RESEARCH ARTICLE
JIVA 23 (3) December 2025
https://doi.org/10.55296/JIVA/23.3.2025.30-39

JIVA
OFFICIAL JOURNAL OF INDIAN VETERINARY ASSOCIATION, KERALA

Received on 16.10.2025
Accepted on 03.11.2025

MOLECULAR IDENTIFICATION AND PHYLOGENETIC CHARACTERIZATION OF *CANDIDATUS MYCOPLASMA HAEMATOVIS* IN A CROSSBRED BEETAL GOAT KID

George Amal[1], Rose Sherin[2], Y. Ajith[1]*, Gireesh S. Sangeetha[1], Mathew K. Manju[1], V. Beena[3], Unny N. Madhavan[1], Siju Susan Jacob[4], Sasi Adithya[2], V.J. Udayasree[1], George Arun[1], Santhosh K. Anita[2], Rose Mary Sunny[2] and P. V. Tresamol[2]

[1] Department of Veterinary Clinical Medicine, Ethics and Jurisprudence,
College of Veterinary and Animal Sciences (CVAS), Kerala Veterinary and Animal Sciences University (KVASU), Mannuthy, Thrissur, Kerala, India-680651
[2] Teaching Veterinary Clinical Complex, CVAS, KVASU,
Mannuthy, Thrissur, Kerala, India-680651
[3] Centre for Animal Adaptation to Environment and Climate Change Studies (CAADECCS), KVASU, Mannuthy, Thrissur, Kerala, India-680651
[4] ICAR - National Institute of Veterinary Epidemiology and Disease Informatics,
Bengaluru, Karnataka, India-560064

* Corresponding author Email: ajithy@kvasu.ac.in; Contact number: +91 8891981301;
ORCID id: 0000-0001-7691-9986

ABSTRACT

Haemotropic *Mycoplasma* species (haemoplasmas) are erythrocyte-associated bacteria that can induce anaemia in domestic animals. Among these, *Candidatus Mycoplasma haematovis* has rarely been reported in goats worldwide and has not been molecularly confirmed in India. A four-month-old male crossbred Beetal goat kid was presented to the Small Ruminant Unit of the Teaching Veterinary Clinical Complex, Mannuthy, with a history of intermittent diarrhoea for two weeks. In addition, there was mortality of one kid from the same household manifesting similar clinical signs. The affected animal had previously been treated by a field veterinarian for suspected theileriosis based on the presence of intra-erythrocytic inclusions on blood smear examination and was administered buparvaquone. Complete blood count revealed anaemia and thrombocytopenia, whereas conventional smear examination failed to detect any haemoparasites. Genus-specific PCR screening, however, indicated the presence of haemotropic *Mycoplasma* and *Anaplasma/Ehrlichia* spp. Sequencing and phylogenetic analysis of the *Mycoplasma* 16S rRNA gene amplicon confirmed the organism as *C. M. haematovis*. The sequence (GenBank accession no. PX133059) showed 98.6% similarity with a goat isolate from China. The kid was successfully treated with intravenous oxytetracycline, supportive

fluid therapy, and tick control, resulting in complete clinical recovery without recurrence during a three-month follow-up. This report provides the first molecular confirmation of *C. M. haematovis* infection in a goat from India. The present case study also highlights the limitations of blood smear microscopy, and the diagnostic value of PCR in haemoparasites detection.

Keywords: Anaemia; *Candidatus Mycoplasma haematovis*; Haemoparasitic co-infections; Goat; PCR; Phylogeny

INTRODUCTION

Haemotropic *Mycoplasma* species (haemoplasmas) are uncultivable, cell wall–deficient, pleomorphic bacteria that parasitize erythrocytes, resulting in subclinical infections, haemolytic anaemia, poor growth, or immunosuppression in mammals. Originally, haemotropic *Mycoplasma* were classified under the genera *Eperythrozoon* and *Haemobartonella* within the order Rickettsiales. They were subsequently reclassified as a distinct cluster within the genus *Mycoplasma* due to their close phylogenetic and phenotypic relationship with mycoplasmas, particularly those in the *pneumoniae* group (Messick et al., 2002, Neimark et al., 2002, Tasker et al., 2003).

