

MYCOPLASMOSIS IN CAMELIDS WITH OWN INVESTIGATIONS

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ABSTRACT

Very little is known about mycoplasma infections in camelids and therefore comprehensive research is needed. *M. haemolamae* which was formerly known as *Haemobartonella* and *Eperythrozoon* can cause severe disease in NWCs with anaemia, weight loss and depression. The newly classified bacterial species can also cause intrauterine infection of the foetus. Only PCR methods can distinguish between *M. haemolamae* and *A. marginale* infections. Haemotrophic mycoplasmas are transmitted by insect vectors. So far *M. haemolamae* has not been detected in OWCs but research is ongoing at CVRL to test dromedaries which suffer from unidentified anaemia and weight loss.

Several classical mycoplasmas have been isolated from dromedaries from different organs exhibiting lesions but it is not clear if these mycoplasmas were solely responsible for these changes. In NWCs no classical mycoplasmas have been isolated so far but antibodies to different known bovine and caprine strains have been reported.

In a recent respiratory disease outbreak in Iran which occurred during a cold spell in dromedaries, antibodies against Adenovirus and BRSV were found in connection with 4 different unidentified mycoplasmas. From these investigations it was hypothesised that classical mycoplasmas may disease camelids in connection with concurrent viral diseases.

Key words: Classical mycoplasmas, dromedaries, haemoplasmas, mycoplasmosis, NWCs

Classification and Aetiology

Little is known about the role of mycoplasmas in the aetiology of camelids, which may be explained by the lack of research done on these bacterial species.

Over the last decade classification of certain bacterial families has been dramatically changed based on the development of molecular techniques. This refers also to *Mycoplasmataceae*. Species of the Order *Rickettsiales* known as *Haemobartonella* and *Eperythrozoon* have been reclassified as belonging to *Mycoplasmataceae*. These bacteria are now named 'haemotrophic' mycoplasmas or 'haemoplasmas'. We now differentiate between "classical" and "haemotrophic" mycoplasmas and both can cause disease in camelids (Fig 1).

Mycoplasmas are the smallest organisms capable of autonomous replication; they have no cell wall and a reduced genome. Haemotrophic mycoplasmas are not only without cell wall, they are also non-cultivable bacteria. These are facultative, intracellular, erythrocytic bacteria. They are attached to red blood cells of mammals, leading to acute and/or subclinical diseases.

Mycoplasmas belong to the class *Mollicutes* with 4 Orders with various families and genera and more

than 200 species described to date. *Mollicutes* affect all vertebrates (including humans) while some species occur also in plants and arthropods. The list is not exhaustive since new species are constantly added like the haemotrophic organisms.

The genus *Rickettsiae* includes many species associated with human disease, including those with the Spotted Fever and Typhus groups and various authors have identified antibodies in camelids to *Rickettsiae* (*R. prowazekii*, *R. rickettsii*, *R. mooseri* and *R. conorii* with no disease or losses in camels (Wernery *et al*, 2013, in press).

Only recently haemoplasmas have not only been found in New World Camelids (NWCs), but also in many other animal species like in captive cervids, *Mycoplasma* (*M.*) *ovis* (Grazziotin *et al*, 2011); California sea lions "*Candidatus M. haemozalophi*" (Volokhov *et al*, 2011); bovines, "*Candidatus M. haemobos*" (Hornok *et al*, 2011). With PCR technology new haemoplasmas will be detected over the next years. For the first time, there is molecular evidence on the transplacental transmission of haemoplasmas in bovines (Hornok *et al*, 2011) and NWCs (Tornquist *et al*, 2011). Table 1 shows the most important mycoplasmas of veterinary importance.

