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Short Communication

TRICHOMONOSIS IN DROMEDARY BULLS

Ulrich Wernery[1], Saritha Sivakumar[1], Klaus Henning[2], Helmut Hotzel[2], Anne Busch[2] and Rolf Karl Schuster[1]

[1]Central Veterinary Research Laboratory, Dubai, UAE

[2]Friedrich-Loeffler-Institut (FLI), Institute of Bacterial Infections and Zoonoses, Jena, Germany

ABSTRACT

During a routine health check of 12 breeding dromedary bulls using microscopy two of them were found to harbour trichomonad flagellates in their preputial washings. A PCR conducted on one of the samples did confirm trichomonads but not *Tritrichomonas foetus*. DNA sequencing identified the organism as *Tetratrichomonas* species. After repeated careful washings of the prepuce with oxidised water resampling revealed negative results.

Key words: Dromedary, PCR, sequencing, *Tetratrichomonas*, Trichomonosis

Trichomonads are small protozoan organisms which can be detected in many wildlife species and domestic animals. Usually, they are non-pathogenic commensals or cause relatively mild diseases. A clinically significant representative of this group is the species *Tritrichomonas foetus*, the causative agent of bovine trichomonosis. Trichomonosis is a venereal disease of cattle primarily characterised by early foetal death and infertility which may also result in extended calving intervals. Trichomonosis occurs worldwide (Tenter, 2006). *Tritrichomonas foetus* has three flagellas at its anterior end and an undulating membrane which makes it very motile. When cows are bred naturally by an infected bull, 30 to 90 % may become infected. Transmission of the parasite may also occur when semen from infected bulls is used for artificial insemination even when semen was frozen (Merck, 2016).

Tritrichomonas foetus is killed by drying or high temperature. Therefore, preputial washes have to reach the laboratory within 48 hours after collection. Various imidazole preparations have been used in bovines to treat bulls, but re-infection after treatment occurs. Successful treatment is measured by repeated sampling at least 3 times after treatment. No information is available of treatment in camel bulls. Literature on trichomonosis in camels is rare and there is only one report on this disease in a dromedary breeding herd from which a flagellate was isolated from 24 out of 48 dromedaries suffering from endometritis (Wernery, 1991). Here, we report a trichomonad infection in two dromedary breeding bulls.

Materials and Methods

In preparation of the 2018/2019 camel breeding season the Camel Reproduction Centre in Dubai, United Arab Emirates (UAE), performed a health check of 12 breeding bulls in late August 2018. For this, a canine urinary catheter was inserted in the distal urethra and 30 to 50 ml of a sterile PBS was introduced. After gentle massage of the prepuce the fluid was recovered with the same syringe, transferred into 50 ml Falcon tubes and sent for bacteriological and parasitological examination to the Central Veterinary Research Laboratory. At the lab the samples were centrifuged at 2,500 rcf for 3 minutes and 100 µl of sediment was microscopically examined at magnification of 100 x and 400 x for moving flagellates. In order to see morphological particularities Giemsa stained smears were examined at 600 x magnification. Isolation experiments were performed using a commercial test kit (InPouch™

Biomed Diagnostics, Inc., White City, USA) and by co-cultivation with BGM-cells (Henning and Sager, 2007).

Treatment of infected dromedary bulls was carried out by repeated careful washings of the prepuce with oxidised water (MicrocynAH, Petaluma, USA).

DNA extraction, PCR and DNA sequencing

Genomic DNA was extracted using the High Pure PCR Template Purification Kit (Roche Diagnostics, Mannheim, Germany) according to the instructions of the manufacturer. PCR for detection of

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DNA of trichomonads was carried out with primers TFR 1 and TFR 2 (Felleisen, 1997) using a modified programme. After an initial denaturation at 96°C for 60 s 35 cycles of denaturation (96°C for 15 s), annealing (67°C) and extension (72°C) followed resulting in a ca. 370 bp amplicon. Analysis was done by electrophoresis on a 1.5% agarose gel, staining with ethidium bromide and visualisation under UV light. After purification with QIAquick Gel Extraction Kit (Qiagen, Hilden Germany) the amplicon was sequenced with BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) and primers TFR 1, TFR 2, TETRI-1 (5´-TTA TTA TTT GCT TTC TGT GG-3´) and TETRI-2 (5´-CTT TGA ATG CAA ATT GCG C-3´). Sequencing products were analysed using a Genetic Analyzer ABI Prism 3130 (Applied Biosystems).

Data analysis

Species identification was carried out by BLAST search (http://www.ncbi.nlm.nih.gov/blast). Sequence of the TFR 1/2 amplicon representing part of rRNA operon was deposited at GenBank database (Acc. no. MN065772). Phylogenetic tree was constructed with Geneious Tree Builder using the Tamura-Mei Genetic Distance Model and Neighbor Joining algorithm with *Tritrichomonas foetus* as outgroup (Kearse *et al,* 2012).

