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NO EVIDENCE OF *Mycoplasma haemolamae* AND *Anaplasma marginale* IN ANAEMIC DROMEDARIES IN THE UNITED ARAB EMIRATES

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ABSTRACT

Haemotrophic mycoplasmosis or haemoplasmosis, caused by the haemotrophic mycoplasma species *Candidatus Mycoplasma haemolamae* (formerly *Haemobartonella*), has been described in both Old World camels (OWCs) and New World camels (NWCs) from different countries. Camelid anaplasmosis (formerly Ehrlichiosis) caused by *Anaplasma marginale* has been described in OWCs only. Knowledge of these pathogens in camels in the United Arab Emirates (UAE) is missing. We investigated 55 clinically healthy, but slightly anaemic dromedaries in the UAE for the occurrence of haemotrophic mycoplasmas and *Anaplasma marginale* using blood smear investigations and polymerase chain reaction (PCR). In the blood smears, neither of the two pathogens was detectable and the investigations using PCR methods did not reveal any DNA from *Cand. M. haemolamae* or *A. marginale* in 55 slightly anaemic UAE dromedaries. So far, the cause of anaemia in those dromedaries remains to be further analysed.

Key words: *Anaplasma marginale,* Arabian Peninsula, ticks, camelids, *Candidatus Mycoplasma haemolamae*

Haemotrophic mycoplasmas or 'haemoplasmas' cause epi-or intra-erythrocytic infections in different animal species. Formerly, classified within the genera *Haemobartonella* and *Eperythrozoon* (order *Rickettsiales*), they are now belonging to the class *Mollicutes* with 4 orders, various families and genera and more than 200 species described to date. New species are constantly added as new haemotrophic organisms. For example, only recently haemoplasmas have been found in captive cervids (*Mycoplasma ovis;* Grazziotin *et al,* 2011), California sea lions (*Candidatus M. haemoza lophi;* Volokhov *et al,* 2011), bovines (*Candidatus M. haemobos*), horses (Dieckmann *et al,* 2010; Hoelzle *et al,* 2010) and New World Camels (NWCs) (Kaufmann *et al,* 2007; Tornquist *et al,* 2011). Infections with haemotrophic mycoplasmas may cause severe clinical signs such as anaemia, ill-thrift, weight loss, stunted growth, and development of acute or recurrent infections as well as no clinical signs. There is molecular evidence of transplacental transmission of haemoplasmas in bovines (Hornok *et al,* 2011) and in NWCs (Tornquist *et al,* 2011). Indication of haemotrophic mycoplasma infections is often based on microscopic findings of stained blood smears but recently molecular biological tools are used making diagnosis more reliable (Wernery, 2012).

Anaplasmosis is a disease of domestic and wild animals caused by intracellular organisms belonging to the genus Anaplasma, family *Anaplasmataceae,* order *Rickettsiales*. Four intra-erythrocytic species of Anaplasma are currently differentiated: *A marginale, A. centrale, A.caudatum* in cattle and *A. ovis* in sheep and goats. *A. marginale* occurs worldwide in tropcical and subtropical regions in cattle and is transmitted by several hard tick species. It causes a major constraint to livestock production, e.g. in African countries. Clinical signs of anaplasmosis caused by *A. marginale* are similar to infections with haemoplasmas and have been described in Old World Camels (OWCs) and NWCs as camelid anaplasmosis (Wernery *et al,* 2014). Both haemoplasmas and anaplasmas can be transmitted by ticks and most likely also mechanically by biting flies, such as tabanids and stable flies, and lice as proven for the transmission of bovine haemoplasmas (Hornok *et al,* 2011).

Knowledge on the occurrence of haemotrophic mycoplasmosis and camelid anaplasmosis in dromedaries in the United Arab Emirates (UAE) is missing. However, due to the occurrence of the hard tick *Hyalomma dromedarii* infesting dromedaries in the UAE, and anaplasmosis occurring in wildlife and cattle (U. Wernery, personal communication)

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we hypothesised that the causing pathogens may also occur in the UAE. The aim of this study was to investigate if dromedaries from the United Arab Emirates (UAE) showing slight anaemia are infected with *Cand. M. haemolamae* or *A. marginale* or both.