In small ruminants, *Mycoplasma ovis* is the predominant species, whereas *Candidatus Mycoplasma haematovis* (formerly *Eperythrozoon ovis*) has also been identified in sheep and goats (Windsor, 2022). The pathogenicity of haemoplasmas in small ruminants is variable; infections with *M. ovis* are often asymptomatic or mild, but chronic cases may result in anaemia due to erythrocyte membrane deformation, increased fragility, and erythrophagocytosis (Paul et al., 2020). The bone marrow has been suggested as a key site for pathogen replication before parasitemia becomes detectable (Smith and Sherman, 2022). Clinical disease, although uncommon in goats, primarily affects young animals and typically manifests as fever, anaemia, jaundice, poor growth, and, in severe cases, mortality (Mahran and Ghattas, 2016; Urie et al., 2019). Haematological findings often include macrocytic haemolytic anaemia with anisocytosis and poikilocytosis (Mahran and Ghattas, 2016). Haemoplasmas are primarily transmitted by haematophagous arthropods, including flies, midges, mosquitoes, ticks, and lice, which are common in livestock environments. Mechanical transmission may also occur during husbandry practices involving blood exposure, such as vaccination, ear tagging, and castration (Windsor, 2022). Environmental factors and management practices further influence the epidemiology of haemoplasma infections in small ruminants.

While *M. ovis* is widely reported, *C. M. haematovis* has received comparatively little attention, with few molecular confirmations globally, including in China. Its clinical significance, host range, and genetic diversity remain poorly characterized. Notably, there are no previous reports of *C. M. haematovis* in goats from India, despite the region being endemic for various arthropod-borne pathogens. This knowledge gap is important, as infections may remain undetected when diagnosis relies solely on conventional blood smear examination, which lacks the sensitivity of molecular methods. The present study provides the first molecular confirmation of *C. M. haematovis* in a goat from India, supported by 16S rRNA gene sequencing and phylogenetic analysis. This finding expands the known geographic distribution of the pathogen and underscores the importance of molecular surveillance for accurate detection, epidemiological assessment, and effective disease management in small ruminants.

MATERIALS AND METHODS

Clinicopathological assessment

The study was conducted at the Small Ruminant Unit and Molecular Diagnostic Laboratory of the Teaching Veterinary Clinical Complex (TVCC), Mannuthy. A 4-month-old male crossbred Beetal goat kid, weighing 7.1 kg, was presented with a history of intermittent diarrhoea for the past two weeks. There was mortality of one kid from same household manifesting similar clinical signs. The affected animal had earlier been treated by a field veterinarian for suspected theileriosis, based on intra-erythrocytic inclusions observed on blood smear, and was administered buparvaquone. On clinical examination, the kid appeared dull, pale, underconditioned, and mildly dehydrated. Rectal temperature (102.1°F) and heart rate (76 beats/min) were within normal physiological limits. Tick infestation was noticed upon physical examination. Blood samples were collected aseptically from the jugular vein into EDTA vials for haematological analysis using an automated 3- part haematology analyzer (Mythic-11, Orphee, Switzerland), and DNA extraction. Faecal samples and peripheral blood smears (Field-staining method) were examined for gastrointestinal parasites and haemoparasites, respectively.

Molecular identification and phylogenetic analysis

The total genomic DNA was extracted using the DNeasy® Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. PCR screening was performed for major haemoparasites, including *Theileria*, *Babesia*, *Trypanosoma*, *Anaplasma/Ehrlichia*, and haemotropic

Mycoplasma spp., using standard procedures (Table 1) in an automated thermal cycler (Eppendorf Mastercycler® Nexus Gradient). PCR products were electrophoresed on 1.5% agarose gels and visualized under UV illumination using a BIO-RAD ChemiDoc™ XRS+ gel documentation system. Mycoplasma-specific amplicons were purified and sequenced bidirectionally at GeneSpec, Kochi. Sequences were analyzed using BLAST against the NCBI nucleotide database. Phylogenetic analysis was performed in MEGA version 10.2 using the Maximum Likelihood method with the Kimura 2-parameter model. *Anaplasma marginale* was used as the outgroup.

RESULTS

The haematological parameters revealed severe microcytic hyperchromic anaemia and thrombocytopenia with normal total leukocyte count with relative lymphocytosis, and granulopenia (Table 2). These alterations were consistent with severe haemoparasitic infection-induced bone marrow suppression or immune-mediated destruction. Conventional Giemsa-stained blood smear examination at the Clinical Diagnostic Laboratory, University Veterinary Hospital, Mannuthy, failed to detect haemoparasites, and faecal

Table 2: Haematological findings of the goat infected with *Anaplasma/Ehrlichia* and haemotropic *Mycoplasma* spp.