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Mycoplasmosis in Camelids

Haemotrophic mycoplasma infections

Haemotrophic mycoplasmas, formerly known as *Haemobartonella* and *Eperythrozoon* species of the order *Rickettsiales* have been identified in NWCs in Europe (Kaufmann *et al*, 2007) and in America (Almy *et al*, 2006). These organisms are named *Mycoplasma* (*M.*) *haemolamae* (Table 1). It seems that haemoplasmas are now a well-known bacteria group in America and emerged also in Europe where more and more NWCs are kept. Haemotrophic mycoplasmosis has frequently been identified in young llamas (McLaughlin *et al*, 1990; Semrad, 1994). Juvenile llamas, from weaning to several years old, have been found to have apparent immunodeficiency disorders. Such llamas have a history of weight loss and stunted growth and develop acute or recurrent infectious conditions. Affected llamas usually die or are euthanised because of grave prognosis. In these cases, infections with uncommon pathogens or opportunistic microorganisms are often detected. During necropsy, severe fibrinous polyserositis involving the thoracic and abdominal organs, moderate diffuse non-suppurative interstitial pneumonia, splenic hyperplasia, necrotising enteritis, widespread vascular thrombosis and anaemic infarcts in the liver are observed. *Mycoplasma*- like

organisms have frequently been diagnosed in these immunodeficient llamas. There is an indication that these organisms are responsible for the anaemia, which often accompanies this ailment. These parasites are attached to the surface of red blood cells of the affected llamas and are often found in clusters, usually towards the edge of the cell (Fig 2) (Wernery *et al*, 1999).

Much progress has been made in the study of the haemotrophic mycoplasmas in camelids, and diagnostic testing has been greatly improved over the last few years. A PCR-based assay has been developed which was made available for diagnostic testing by the Veterinary Diagnostic Laboratory at Oregon State University's College of Veterinary Medicine (Tornquist, 2006; 2008). Specificity for *M. haemolamae* was shown by failure to identify other mycoplasmas species like *M. haemosuis*, *M. haemofelis*, and *M. genitalium*. All studies have elucidated, that many infections are subclinical, and clinical signs of infections with these organisms can vary widely. Clinical infections are associated with fever, mild to marked anaemia, depression, icterus, infertility, oedema, poor growth rate and mild to severe hypoglycaemia. It is not yet investigated, if these bacteriae may cause or serve as co-factors in some forms of immune suppression.

Table 1. *Mycoplasmatataceae* of veterinary importance and their diseases.

Family	Host	Genus (No. of species)	Important species (many more have been identified)
Mycoplasmatataceae	Cattle, sheep, goats	<i>Mycoplasma</i> (12)	<i>M. mycoides</i> subsp. <i>mycoides</i> (2x) , <i>M. agalactiae</i>
		<i>Acheloplasma</i> (1)	<i>M. mycoides</i> subsp. <i>capri</i> , <i>M. bovis</i>
		<i>Ureaplasma</i> (1)	<i>M. capricolum</i> subsp. <i>capripneumoniae</i> , <i>M. conjunctivae</i>
			<i>M. capricolum</i> subsp. <i>capricolum</i> , <i>M. ovis</i> <i>M. sp. bovine group 7</i>
	Horses	<i>Mycoplasma</i> (11)	<i>M. felis</i>
		<i>Acheloplasma</i> (8)	<i>M. equirhinis</i>
	Dogs and cats	<i>Mycoplasma</i> (15)	<i>M. canis</i> , <i>M. haemofelis</i> (<i>Haemobartonella felis</i>)
		<i>Acheloplasma</i> (1)	<i>M. cynos</i> , <i>M. gatae</i>
		<i>Ureaplasma</i>	<i>M. felis</i>
	Swine	<i>Mycoplasma</i> (13)	<i>M. hyopneumoniae</i> , <i>M. suis</i> (<i>Eperythrozoon suis</i>)
		<i>Acheloplasma</i> (5)	<i>M. hyorhinis</i>
		<i>Ureaplasma</i> (1)	<i>M. hyosynoviae</i>
	Camelids	<i>Mycoplasma</i> (2)	<i>M. haemolamae</i> (<i>Haemobartonella spp.</i>)
		<i>Acheloplasma</i> (2)	<i>M. arginini</i>
		<i>Ureaplasma</i> (?)	<i>A. laidlawii</i> , <i>A. oculi</i>
	Domestic birds	<i>Mycoplasma</i> (17)	<i>M. gallisepticum</i>
		<i>Acheloplasma</i> (2)	<i>M. meleagridis</i>
<i>Ureaplasma</i> (2)		<i>M. synoviae</i>	

