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INTRAVENOUS INFECTION OF DROMEDARY CAMELS WITH *Anaplasma marginale*

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ABSTRACT

Three dromedary camels were infected with the Rickettsia, *Anaplasma marginale*. One American Type Culture Collection strain was intravenously injected into the jugular veins of two adult dromedary camels. Additionally, EDTA blood from a bovine bull naturally infected with *Anaplasma marginale* was also intravenously injected into the jugular vein of a third camel. All three camels were tested for *A. marginale* using PCR, Giemsa staining and serology. All tests were negative and the dromedary camels remained healthy throughout the study.

Key words: *Anaplasma marginale* infection, dromedary camels

Anaplasmosis, formerly known as "gall disease" refers to a bacterial disease of ruminants and human beings, caused by obligate intraerythrocytic pathogens. These bacteria belong to the order Rickettsiales family *Anaplasmataceae*, genus *Anaplasma*. The second and third family within Rickettsiales are named Orientia and Midichloriaceae, which infect human beings. The bacterial classification of Rickettsiales is complex and continuously evolving. It follows hierarchical systems which consist of morphological, physiological, staining, biochemical and nowadays DNA homology criteria. Most of these obligate bacterial species – totalling more than 100 are either unclassified, uncultured or "candidatus", awaiting formal classification, (Wernery, 2022).

The family *Anaplasma*taceae comprises of 5 genera which are:

Anaplasma
Ehrlichia
Neorickettsia
Aegyptianella and
Wolbachia

Only few species of the genus *Anaplasma* are pathogens of veterinary importance, which are listed by Markey *et al* (2013) and four intraerythrocytic species of *Anaplasma* are included in the list of bacterial names with standing in nomenclature (Euzéby, 2005).

Anaplasma (A.) marginale, A.centrale in cattle and *A. ovis* in sheep and goats.

Although (A.) marginale, A.centrale, A. platys, A. phagocytophilum, A. canis, A.ovis and A. canditatus cameli have been identified in Old World Camels (OWCs) by several researchers using molecular biological techniques, none of the investigated camels exhibited clinical signs indicating a possible asymptomatic carrier status (Wernery, 2022).

To determine whether dromedaries develop disease caused by *Anaplasma* species, three dromedaries kept at the Central Veterinary Research Laboratory (CVRL), Dubai were infected with one strain of *A. marginale* received from American Type Culture Collection, USA and with 1.8ml EDTA blood from a Pakistani bovine bull naturally infected with *A. marginale*.

Materials and Methods

Dromedary camel

The *Anaplasma marginale* Theiler strain VR-1436, ordered from ATCC, USA was intravenously injected into two female adult dromedary camels housed at CVRL. The strain was sent on dry ice to CVRL in vials containing 1ml of bovine blood diluted in 5ml NaCl in each vial. Both camels received 5 ml of this diluted suspension into their jugular veins. A small aliquot of 100µl was retained for PCR testing and microscopic staining. Twenty-four hours before injection baseline EDTA blood samples were tested by PCR and serum samples were tested using an antibody ELISA. Following infection, blood

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samples were collected daily and tested for the presence of *A. marginale* via stained blood smears and PCR. Antibody response was evaluated using the ELISA.

A third dromedary camel housed at CVRL premises was injected intravenously with 1.8ml EDTA blood collected from a Pakistani bovine bull, naturally infected with the pathogen (Fig 1).

Diagnostic testing for this dromedary camel blood followed the same procedure as mentioned above: daily microscopic staining and PCR were performed for 14 days. The ELISA test was performed three times (one prior to infection and twice post-infection).