Materials and Methods

Sample collection

In total 55 EDTA blood samples were taken from the jugular vein of slightly anaemic dromedaries. They were chosen randomly over a period of four weeks from a total of 428 dromedaries due to their slight anaemia. Blood samples are regularly taken for a general health check. Most camels in the UAE are infested with *H. dromedarii*. Twelve of the dromedaries were from dairy camels more than 10 years old and the remaining originated from 2 to 8 year-old racing dromedaries. These animals were tested antibody-negative against *Trypanosoma evansi* using an in house indirect antibody enzyme linked immuno assay (iELISA). All tested camels were otherwise healthy and in good condition. The EDTA-blood of the dromedaries was tested on the day of blood collection for red blood cells (RBC), haemoglobin (Hb), platelets (PLT) using an automated haematology analyser (Sysmex, Japan), whereas, iron was tested in an automated biochemistry analyser (Hitachi, Germany (Roche)). Blood smears were Giemsa stained and checked under microscope using a 1000 magnification.

DNA extraction

The remaining samples were then frozen overnight and DNA extracted on the following day with the QiagenDNeasy blood and tissue kit (Qiagen Ltd., Crawley, United Kingdom) according to the manufacturer's instruction. Briefly, 200µl of blood mixed with 20µL of proteinase K. After adding 200µl of Buffer AL the mixture was vortexed and incubated at 56°C for 10min. Then, 200µL ethanol (96–100%) was mixed with the sample and it was loaded into a DNeasy Mini Spin Column for purification and DNA was eluted in 50µL of elution buffer and stored at 4°C. The extracted DNA of all 55 dromedaries were dispatched to the Institute of Comparative Tropical Medicine and Parasitology in Munich, Germany, for DNA detection of *M. haemolamae* and *A. marginale* by molecular methods. After arrival, the quality and quantity of DNA were checked with a spectrophotometer (NanoDrop®1000, Peqlab, Erlangen, Germany).

Real-time Polymerase Chain Reaction (PCR)

Cand. Mycoplasma haemolamae and *A. marginale* were detected with species specific real-time PCRs using TaqMan® probes (Meli *et al,* 2010, Carelli *et al,* 2007). Both real-time PCRs were run on an AB7500 fast real-time PCR cycler (Applied Biosystems, Darmstadt, Germany) in a total 15µl reaction volume: 10 µl of the Universal fast TaqMan® Mastermix (Applied Biosytems), 1.0 µl of molecular grade water, 1.8µl of the respective forward and reverse primers (10µM), 0.4µl of the respective probe (10µl) and 5µl of the extracted DNA. The temperature protocol was the same for both real-time PCRs: an initial activation step at 95°C for 20 sec, followed by 40 cycles of 95°C, 3 sec. and 60°C, 30 sec. In every PCR run, a negative control (molecular grade water) and a positive control was used. The *A. marginale* positive control was genomic DNA extracted from a cell culture isolate, and for *Cand. M. haemolamae*, DNA from a linearised plasmid containing the sequence amplified by the real-time PCR assay was used, provided by Marina Meli, Zurich.

Results

The mean values of 4 blood parameters of 55 dromedaries are shown and compared with reference values in Table 1.

Table 1. Mean values of selected blood parameters in the 55 dromedaries investigated.

Parameters	Units	Reference Values*	Found Values**
Red Blood Cells	10^{12}/L	7 – 10.5	6.7 ± 0.6 SD
Haemoglobin	g/dl	10.5 – 14.5	9.4 ± 0.7 SD
Platelets	10^{9}/dl	270 – 600	248 ± 100 SD
Iron	µmol/l	15 – 27	12 ± 10 SD

* Wernery *et al* (1999) ** SD = Standard Deviation

All four parameters red blood cells (RBC), haemoglobin (Hb), platelets (PLT) and iron were slightly decreased when compared with reference values of dromedaries from the UAE (Wernery *et al,* 1999). No evidence of intraerythrocytic inclusion bodies was detected in any of the blood smears. Furthermore, in none of the 55 EDTA blood samples DNA of *M. haemolamae* nor of *A. marginale* were detected.