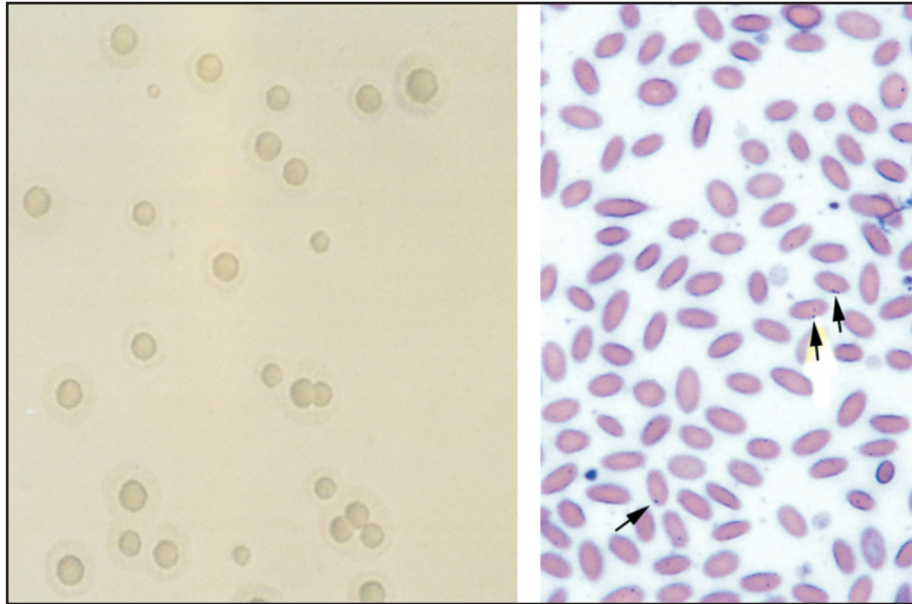


Fig 1. Classical mycoplasmas on selective agar (left) and haemotrophic mycoplasmas on camelids' erythrocyte surface as bluish spots (arrows directed to bluish spots).

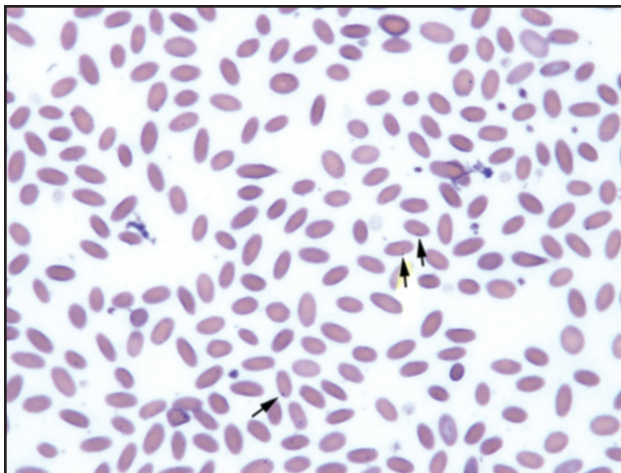


Fig 2. *Mycoplasma haemolamae* in a young llama suffering from immune-deficiency disorder, bacteria attached to surface of red blood cells (arrows directed to bluish spots). (Giemsa stain, courtesy of Dr. Tornquist, USA).