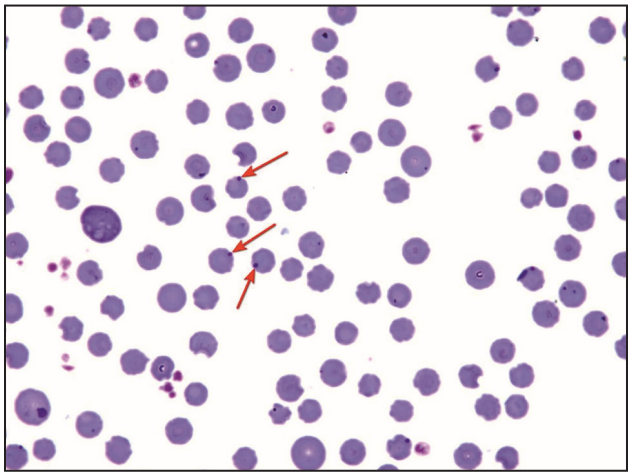

Fig 1. Blood smear stained with Giemsa showing *Anaplasma marginale* in the bovine erythrocytes (arrow, x 100 oil)

Stain

EDTA blood was drawn from the jugular veins of all three dromedaries, carefully mixed and prepared as thin blood smears on glass slides and stained with Giemsa. The stained slides were examined on oil immersion light microscope at a magnification of 1000 (100x objective).

Serology

For the investigation whether antibodies against *A. marginale* were produced, a commercial competitive ELISA from US was used (VMRD), which detects antibodies against *A. marginale, A.centrale* and *A.ovis*.

The serum of all three dromedary camels were tested three times, one time before infection and twice after infection.

PCR

PCR test was performed according to Stuen *et al* (2003).

Genomic DNA was extracted from whole blood samples using the QIAsymphony DNA Mini Kit (Cat. No. 931236; Qiagen) on the QIAsymphony automated platform, according to the manufacturer's instructions.

The extracted DNA was then subjected to polymerase chain reaction (PCR) using species specific oligonucleotide primers (16S ANA-F and 16S ANA-R) targeting a fragment of the *Anaplasma* 16S rRNA gene, as previously described by Stuen *et al* (2003), with modifications to the thermal cycling conditions. The cycling protocol consisted of an initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 61°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. The primer pair amplifies a product of 421 base pairs (bp), excluding the primer-binding regions. PCR amplicons were separated by agarose gel electrophoresis, and the presence of a target fragment of approximately 468 bp, inclusive of primer sequences, was evaluated against a positive control.

Results

No *A. marginale* organisms were detected in any of the Giemsa-stained blood smears and all PCR assays for *A. marginale* remained negative across all sampling days. Furthermore, no antibodies against *A. marginale* were detected by cELISA. However, PCR confirmed the presence of *A. marginale* in the incoclum and *A. marginale* bacteria were clearly visible in the bovine bull's EDTA blood (Fig 1).

Discussion

Rickettsiae are obligate bacteria, that replicated inside the host cells particularly within endothelial cells of small blood vessels and blood cells. Their requirement for an invertebrate vector distinguishes these microorganisms from other bacterial species. Transmission typically occurs as a result through the bite of an infected arthropod, primarily ticks. Rickettsiae are Gram-negative coccobacilli or short rods, that cannot grow in cell free media; they require living host cells for their replication and are cultured in cell lines or in yolk sac of embryonated hen eggs.

Anaplasmosis occurs in tropical and sub-tropical regions worldwide including South and Central America, USA, Southern Europe, Africa, Asia and Australia. At least 17 different tick species have been reported to transmit this pathogen to human beings and animals.

Bovine anaplasmosis, caused by *A. marginale* is an economically important tick-borne disease of

cattle, which invades cattle red blood cells (Fig 1) and causes anaemia, fever and weight loss. 40-50% of red blood cells can become infected; however, haemoglobinaemia or haemoglobinurea do not typically occur and which helps in differentiating anaplasmosis from babesiosis. In camelid literature, clinical anaplasmosis disease has not been reported. The primary vector in cattle is the hard tick *Rhipicephalus* spp., which does not infest dromedary camels in the UAE.

Furthermore, Perveen *et al* (2021), examined 562 *Hyalomma* ticks collected from different ruminants including camels from different areas of the UAE and detected no *Anaplasma* bacteria via PCR.

These findings suggest, that camel anaplasmosis is absent in the UAE due to the lack of a suitable vector. Moreover, our experimental infection demonstrates that dromedary camels are refractory to *A. marginale* infection, showing no signs of bacterial propagation, PCR positivity or seroconversion. Dromedary camels appear to be innately resistant to *Anaplasma marginale*.

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