animal), a metabolic stimulant (butophosphan, 3 ml/animal), an antihistamine (chlopheniramine maleate, 0.4 mg/kg/day) and a multivitamin were administered as supportive therapy once daily for three consecutive days. Three animals (Animal No 1, 3 and 4) fully recovered with the treatment, however one animal (Animal No. 2) died despite the continuous care, emphasising the importance of early and accurate diagnosis of the disease, followed by timely institution of therapy.

DISCUSSION

According to Banker *et al.*, (2020), the recommended therapeutic protocol for caprine theileriosis involves the combined administration of buparvaquone and oxytetracycline. However, Sykes and Papich (2021) identified buparvaquone as the drug of choice due to its specific mode of action on *Theileria* spp., where it inhibits cytochrome *b*, an enzyme essential for mitochondrial electron transport. Despite its proven efficacy, the use of buparvaquone in Sri Lanka is constrained by limited availability and high cost, rendering it unaffordable for small-scale livestock owners. Consequently, the affected animals in this study were treated with a combination of oxytetracycline and imidocarb dipropionate, which provided a pragmatic alternative under field conditions.

Molecular characterisation through phylogenetic analysis would have been valuable for definitive species identification of the PCR-detected *Theileria luwenshuni*. However, in this case the DNA sequencing was not undertaken due to logistical and financial constraints, as the primary objective of this investigation was the rapid diagnosis and prompt initiation of treatment at the field level. Limited access to advanced molecular diagnostic facilities and dependency on external institutions also restricted further genomic confirmation. However, another extensive study is ongoing to screen the goats from same Veterinary Surgeon's Division for all pathogenic *Theileria* species where phylogenetic analysis will be carried out to confirm the infection with each species.

Effective tick control remains a cornerstone in the prevention and management of theileriosis in goats. Given that the animals in the present study were maintained under semi-intensive management systems, recurrent exposure to tick vectors was unavoidable. Therefore, integrated tick control programmes should be prioritised, emphasising farmer education on the importance of maintaining hygienic farm environments and implementing biosecurity practices to reduce vector habitats. Moreover, appropriate and judicious use of acaricides is essential to prevent the emergence of resistance among tick populations. Concurrently, improving nutritional management is critical to enhance host immunity, enabling animals to better tolerate or resist infections, particularly those associated with low parasitemia.

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