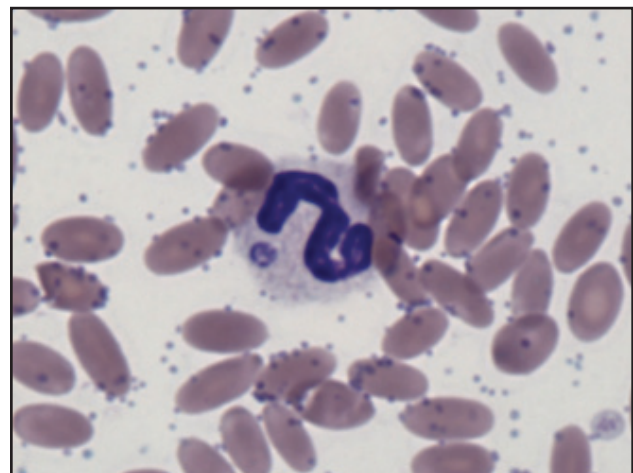


Fig 3. Infection with *M. haemolamae* and *A. phagocytophilum* (morula in cytoplasm of a neutrophil, courtesy of Dr. K. Lascola, USA). M. h. = *Mycoplasma haemolamae* & A. p. = *Anaplasma phagocytophilum*.

An interesting observation was made by Almy *et al* (2006). The authors examined blood smears of a 4day-old alpaca cria, which revealed massive erythrocyte parasitism by *M. haemolamae*. Blood was collected from both the nonparasitaemic dam and the cria. Both were positive in the PCR for this parasite indicating a possible in utero transmission.

Kaufmann *et al* (2007) were the first to report *M. haemolamae* infections in alpacas in Europe. However, the authors could not elucidate if the infection was brought from Peru or if it originated from Switzerland, although no arthropod vectors were evident on the pastures where the alpacas were

grazing yearlong. As mentioned before, a novel haemoplasma species in California sea lions was recently described by Volokhov *et al* (2011). The 16S rRNA-based phylogenetic analysis demonstrated that this novel haemoplasma species was phylogenetically most closely related to the known haemoplasma species *M. haemolamae* described in alpacas (Almy *et al*, 2006; Lascola *et al*, 2009).

It is worthwhile mentioning that *M. haemolamae* infections are indistinguishable from *Anaplasma* (*A.*) *marginale* infections when stained blood smears are investigated. Also *A. marginale* which has been described to occur in camelids (Ajayi *et al*, 1984;

Alsaad, 2009) parasitise in red blood cells. These publications as well as the report from Nazifi *et al* (2009) who described haematrophic mycoplasmas in Iranian dromedaries should be carefully evaluated because no PCRs were applied for the diagnosis and differentiation of diseased dromedaries.

Furthermore, double infections of *A. phagocytophilum* and *M. haemolamae* have been described (Lascola *et al*, 2009). However, *A. phagocytophilum* infection (tick-borne fever) and haemoplasmosis can easily be differentiated from each other because *A. phagocytophilum* parasitise in white blood cells (Fig 3).

Several diseases are caused by this organism like tick-borne fever in ruminants, equine granulocytic anaplasmosis and human granulocytic anaplasmosis (Nieder *et al*, 2012).

Infection with *A. phagocytophilum* should be named “camelid granulocytic anaplasmosis” (camelid tick-borne fever) and infection with *A. marginale* “camelid anaplasmosis”.

The mode of transmission of *M. haemolamae* has not yet been determined in camelids although biting insect vectors are suspected (Anonymous,

2011). Blood sucking arthropods like horn fly (*Haematobia irritans*), stable fly (*Stomoxys calcitrans*) and two species of horse flies (*Tabanus bovinus*, *T. bromius*) were identified for the transmission of bovine haemolamas (Hornok *et al*, 2011). The transplacental transmission has been confirmed in bovines and NWCs (Hornok *et al*, 2011; Tornquist *et al*, 2011).

Classical mycoplasma infections

As reported *M. haemolamae* does not only cause a subclinical infection but also a severe disease contrary to classical mycoplasmas. Although pathogenic for several animal species, they do not seem to play an important epidemiological role in camelids.

Basic differences of opinion exist whether camelids are susceptible to contagious bovine pleuropneumonia (CBPP) caused by *M. mycoides*. Most opponents believe that the proponents have confused pulmonary changes due to *Pasteurella* with those due to *M. mycoides*. Walker (1921) was not able to elicit this pulmonary disease through the subcutaneous application of “virulent lymph”. Samartsev and Arbuzov (1940), Hutyra *et al* (1946), Curasson (1947) and Turner (1959) are of the opinion

Table 2. Literature survey regarding anaplasma and haemoplasma infections in OWCs and NWCs.