Parameters	Observation	Reference range
TLC (10^3/ µL)	6.8	4-13
Lymphocyte (10^3/ µL)	5	2-9
Monocyte (10^3/ µL)	0.3	0-0.55
Granulocyte (10^3/ µL)	1.5	1.7-5.4
Lymphocyte (%)	73.6	50-70
Monocyte (%)	5	0-4
Granulocyte (%)	21.4	30-48
TEC (10^6/ µL)	0.77	8-18
Haemoglobin (g/dL)	2.1	8-12
PCV (%)	1.2	22-38
MCV (fL)	15.6	16-25
MCH (pg)	27.3	5.2-8
MCHC (%)	175	30-36
Platelets (10^3/ µL)	200	300-600

Haematologic reference ranges, 11th edition. The Merck Veterinary Manual
TLC – Total Leukocyte Count, TEC – Total Erythrocyte Count, PCV -Packed Cell Volume, MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration

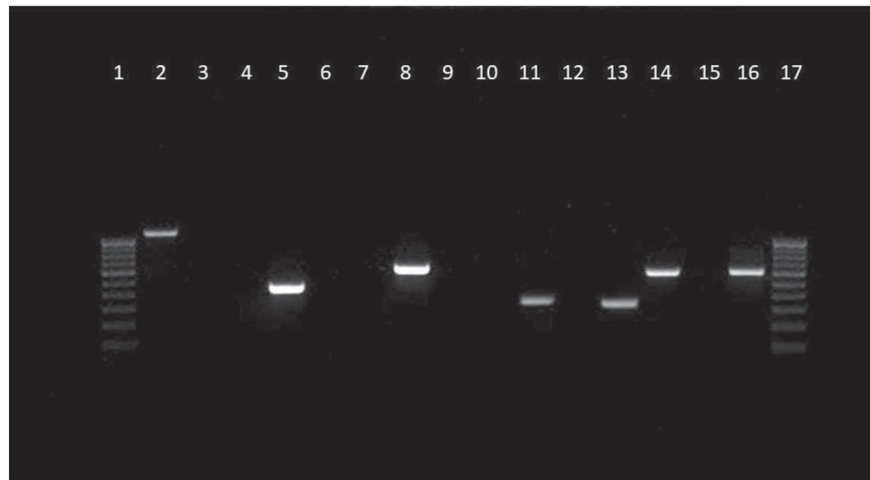

Fig. 1: PCR products after agarose gel electrophoresis
Lane 1: 100 bp DNA ladder
Lane 2-4: Theileria spp. *– Positive control, Negative control, Sample*
Lane 5-7: Babesia spp. *– Positive control, Negative control, Sample*
Lane 8-10: Trypanosoma spp. *- Positive control, Negative control, Sample*
Lane 11-13: Anaplasma spp.*- Positive control, Negative control, Sample*
Lane 14-16: Mycoplasma spp. *- Positive control, Negative control, Sample*
Lane 17: 100 bp DNA ladder

analysis revealed no gastrointestinal parasitic ova or oocysts. However, PCR amplification confirmed the presence of both *Anaplasma/Ehrlichia* and haemotropic *Mycoplasma* spp. (Fig. 1).

Sequencing of the Mycoplasma-specific amplicon identified it as *C. M. haematovis* (GenBank accession no. PX133059), showing 98.6% similarity with a goat isolate from China (KU983749). Phylogenetic analysis revealed clustering of the present isolate within the *C. M. haematovis* clade (bootstrap = 66), closely related to isolates from Hungary (EU828580), Japan (JF931131), and Egypt (LC848325 and PQ894442), and distinct from *Mycoplasma ovis* and other *Mycoplasma* spp., with *Anaplasma marginale* as the outgroup (Fig. 2).

