Epidemiology of *Candidatus* Mycoplasma haemolamae' infection in South American camelids in Central Europe

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Abstract

In this study we describe the prevalence of *Mycoplasma haemolamae* infection in South American camelids (SAC) located in central Europe (Switzerland and Germany). A newly developed real-time PCR assay was applied to detect *M. haemolamae* in SAC blood. Samples were collected from 225 llamas and alpacas and the prevalence was found to be 18.7% (n=42). Camelids bred domestically and imported from overseas were PCR-positive for *M. haemolamae*, though imported animals were more frequently positive. Older animals were significantly more likely to be infected compared to younger ones, but neither species, gender or health state were found to be statistically associated with infection. The study thereby raises questions about possible transmission routes between animals. Even though *M. haemolamae* doesn't seem to be of high pathogenicity, it also needs to be considered as a possible cause of disease in SAC in Europe.

Key words: haemotropic mycoplasma, infectious disease, real-time PCR, South American camelids

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Introduction

Mycoplasma haemolamae is an opportunistic bacterial pathogen specific to South American camelids (SAC). *Haemotropic mycoplasmas* (haemoplasmas) were first reported in South American camelids (SAC) in 1990 (McLaughlin et al., 1990; Reagan et al., 1990). *M. haemolamae* can be asymptomatically carried in camelids. Clinical infection results in erythrocyte parasitism, which can coincide with the display of clinical signs including fever, anaemia, lethargy, anorexia, hypoglycaemia and reduced weight gain (McLaughlin et al., 1990). *In vitro* cultivation of this organism has not yet been achieved and the term '*Candidatus* Mycoplasma haemolamae' was proposed for this species. Molecular methods provide a basis for the detection and characterisation of haemoplasmas of different mammalian species (Messick et al. 2002). Recently, a real-time PCR was developed to allow for the specific detection of *M. haemolamae* from camelid blood samples (Tornquist et al. 2010).

The epidemiology of *M. haemolamae* infection in camelids is not well understood. A study examining alpacas in Peru and Chile found in-herd prevalence rates ranging from 9.26% to 19.3% (Tornquist et al. 2010). The route of transmission is not known for this species; however, vectors such as insects and blood-sucking arthropods are suspected to play a role in horizontal transmission (Seneviratna et al., 1973; Willi et al., 2007; Woods et al., 2004). Vertical transmission also appears likely as crias have been found to be infected a short period after birth (Almy et al., 2006; Fisher and Zinkl, 1996). Pathogenicity of this species is a function of its ability to adhere to erythrocytes. However, infection can proceed without symptoms and there are

asymptomatic carriers in SAC (Tornquist et al., 2010).

In Switzerland samples of SAC were analysed for the presence of haemoplasmas by a conventional PCR developed for the detection of feline haemotropic mycoplasma species (Tasker et al., 2003). In 2006 haemoplasmas were identified in two alpacas referred for necropsy to the Centre for Fish and Wildlife Health at the University of Berne. The amplified PCR products showed 99% identity to the sequences published for *M. haemolamae* (GenBank Accession No. GU047355) (Meli et al., 2010). As this was the first report of SAC haemoplasmas in Europe, there was great interest in uncovering further knowledge of the epidemiology in Europe. A study was initiated aiming at determining the prevalence of haemoplasma infections in SAC in Europe.

Animals, material and methods

Animals and blood sampling

Blood samples from 225 SAC were included: 194 of these animals, all of them from Switzerland, contributed to a preliminary study (Kaufmann et al., 2010). The SAC were housed on farms in Switzerland (n=194) and Germany (n=31). Animals were either sampled on-site at farms (n=144) during a study about the prevalence of infectious diseases in SAC, or at the Vetsuisse Faculty, University of Berne, or on-site at farms because of clinical suspicion of illness (n=81). From all animals, if available, the following parameters were recorded: age, gender, species (llama or alpaca), origin (imported from South America or born in Europe).