Species	Author	NWC/ OWC	Year	Country	Serol.% Prevalence	Bacteria detection
<i>Anaplasma</i>	Monteverde	OWC	1937	Somalia	-	40%
	Anonymous	OWC	1939		-	-
	Anonymous	OWC	1960		-	-
	Ristic and Kreier	OWC	1974		-	-
	Ristic	OWC	1977		-	-
	Anonymous	OWC	1981	Somalia	-	4.4%
<i>A. marginale</i>	Ajayi <i>et al</i>	OWC	1984	Nigeria	10.7	-
	Wernery <i>et al</i>	OWC	2007	UAE	0.5	-
	Alsaad	OWC	2009	Iraq	-	52 positive
<i>A. phagocytophilum</i>	Barlough <i>et al</i>	NWC	1997	USA	-	1 llama
	Wernery <i>et al</i>	NWC	1999	UAE	-	1 guanaco
	Wernery <i>et al</i>	OWC	2001	UAE	-	3 positive
<i>Mycoplasma haemolamae</i>	McLaughlin <i>et al</i>	NWC	1990	USA	-	14.1%
	Semrad	NWC	1994	USA	-	1 llama
	Almy <i>et al</i>	NWC	2006	USA	-	1 cria
	Kaufmann <i>et al</i>	NWC	2007	Switzerland	-	4 positive
	Marsh	NWC	2010	USA	-	-
	Al-Khalifa <i>et al</i>	OWC	2009	Saudia Arabia	-	10%
	Nazifi <i>et al</i>	OWC	2009	Iran	-	?
<i>A. ruminantium</i>	Karrar <i>et al</i>	OWC	1963		-	-
	Karrar	OWC	1968	Sudan	-	-

- = Not tested or negative

that camels are not susceptible to contagious bovine pleuropneumonia, though they have not provided any supporting scientific proof. However, those scientists who purport that camels are susceptible to this disease have not supplied any proof of their theory either. This group includes Vedernikoff (1902) and Kowalevsky (1912) who supposedly have often observed respiratory CBPP among Bactrian camels in Kazakhstan. This has also been reported by Davies (1946).

Bares (1968), who reported finding very low (non-specific?) antibody titers using complement fixation against *M. mycoides* in dromedary sera from Chad, is of the opinion that dromedaries are most likely not susceptible to CBPP, and that they play no role in the epizootiology of this disease. All earlier publications implicating *M. mycoides* as a cause of pulmonary changes in the camel should therefore be interpreted with reservation.

Paling *et al* (1978) identified antibodies against contagious caprine pleuropneumonia (*Mycoplasma* strain F38) in 49% of the dromedary sera examined in Kenya. The significance of these results is unclear since the causative agent was not isolated.

Although it has not yet been possible to isolate

M. mycoides from pulmonary lesions in camels, other *Mycoplasma* species have been cultivated from the respiratory tracts of healthy dromedaries. In Egypt, Refai (1992) was able to identify the following isolates from the anatomical sites given:

- Mycoplasma arginini*: nose
lung
mediastinal lymph nodes
- Acheloplasma laidlawii*: respiratory tract
- Acheloplasma oculi*: nose

Elfaki *et al* (2002) examined 100 dromedaries with pneumonic lesions and isolated *M. arginini* from 8.8%. The camels from which these *mycoplasmas* were cultured had developed an interstitial pneumonia. However, it was not elucidated, if *M. arginini* was responsible for these lesions. El-Metawally *et al* (2010) examined slaughtered female dromedary camels in Egypt for the presence of classical *mycoplasma* in different organs. Tissue samples were taken from lung, uterus, cervix, vagina and mammary gland for *mycoplasma* cultivation. *M. arginini* was isolated in 6% of lungs and 2% of vagina samples. Although pathological changes were observed in lungs a relationship between the lesions and the isolates could not be made. Suheir *et al* (2005) isolated *M. arginini* and *A. laidlawii* from camel milk in Sudan but were also not able to conclusively emphasise their pathogenic role in camel mastitis.