Considering the confirmed co-infection, a combined therapeutic approach was applied. Blood transfusion was recommended but declined by the owner. The kid was stabilized with isotonic fluid therapy to correct dehydration. Oxytetracycline (Steclin, 50 mg/mL; Zenex Animal Health, India) was administered intravenously at 20 mg/kg body weight once daily for five days, diluted in normal saline as a constant rate infusion (CRI). Ivermectin (Neomec 1% w/v; Intas Pharmaceuticals Ltd.) was administered subcutaneously at 0.2 mg/kg body weight to control ticks. A multivitamin-mineral supplement (Chelated Agrimin®

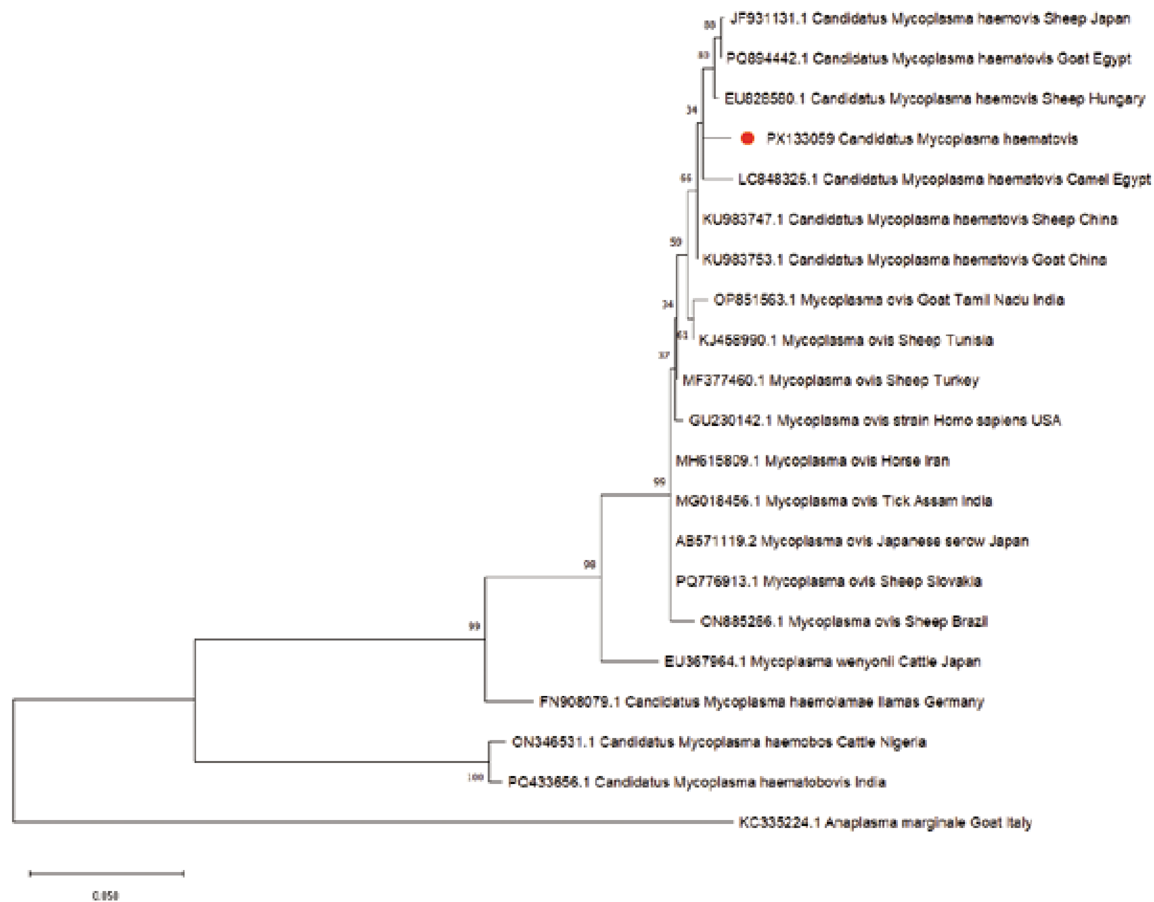

Fig. 2: Phylogenetic tree of *C. M. haematovis* isolate based on 16s *rRNA* partial sequence

Forte; Virbac India) was provided orally at 25 g/day. Following therapy, the kid fully recovered, with normalization of appetite, activity, and faecal consistency. No recurrence of clinical signs or mortality was reported during a three-month telephonic follow-up.

Discussion

The present study provides the first molecular confirmation of *C. M. haematovis* in a goat in India and demonstrates its occurrence in co-infection with *Anaplasma* spp., highlighting both diagnostic challenges and complex pathophysiological interactions. Haemotropic *Mycoplasma* spp. are recognized causes of haemolytic anaemia in small ruminants, acting through erythrocyte adhesion and immune-mediated destruction. The severe anaemia observed in this case aligns with these mechanisms and may be exacerbated by the pathogen's capacity to evade or suppress the host immune response, particularly inflammatory pathways (Askar et al., 2021). Immune responses to haemoplasmas can vary depending on host factors and environmental conditions. The concurrent

detection of *Anaplasma* spp. is equally significant. These pathogens infect both erythrocytes and leukocytes, leading to haemolysis and altered leukocyte dynamics (Smith and Sherman, 2022). Co-infection with haemoplasmas and *Anaplasma* can intensify anaemia severity through additive or synergistic red cell damage. The haematological profile of the present case, severe anaemia with normal total leukocyte count, relative lymphocytosis, granulopenia and thrombocytopenia supports this explanation. Collectively, these abnormalities indicate that the co-infection may have intensified immune dysregulation beyond what would be expected from haemoplasmosis alone.