Blood samples were collected from the jugular vein of each animal. They were collected in EDTA-coated and uncoated serum tubes and either processed within 24 hours or stored at -20° C until further processing. Packed cell volume (PCV) and total protein, when possible, were determined within 24 hours. Haematology was performed either by ADVIA 120®, Bayer (Clinical Laboratory, Department for Clinical Veterinary Medicine, University of Berne) or using the Cell-Dyn 3500 hematology analyser, Abbott (Clinical Veterinary Laboratory, University of Zurich). Total protein was determined by automated analysis from serum using Hitachi 912, Roche Diagnostics (Clinical Laboratory, Department for Clinical Veterinary Medicine, University of Berne) or Cobas-Integra 800, Roche Diagnostics (Clinical Veterinary Laboratory, University of Zurich) according to standard procedures recommended by the International Federation of Clinical Chemistry (IFCC).

Identification of *M. haemolamae* infection by real-time PCR

DNA was purified from EDTA-anticoagulated blood samples as described elsewhere (Meli et al., 2010). Samples were stored at -20°C until further analysis. The DNA was analysed by a real-time PCR assay developed for specific detection of M. haemolamae (Meli et al., 2010).

Statistics

The software Statview 4.57 (Abacus Concepts, Berkeley USA) was used for statistical analyses. Differences in PCR status (positive or negative for *M. haemolamae*) between imported and domestically bred animals; females and males; and alpacas and llamas were evaluated using the chi square test. Differences were considered significant when probabilities (*P*) were less than 0.05. To analyse whether there was an influence of the age on PCR status, the Mann-Whitney *U* test (PCR-positives vs. PCR-negatives) was used. The same test was applied to detect differences in age distribution, PCV and total protein values between the groups mentioned above.

Results

Blood samples of 225 SAC were used for this study. The 225 SAC consisted of 169 alpacas and 56 llamas, 152 of them were female and 61 male (in 12 animals the gender was not known). The youngest were newborn crias and the oldest were 20 years old (median 3.1 years, 25% quartile 1.2, 75% quartile 6.1, age distribution with left kurtosis). The exact date of birth could only be ascertained for 172 SAC. Seventy-eight animals were bred domestically in Europe, while 38 were imported from South America, Peru or Chile. For the remaining 109 SAC, the place of birth could not be determined with accuracy. The group of imported animals showed a higher mean age than the group of SAC born in Europe (5.6 +/- 2.7 vs. 3.4 +/- 3.6 years respectively).

Forty-two of the 225 blood samples were real-time PCR-positive for *M. haemolamae* (18.7 %; 95% interval of confidence: 13.6% to 23.8%). The frequency of PCR-positive and PCR-negative animals in relation to species, gender and origin is shown in Table 1. No differences were seen in the frequency of positive animals between alpacas and llamas, females and males, respectively. Of 78 SAC born in Europe, 14 (17.9%) were PCR-positive, while 10 (26.3%) of 38 imported animals were found to be positive. The difference between the groups was not statistically significant ($P = 0.33$).

Table 1. Frequencies of PCR positive animals for '*Candidatus* Mycoplasma haemolamae' in the groups gender, species, origin and health state

		Negative	**Positive**	**Total**	**% PCR positive**	**Total counts per variable**
Gender	Female	123	28	151	18.5	
	Male	50	12	62	19.4	213
Species	Alpaca	138	31	169	18.3	
	Llama	45	11	56	19.6	225
Origin	Import	28	10	38	26.3	
	Europe	64	14	78	17.9	116
Health state	Healthy	117	27	144	18.8	
	Sick	40	12	52	23.1	196
Total samples		183	42	225	18.7	225

PCR-positive animals were significantly older than PCR-negative SAC (median of age 4.4 years, 25% quartile 2.9, 75% quartile 8.2 *vs*. median of age 3.0 years, 25% quartile 1.1, 75% quartile 5.4, respectively) (Figure 1). Nevertheless, some young animals under one year of age were already positive.