Discussion

Candidatus M. haemolamae has been identified in NWCs and in OWCs (Wernery *et al,* 2014). Haemoplasmas are now a well-known bacteria group in North and South America and emerging also

in Europe where more and more NWCs are kept. Haemotrophic mycoplasmosis has frequently been identified in young llamas and alpacas (Crosse *et al,* 2012; Lascola *et al,* 2009; Tornquist, 2006). Juvenile llamas from weaning to several years old have been found to have apparent immunodeficiency disorders. Such animals have a history of weight loss, stunted growth and develop infectious conditions. Such animals usually die or are euthanised because of unfavourable prognosis. *Mycoplasma*-like organisms have frequently been diagnosed in immunodeficient NWCs and there is an indication that these organisms are responsible for anaemia which often accompanies this ailment. However, there are also reports of NWCs infected with haemoplasmas without showing clinical signs. In southern England, 29% alpacas were found positive using PCR or denaturing gradient gel electrophoresis (DggE) without being anaemic (Crosse *et al,* 2012). Almy *et al* (2006) reported a case of a *Cand. M. haemolamae* infection in a 4 day-old cria suggesting *in utero* transmission. Much progress has been achieved in the study of haemotrophic mycoplasmas in camelids and diagnostic testing has been greatly improved over the last few years due to the introduction of a real-time PCR assay (Meli *et al,* 2010), since it is difficult to differentiate *Cand. M. haemolamae* from *Anaplasma* species by light microscopy.

Natural infection with *Mycoplasma* spp. was observed in 67 adult Iranian dromedaries (Nazifi *et al,* 2009) in Giemsa stained blood smears. In infected camels, the number of RBCs, Hb and haematocrit (packed cell volume) was significantly decreased and a normocytic and normochromic anaemia was observed with lower serum glucose concentration, whereas Al Khalifa *et al* (2009) in Saudi Arabia found a much lower infection rate. Anaemia has been frequently detected in dromedaries in the UAE and is of great concern because it may have a negative effect on racing performance. In the cases of the present study which were negative for *T. evansi* in the antibody ELISA, some blood parameters were slightly decreased as shown in the Table 1, but none of the 55 blood samples were positive in the PCR. Beside the 4 *Anaplasma* species mentioned earlier, *A. phagocytophilum* has also been diagnosed in NWCs and OWCs (Wernery *et al,* 2001; Barlough *et al,* 1997), but these organisms are found in white blood cells as 'morulae' which can be easily differentiated from *Anaplasma marginale* and *M. haemolamae* (Wernery, 2012).

In the older literature describing camelid anaplasmosis due to *A. marginale* in dromedaries, only subclinical cases or infections in healthy dromedaries were reported (Anonymous, 1939; Anonymous 1960; Maurice *et al,* 1967). Ristic and Kreier (1974), Ristic (1977) and Ajayi *et al* (1984) found antibodies to *A. marginale* in 10.7% (3/28) of Nigerian camel sera using 3 different serological tests. Recently, Wernery *et al* (2007) tested a total of 1,119 sera from dairy camels with a competitive ELISA that detects antibodies to *A. marginale, A. centrale* and *A. ovis* (Veterinary Medical Research and Development, Pullman, USA). With this cELISA only 5 antibody – positive animals (0.5%) were found, although the camels tested had *Hyalomma dromedarii* infestations. However, Alsaad (2009) reported a disease in dromedaries in Iraq caused by *A. marginale.* During that outbreak, 52 dromedaries, naturally infected with *A. marginale*, showed signs of pale mucous membrane, loss of appetite, emaciation, coughing, lacrimation and rough hair coat. A macrocytic, normochromic anaemia was diagnosed, and Anaplasma spp. detected as spherical granules near the periphery inside infected RBCs. A significant decrease of RBCs, HB and PCV was also noticed and several ticks were detected on the body.

In conclusion, none of the 55 blood samples from slightly anaemic dromedaries showed any evidence of *A. marginale* and/or haemotrophic mycoplasmas, neither in the Giemsa-stained blood smears nor by PCR methods. It seems that both diseases may so far, do not play a role in anaemia in dromedaries of the UAE.

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