In the Andean region of Peru, 757 alpacas, llamas and vicuñas were tested with the indirect haemagglutination test against *mycoplasmas* (Hung *et al*, 1991). The animals did not show any clinical signs but revealed antibodies against the following *mycoplasma* species: *M. mycoides* subsp. *mycoides* LC; 554 alpacas: 5% positive; 141 llamas: 15.6% positive; *M. capricolum* and F38 biotype; 0.9% alpacas and 0.2% llamas positive. Only one reactor was diagnosed to *M. m. mycoides* LC and *M. capricolum* in vicuñas, and none of the entire flock had antibodies against *M. mycoides* subsp. *capri* or *M. agalactiae*.

Table 3 highlights the international literature on classical mycoplasmosis in camelids.

Laboratory diagnosis

Diagnosis of *M. haemolamae*

For the laboratory diagnosis of haemotrophic *mycoplasmas*, unclotted blood and eventual tissue samples should be dispatched to the laboratory. Haemotrophic *mycoplasmas* do not grow on agars.

Animals with high parasitaemias can be

Table 3. Literature survey of classical mycoplasmosis in camelids.

Author	Year	Mycoplasma species
Vedernikoff	1902	<i>M. mycoides</i> positive
Kowalevsky	1912	<i>M. mycoides</i> positive
Samartsev and Arbuzov	1940	<i>M. mycoides</i> neg.
Hutyra <i>et al</i>	1946	<i>M. mycoides</i> neg.
Davies	1946	<i>M. mycoides</i> positive
Curasson	1947	<i>M. mycoides</i> neg.
Turner	1959	<i>M. mycoides</i> neg.
Bares	1968	Serology positive <i>M. mycoides</i> very low
Paling <i>et al</i>	1978	Serology 49% F38
Hung <i>et al</i>	1991	Llama, alpaca, vicuña serology positive against different species
Refai	1992	<i>M. arginini</i> <i>A. laidlawii</i> culture <i>A. oculi</i>
Suheir <i>et al</i>	2005	<i>M. arginini</i> culture <i>A. laidlawii</i>
Elfaki <i>et al</i>	2002	Serology 9% <i>M. arginini</i>
El-Mutwally	2010	<i>M. arginini</i>
Wernery and Kinne	2012	Several unidentified strains

Table 4. Laboratory diagnosis of anaplasmosis and mycoplasmosis.

Disease	Laboratory diagnosis	Appearance of agent in Giemsa-stained smears
Camelid anaplasmosis and mycoplasmosis	Wright-Giemsa, acridine orange blood smears. PCR, Serology	Reddish-violet-purple cocci to ring-shaped bodies in RBCs or WBCs
(<i>Anaplasma marginale</i> , <i>Anaplasma phagocytophilum</i> , <i>Mycoplasma haemolamae</i>)		cave: Differentiation between <i>M. haemolamae</i> and <i>A. marginale</i> only by PCR

diagnosed through microscopic detection of the bacteria in Wright-Giemsa stained blood smears. They appear as basophilic punctate to ring-shaped bodies (Fig 2) on the surface of erythrocytes. Low parasitaemia is often difficult to diagnose, as organisms resemble Howell-Jolly bodies (Wernery *et al*, 1999) or background debris. It cannot be differentiated from *A. marginale* infection with this method. Organisms stain well with acridine orange but require a fluorescent microscope for viewing (Marsh, 2010).

