**ABSTRACT**

Mycoplasma ovis and ‘Candidatus Mycoplasma haemovis’ are hemotropic bacteria (hemoplasmas) that may cause anemia in sheep, goats, deer and antelope. Acute infection in sheep may result in a severe hemolytic anemia while animals that are chronically infected may be asymptomatic. These organisms have zoonotic potential. Molecular tests are the most efficient methods for hemoplasma detection. The aim of this study was to investigate the presence of hemoplasma infection in 10 female sheep (2-3 years old), which were the subjects of an unrelated orthopedic implant study. Blood samples were collected for CBC and chemistry panel. Since hemoplasmas were observed on the blood smear of 1 of these sheep, molecular testing was pursued for all animals. Total DNA was extracted from EDTA blood samples for screening of hemoplasmas by quantitative PCR (qPCR) and a conventional PCR (cPCR) was used for amplification and sequencing of the near-entire 16S rRNA gene. The hematocrit of 1 sheep was slightly below (26.1%) the reference range while the single bacteremic sheep was hypoglycemic (<22 mg/dL). qPCR resulted in 2 positive and 3 suspect samples; cPCR resulted in 3 positive samples, but when re-amplified resulted in 8 DNA fragments with expected size (approximately 1.3 Kb). Sequence analysis of the positive samples revealed 2/10 of the sheep were infected with M. ovis only, while 6/10 were co-infected with M. ovis and ‘Candidatus Mycoplasma haemovis’. Our results suggest that the hemoplasma infection in most of these sheep was subclinical with an occurrence of 80%. The importance of detecting chronic hemoplasma infection in research animals is that the infection might confound experimental results and that manipulation of these animals could lead to overt disease.

**RESULTS**

CBC and chemistry results were unremarkable, except for one sheep that was slightly anemic (26.1%, reference: 27-45%) and another one that was hypoglycemic (<22 mg/dL, reference value: 50-80 mg/dL).

Only the hypoglycemic sheep had hemoplasmas visualized by light microscopy (Fig. 2).

cPCR resulted in 3 positive samples (Fig. 3) while qPCR resulted in an additional 4 positive samples (total of 7) and 3 suspect samples (Fig. 4A).

Re-amplification of the nearly complete 16S rRNA gene using a cPCR developed in our lab resulted in a unique M. ovis sequence data for this gene resulted in 8 positive samples (Fig. 5B).

Direct sequencing of PCR products showed that 2/8 of the sheep were infected with M. ovis only, while sequencing chromatograms findings suggested that 6/8 were co-infected with M. ovis and ‘Candidatus M. haemovis’.

PCR products with the expected size (1,342 bp) were purified and cloned from all positive samples (8/10) in order to confirm the co-infection.

Sequencing of multiple clones (2-10), revealed both M. ovis and ‘Candidatus M. haemovis’ in all positive samples.

**CONCLUSION**

As expected, qPCR designed as a screening assay has greatest sensitivity for detection of hemoplasmas in blood samples from sheep when compared to cPCR.

Co-infection with M. ovis and ‘Candidatus M. haemovis’ was previously described in sheep1; however, the applied PCR protocols based on the 16S rRNA gene fail to distinguish between these two species without the need for cloning and sequencing steps.

We found that 80% of the sheep were infected with hemoplasmas, which is in agreement with previous reports in sheep (52-81%) based on PCR detection4; however, most of these studies were performed in sheep having clinical signs of hemoplasmosis while our results suggest that the infection was subclinical.

Chronic infection with hemoplasmas in these research animals is common and may represent a confounding variable in research data, especially if these animals develop overt disease due to manipulation and/or stress.

**REFERENCES**