The initial misdiagnosis of theileriosis based on blood smear examination is unsurprising, as haemoplasma inclusions can morphologically resemble piroplasms. This underscores the limitations of cytology for differentiating haemoparasites and highlights the superiority of multiplex PCR in endemic regions where multiple pathogens co-circulate (Paul et al., 2020). Although smear microscopy remains useful for rapid screening, it is neither sensitive nor specific enough for definitive diagnosis, especially in mixed infections (Wangai et al., 2011). Molecular analysis revealed that the partial 16S *rRNA* gene sequence of the present isolate shared 98.6% similarity with a goat isolate from China, indicating approximately 1.4% sequence divergence. While modest, this variation suggests genetic diversity among *C. M. haematovis* strains across geographic regions and supports the molecular novelty of the Indian isolate. Such findings emphasize the need for further sequencing-based epidemiological studies to better understand strain evolution and host adaptation in small ruminants.

Overall, this study highlights the clinical and epidemiological significance of mixed haemoparasitic infections in goats and reinforces the critical role of molecular diagnostics for accurate identification and surveillance in tropical endemic regions like Kerala. Although its zoonotic potential remains unconfirmed, the emergence of C. M. haematovis in goats may have public health implications, warranting further investigation into its transmission and cross-species risk.

SUMMARY

The detection of co-infection with *C. M. haematovis* and *Anaplasma* spp. in a goat presenting with severe anaemia, relative lymphocytosis, granulopenia, and thrombocytopenia underscores the importance of multiplex molecular diagnostics in small ruminant practice.

The haematological alterations observed suggest potential synergistic pathogenic effects between these two haemoparasites, contributing to disease severity. Clinicians in endemic regions should consider PCR-based confirmation of suspected cases for definitive diagnosis, and implement combined antimicrobial therapy alongside supportive care. Herd-level molecular surveillance is recommended to evaluate the prevalence of mixed haemoparasitic infections and guide effective control strategies. Furthermore, broader molecular surveillance across goat populations in different regions of India is essential to elucidate the prevalence, strain diversity, and epidemiological significance of *C. M. haematovis* and related haemoparasites.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the facilities provided under the State Plan Projects, Government of Kerala, at the Teaching Veterinary Clinical Complex, College of Veterinary and Animal Sciences, Mannuthy, Kerala Veterinary and Animal Sciences University (KVASU). Financial support for this study was provided by the Startup Research Grant Scheme (Grant No. SRG/2022/000837) of the Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India.

REFERENCES

Allsopp, B. A., Baylis, H. A., Allsoppi, M. T. E. P., Cavalier-Smith, T., Bishop, R. P., Carrington, D. M., and Spooner, P. (1993). Discrimination between six species of *Theileria* using oligonucleotide probes which detect small subunit ribosomal RNA sequences. *Parasitology*, *107*(2), 157-165. https://doi.org/10.1017/S0031182000067263

Askar, H., Chen, S., Hao, H., Yan, X., Ma, L., Liu, Y., & Chu, Y. (2021). Immune evasion of Mycoplasma bovis. *Pathogens*, *10*(3), 297.https://doi.org/10.3390/pathogens10030297

Criado-Fornelio, A., Martinez-Marcos, A., Buling-Saraña, A., and Barba-Carretero, J. C. (2003). Presence of *Mycoplasma haemofelis*, *Mycoplasma haemominutum* and piroplasmids in cats from southern Europe: a molecular study. *Veterinary Microbiology*, *93*(4), 307-317. https://doi.org/10.1016/S0378-1135(03)00044-0

Hilpertshauser, H., Deplazes, P., Schnyder, M., Gern, L., and Mathis, A. (2006). *Babesia* spp. identified by PCR in ticks collected from domestic and wild ruminants in southern Switzerland. *Applied and*

