ANIMALS

CANDIDATUS MYCOPLASMA HAEMOLAMAE: FIRST REPORT IN A NEW ZEALAND ALPACA

Disease information

Candidatus Mycoplasma haemolamae (CMhl) is a haemotropic mycoplasma that adheres to the surface of the red blood cells of camelids. It was previously classified in the species Eperythrozoon but reclassified in 2004 as a Mycoplasma species (Messick, 2004). Haemotropic mycoplasmas are also known as haemoplasmas. CMhl is closely related to M. felis and M. ovis, both of which (among other haemotropic mycoplasmas) have previously been reported in New Zealand (Thompson, 1998). The presence of the organism is variably associated with anaemia (Foster *et al.*, 2009), depression, fever and weight loss (Kaufmann *et al.*, 2010). Animals subjected to immune suppression (e.g., stress or concurrent disease conditions) are more likely to show clinical signs and are also more likely to have visible blood parasites on peripheral blood smears.

Treatment of animals is typically with oxytetracyclines but it appears that animals remain chronically infected despite treatment (Tornquist *et al.*, 2009) and subclinical carriers are common. Transplacental or parturient transfer of infection between animals has been reported but transfer is not inevitable (Tornquist *et al.*, 2011). The most common route of transfer between animals is thought to be mechanical, via biting insect vectors. This has been proven in the case of other haemotropic mycoplasmas (Messick, 2004). Iatrogenic mechanical transmission is also possible.

Prevalence estimates in subclinically affected populations vary considerably, with 18.7 percent reported in central Europe and 9–19 percent in Peru and Chile (Kaufmann *et al.*, 2010; Forman, 2009). In a Swedish study (Kaufmann *et al.*, 2010) there was a prevalence of 18.6 percent in the camelid population and 39.1 percent of all farms tested had at least one animal positive for CMhl.

CMhl was first identified in camelids in the USA in 1990 (McLaughlin *et al.*, 1990). The first alpacas were imported into New Zealand in 1986, four years before the first identification of the organism and 15 years before a PCR test was developed in 2001. An MPI import risk analysis for alpacas published in 2010 considered that CMhl was likely to be already present in New Zealand because carrier states and subclinical infections were common (Kaufmann *et al.*, 2011). The risk analysis is available from the MPI website http://www.biosecurity.govt.nz/regs/imports/ihs/risk.

This case report describes the first confirmed finding of Candidatus Mycoplasma haemolamae (CMhl) in New Zealand. This is the first time that PCR testing for the organism has been performed on animals from New Zealand.

CASE REPORT 1

A veterinary laboratory informed the Investigation and Diagnostic Centre that a client had requested testing for CMhl in a herd of alpaca that had been experiencing problems with anaemia over the previous few months. The PCV reference range for adult alpacas is 27–45 percent (Fowler, 2010). One severely anaemic animal with a PCV of 5 percent had been identified by the attending veterinarian and had died. Blood samples taken by jugular venipuncture from this animal and ten others in the same group were available for analysis. These samples were analysed for evidence of anaemia and for copper, selenium, iron and vitamin B12 concentrations. Samples were sent overseas for subcontracted blood testing by PCR to detect CMhl. Parallel samples were tested by Massey University using a generic PCR test capable of detecting haemotropic mycoplasmas. Peripheral blood smears were collected from each animal and examined by veterinary pathologists.

Although no organisms were seen on blood smears, DNA testing for CMhl at Oregon State University detected the organism by both real-time PCR (O'Reilly *et al.*, 2008) and conventional PCR (Tornquist *et al.*, 2009) in one of the 11 blood samples. Both these tests are specific for CMhl as they target a sequence that is unique to the hypervariable region of the CMhl 16S rRNA gene. The animal that produced the positive result had no clinical signs of anaemia (PCV = 41 percent) and its blood smear was unremarkable. Sequencing of the PCR product at Oregon State University was requested but was not possible.