Polymerase Chain Reaction (PCR) is the method of choice especially in blood-smear inconclusive or negative cases and can be performed on EDTA blood or tissue samples. Meli *et al* (2010) developed a real-time TaqMan qPCR assay for detection of *M. haemolamae* in camelids. This assay is based on the 16S rRNA gene and amplified “Candidatus *M. haemolamae*” DNA, but not on DNA from other haemotrophic *mycoplasma* species. The samples should be dispatched to the laboratory in RNA later. Real-time PCRs are also nowadays increasingly used in the diagnosis of *A. marginale* infections. Serological testing *M. haemolamae* is not routinely carried out but there are studies using *M. suis* as an antigen in an indirect haemagglutination test. Serological testing for *A. marginale* antibodies is performed by using an indirect ELISA from VMRD, Inc. (Wernery *et al*, 2007).

The laboratory diagnosis of anaplasmosis and mycoplasmosis is summarised in Table 4.

Diagnosis of classical mycoplasmas

Mycoplasmas are very fragile bacteria and specimens must be kept refrigerated and delivered to a laboratory as soon as possible, optimal within 72 hours. If swabs are taken they must be transported in transport medium. Specimens should be obtained from animals during early stages of the clinical disease to avoid overgrowth with other bacterial species. Mycoplasmas are fastidious organisms, which require selective agars with supplements. Inoculated agar plates are incubated in a humid atmosphere at 37°C and 5% CO₂ and 95% N₂ for at least 6-10 days. Pure cultures of the isolated mycoplasmas should

be obtained to carry out identification methods. Many different identification technologies are available but precise identification of the species often requires sophisticated techniques and cultures are usually submitted to reference laboratories for final identification. Species-specific DNA probes and PCRs have been developed for the identification of some species. As mentioned later, several classical mycoplasmas from dromedaries could not be completely identified due to lack of probes or primers. So far none of the classical mycoplasmas isolated from camels has been identified as a primary cause of disease.

Treatment and control

Long-acting tetracycline is recommended to treat *M. haemolamae* infections, but even repeated injections may not completely eliminate infections. Some authors even recommend a tetracycline treatment for upto 50 days to increase the chance of elimination (McLaughlin *et al*, 1990). Other antibiotics like florfenicol or enrofloxacin were also not capable in clearing the infection and there was no significant difference between the two drug formulations (Tornquist, 2006). Due to the lack of cell wall, mycoplasmas are resistant to β-lactam antibiotics (penicillin and cephalosporin group), which are therefore added to transport media. The classical mycoplasmas are quite sensitive to macrolides (erythromycin, spiramycin and tylosin), tetracyclines, quinolones and chloramphenicol but therapeutic success often depends on the stage of infection. So far, no vaccines for camelid mycoplasmosis are available.

Insect control is another important step to avoid infections with haemotrophic mycoplasmas.

Own investigations

Mycoplasma haemolamae

As reported *M. haemolamae* can be a devastating disease in NWCs but has not been reported to occur in OWCs. Therefore CVRL started a research project in dromedaries in the UAE in spring 2012. EDTA blood from dromedaries with undiagnosed anaemia was tested with RT-PCR for *M. haemolamae*. Fifteen

dromedaries which showed RBC counts of less than $6 \times 10^{12}/L$ and haemoglobin values of less than 8g/dl were tested. No positive cases were detected in dromedaries. However, the number of animals tested is far too small to come to any conclusion if dromedaries may also contract haemoplasma infection.

Classical mycoplasma infections

As reported by Wernery and Kinne (2012), a severe respiratory disease occurred in spring 2011 in Iran spreading to Pakistan involving thousands of dromedaries with a great number of fatalities. It was reported by promed that during this outbreak a severe cold with gusty winds had hit these countries. Thirty four blood samples and nasal swabs were received from diseased Iranian dromedaries. No virus was isolated from these samples but all sera were positive for Adenovirus and Bovine Respiratory Syncytial Virus (BRSV) antibodies. Although no mycoplasma transport medium was used, 5 unidentified mycoplasmas were isolated which were similar to:

<i>M. phocidae</i>	96%
<i>M. anseris</i>	95%
<i>M. alkalescens</i>	95%
<i>M. canadense</i>	95%

It was concluded that classical mycoplasmas may disease dromedaries in combination with concurrent viral infections.

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