Environmental Microbiology, *72*(10), 6503-6507. https://doi.org/10.1128/AEM.00823-06

Mahran, O. M., and Ghattas, S. G. (2016). *Eperythrozoon ovis* infections of sheep and goats in shalateen, abu-ramad and halaeeb, red sea governorate, Egypt. *Egyptian Journal of Agricultural Research*, *94*(2), 397-406. https://doi.org/10.21608/ejar.2016.152484

Messick, J. B., Walker, P. G., Raphael, W., Berent, L., & Shi, X. (2002). *'Candidatus mycoplasma haemodidelphidis'* sp. nov.,*'Candidatus mycoplasma haemolamae'*sp. nov. and *Mycoplasma haemocanis* comb. nov., haemotrophic parasites from a naturally infected opossum (*Didelphis virginiana*), alpaca (*Lama pacos*) and dog (*Canis familiaris*): phylogenetic and secondary structural relatedness of their 16S rRNA genes to other mycoplasmas. *International journal of systematic and evolutionary microbiology*, *52*(3), 693-698. https://doi.org/10.1099/00207713-52-3-693

Neimark, H., Johansson, K. E., Rikihisa, Y., & Tully, J. G. (2002). Revision of haemotrophic *Mycoplasma* species names. *International Journal of Systematic and Evolutionary Microbiology*, *52*(2), 683-683. https://doi.org/10.1099/00207713-52-2-683

Paoletta, M. S., Arias, L. L., De La Fourniere, S., Guillemi, E. C., Luciani, C., Sarmiento, N. F., and Wilkowsky, S. E. (2018). Epidemiology of *Babesia*, *Anaplasma* and *Trypanosoma* species using a new expanded reverse line blot hybridization assay. *Ticks and Tick-borne Diseases*, *9*(2), 155-163. https://doi.org/10.1016/j.ttbdis.2017.08.011

Paul, B. T., Jesse, F. F. A., Chung, E. L. T., Che-Amat, A., Mohd Lila, M. A., Hashi, H. A., and Norsidin, M. J. (2020). Review of clinical aspects, epidemiology and diagnosis of haemotropic *Mycoplasma ovis* in small ruminants: Current status and future perspectives in tropics focusing on Malaysia. *Tropical Animal Health and Production*, *52*(6), 2829-2844. https://doi.org/10.1007/s11250-020-02357-9

Rahman, M., Faruque, M. R., Rahman, M. M., and Chowdhury, M. Y. E. (2022). Epidemiology and molecular detection of *Anaplasma* spp. in goats from Chattogram district, Bangladesh. *Veterinary Medicine and Science*, *8*(3), 1240-1249. https://doi.org/10.1002/vms3.775

Smith, M.C. and Sherman, D.M., 2022. *Goat medicine (3rd ed.)*. John Wiley & Sons.

Tasker, S., Helps, C. R., Day, M. J., Harbour, D. A., Shaw, S. E., Harrus, S. and Gruffydd-Jones, T. J. 2003. Phylogenetic analysis of haemoplasma species: an international study. *J. Clin. Microbiol.* **41**(8): 3877-3880.

Tasker, S., Helps, C. R., Day, M. J., Harbour, D. A., Shaw, S. E., Harrus, S., and Gruffydd-Jones, T. J. (2003). Phylogenetic analysis of hemoplasma species: an international study. *Journal of clinical microbiology*, *41*(8), 3877-3880. https://doi.org/10.1128/jcm.41.8.3877-3880.2003

Urie, N. J., Highland, M. A., Knowles, D. P., Branan, M. A., Herndon, D. R., and Marshall, K. L. (2019). *Mycoplasma ovis* infection in domestic sheep (*Ovis aries*) in the United States: Prevalence, distribution, associated risk factors, and associated outcomes. *Preventive Veterinary Medicine*, *171*, 104750. https://doi.org/10.1016/j.prevetmed.2019.104750

Wangai, L. N., Karau, M. G., Njiruh, P. N., Sabah, O., Kimani, F. T., Magoma, G., & Kiambo, N. (2011). Sensitivity of microscopy compared to molecular diagnosis of P. falciparum: implications on malaria treatment in epidemic areas in Kenya. *African journal of infectious diseases*, *5*(1). https://doi.org/10.4314/ajid.v5i1.66504

Windsor, P. A. (2022). Anaemia in lambs caused by *Mycoplasma ovis*: global and Australian perspectives. *Animals*, *12*(11), 1372 https://doi.org/10.3390/ani12111372