The Massey University haemotropic mycoplasma PCR (Gentilini *et al.*, 2009) gave a strong positive result from the same animal. This conventional PCR targets the hypervariable region of the 16S rRNA gene but is not specific for CMhl.

Sequencing of the PCR product allowed it to be identified as 100 percent homologous with CMhl DNA entered in NCBI Genbank.

The alpaca farm had been identified as being in an area at risk of cobalt deficiency. Cobalt is an essential building block of vitamin B12. Signs of vitamin B12 deficiency are listlessness, weight loss, anaemia and ketosis. Reference levels for healthy alpaca vitamin B12 levels ranged from 70 to 880 pmol/L (95 and 1192 ng/L) in one New Zealand study (Ellison, 2004) and from 150 to 430 pmol/L (203 and 582 ng/L) in an Australian survey of healthy alpacas (Lee *et al* 1999). Four of ten animals sampled from the herd had vitamin B12 concentrations below 70 pmol/L and eight were below the 150 pmol/L level suggested by the Australian study. Concentrations of vitamin B12 in the ten in-contact animals sampled ranged from 46 to 357 pmol/L, with a mean value of 120.

In addition to this apparent trace element deficiency, drench resistance to ivermectin was identified by the attending veterinarian. Animals were found to have large numbers of *Haemonchus contortus* larvae present after drenching with ivermectin at the recommended dose rates. Histopathology reports from the severely anaemic animal that died also identified chronic severe hepatopathy. The most likely cause of this lesion was identified as sporidesmin toxicity (facial eczema). A combination of drench resistance, facial eczema and cobalt deficiency was considered to be the most likely cause of the anaemia previously reported on this farm. Anaemia was not identified in any of the ten in-contact animals sampled during this investigation.

CASE REPORT 2

A second independent investigation on an alpaca farm with clinical signs of anaemia and a 5 percent increase in mortality rate over the previous year showed an association with a number of factors including mineral deficiencies and chronic parasitism that was likely due to drench resistance. Although organisms with characteristics of CMhl were suspected to be present on red blood cells from several individuals in a cohort of alpacas that were in poor condition, PCR tests of all samples were negative at the two independent laboratories mentioned in the first case report. Husbandry changes to correct mineral deficiencies, and amended drenching protocols, resulted in reduced mortality and clinical improvement in the herd.

Discussion

Case report 1 is the first confirmed finding of this organism in an alpaca bred in New Zealand. CMhl has not previously been tested for by PCR in New Zealand animals. Examination of blood smears by light microscopy is not a sensitive method for detecting haemotropic mycoplasmas, because of cyclical bacteraemia and the low numbers of organisms present in subclinical animals (Fowler, 2010). In acutely or chronically affected cats, detection of Mycoplasma haemofelis is possible in less than 50 percent of cases (Sykes, 2010). In a recent New Zealand study (Jenkins *et al*., 2013) the sensitivity of blood smear tests for feline Mycoplasmas was 9.7 percent and specificity 97.8 percent. International studies have reported sensitivities of 0–37.5 percent (Messick, 2004; Sykes, 2010; Tasker, 2010). It is important to ensure that smears are made using blood that has not been exposed to ethylene diamine tetra-acetic acid (EDTA), because EDTA causes the organisms to detach from the red blood cells (Sykes, 2010). In smears where the organisms have detached from the cells they may only be noticed as intracellular debris (Foster *et al*., 2009).

In case report 1, an animal with no clinical signs had a PCR-positive test for CMhl, and in case report 2, ten animals in poor body condition tested negative. Overseas studies have shown that most infections do not cause clinical disease (Kaufmann *et al*., 2010). In cases where clinical disease is noticed, clinical signs include anaemia, depression and icterus (Fowler, 2010).

CONCLUSION

These findings confirm the presence of CMhl in New Zealand alpacas. However, the infected animal in this study showed no clinical signs of disease and had a normal PCV and blood smear. This is consistent with most infections with CMhl. Anaemia cases in alpacas are likely due to a combination of causes such as mineral deficiency, drench resistance, chronic sporidesmin exposure and infectious diseases.

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