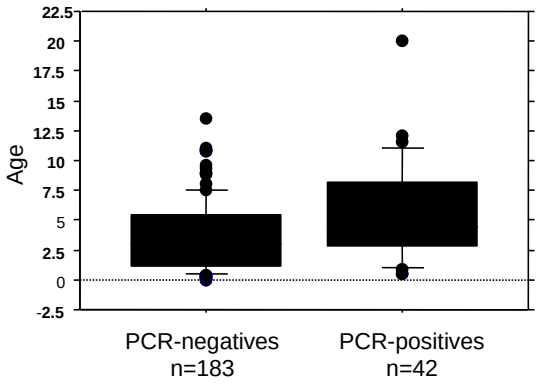

Figure 1. Age distribution in camelids positive or negative for '*Candidatus* Mycoplasma haemolamae' in real-time PCR

PCV values did not differ between PCR-positive and PCR-negative camelids (median = 30 l/l (25% quartile 27.1 l/l / 75% quartile 36 l/l) vs. median = 31 (25% quartile 26.6 l/l / 75% quartile 35.5 l/l), respectively); however, the total protein content was significantly higher in the blood of animals positive for *M. haemolamae* (P = 0.05) (median 70.6 g/dl (25% quartile 66.6 g/dl / 75% quartile 73.5 g/dl) in positive versus median 63.8 g/dl (25% quartile 58.1 g/dl / 75% quartile 71 g/dl) in negative animals).

Discussion

This study presents data about the prevalence of '*Candidatus* M. haemolamae' in Switzerland and Germany following the first detection of infected camelids in Switzerland. The frequency of infection seems to be relatively high, with 18.7% testing positive by real-time PCR.

M. haemolamae was found in both domestically bred and imported animals. However, animals imported from South America showed a slightly but not significantly higher frequency of infection. Tornquist et al. (2010) found infection rates comparable to our study in Peruvian and Chilean llamas and alpacas. In the present study, haemoplasma infection could be detected in SAC born in Europe as well as in crias, indicating that transmission of haemoplasmas occurred *in loco*. As long as the transmission route is not ascertained. indirect transmission by vector as well as horizontal or vertical infection should be considered.

The most significant finding was that PCR-positive animals were older on average than negative ones. It can be hypothesised that older animals had more contact with other infected animals during their lifetimes or had greater exposure to infectious sources. The high frequency of infection in clinically healthy animals, in addition to the fact that available PCV values did not differ between PCR-positive and –negative camelids, suggests that M. *haemolamae* is a weak virulent and opportunistic pathogen in SAC. This is supported by the fact that other mammals, including sheep, can be chronic carriers of

high loads of haemoplasmas without clinical symptoms (Messick, 2004). This coincides with a recent study in llamas and alpacas in South America finding in-herd prevalence rates between 9.3% and 19.3% for clinically healthy SAC (Tornquist et al., 2010). Despite the fact that we did not observe any acute cases, it may be possible that haemoplasmas cause anaemia in acute infections and that these events may pass unnoticed or are misdiagnosed by stock handlers or veterinarians.

In regard to infection state, no significant differences were observed when comparing males to females or llamas to alpacas. Neither of these factors seems to predispose SAC to a higher risk of infection.

Conclusion

In conclusion, this study showed 18.7% of European SAC to be infected with haemotropic mycoplasmas, but that the prevalence was neither influenced by gender, species or origin. These findings add to the knowledge of the epidemiology of *M. haemolamae* in imported and domestically bred South American camelids in central Europe. The discovery that older animals, compared to younger animals, are more likely to be carriers of *M. haemolamae* raises important questions about other unidentified factors involved in the transmission and dissemination of this organism. Furthermore, the detection of *M. haemolamae* in 18.7% of the animals suggests that haemoplasmas need to be considered by veterinarians as a possible cause of disease in SAC in Europe.

Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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