Results and Discussion

Two out of 12 preputial washes in repeated samples in August and September 2018 contained quickly moving *Trichomonas* like flagellates. The oval body in Giemsa stained smears measured 7-9 x 3.5-5 µm and was equipped with an undulating membrane and with four 15 µm long anterior and a shorter 6 µm long posterior flagella (Fig 1). Long term cultivation of the trichomonads failed because of bacterial contamination. A sample which was sent to the National Reference Laboratory for Trichomonosis of Cattle, Friedrich-Loeffler-Institut in Jena, Germany was positive for trichomonads by a PCR according to Felleisen *et al*. (1998). DNA sequencing and data analysis identified the trichomonads as members of the genus *Tetratrichomonas* and excluded the presence of *Tritrichomonas foetus* in the sample.

Fig 2 shows the relatedness within *Tetratrichomonas* spezies with *Tritrichomonas foetus* as out-group. The nearest relatives of isolate 18QT0009 are trichmonads from cow, sheep and goat without valid species names. Clearly defined *Tetratrichomonas* spezies as *T. butteri, T. brumpti* or *T. gallinarum* are more distantly related.

The reproductive biology of Camelidae presents some very important particularities not seen

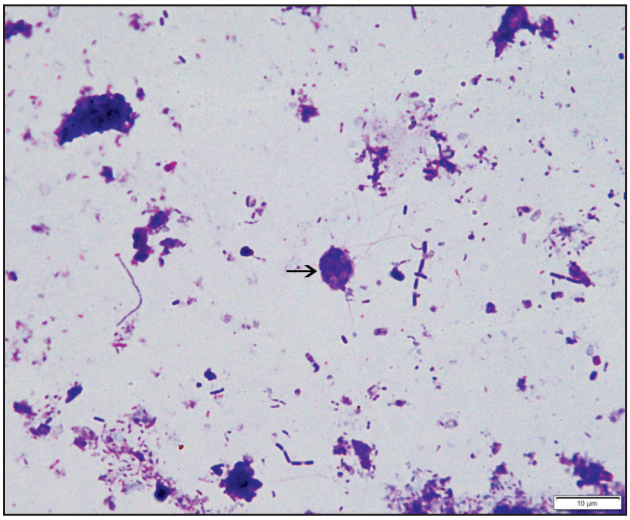

Fig 1. Giemsa stained *Tetratrichomonas* sp. (Arrow) from preputial washing of an adult dromedary bull.

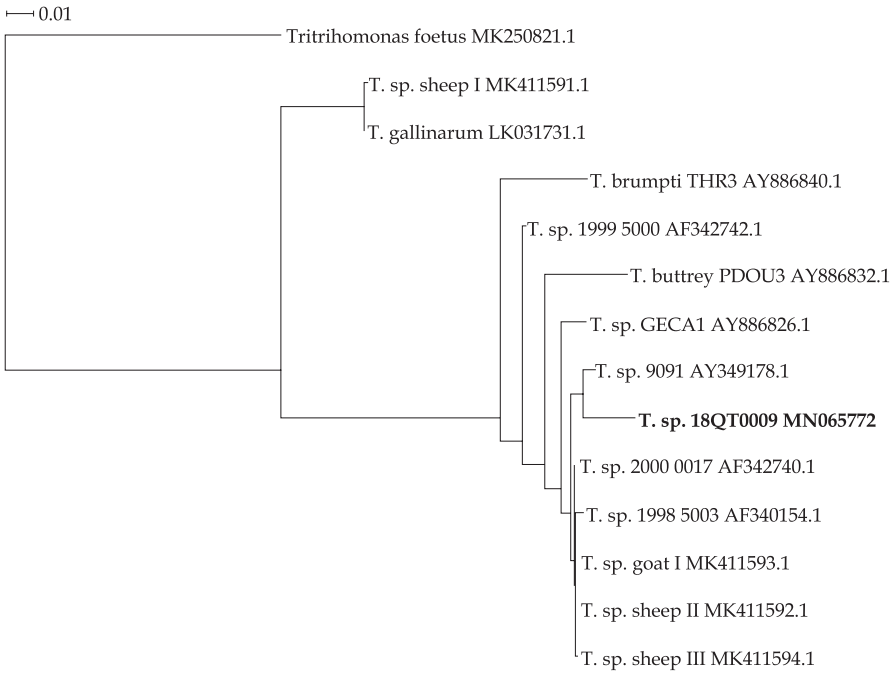

Fig 2. Relatedness within *Tetratrichomonas* isolates according to neighbour joining method.

in other domesticated ruminant species (Tibary and Anouassi, 1997; Wernery *et al,* 2014). Furthermore, infections of the reproductive tracts in Camelidae, as in other domesticated animal species, are the most commonly acquired causes of reproductive failures resulting in infertility (Tibary *et al,* 2006). Research has primarily focussed on dromedaries used for slaughtering with no information on the reproductive status of the tested dromedaries (Merkt *et al,* 1987). A great number of these investigations have been carried out in abattoirs of different countries. Interpretation of presence of bacteria from the reproductive system is difficult, especially when no history is available. In order to evaluate the role of various microorganisms in the development of uterine infections in dromedaries, Wernery *et al* (2014) proposed a classification of bacteria and protozoa as it is done for equines. Hardly, no information about the reproductive tract in camel bulls, especially about trichomonad infections, is available. During a routine inspection, a trichomonad protozoon was detected and subsequently investigated. Although camels are not ruminants the isolate 18QT0009 is closely related to trichomonads from ruminants. In this group it seems to be an own type which we have designated as "genotype camel". The presence of *Tritrichomonas foetus* in the sample could be definitely excluded.

It is not clear whether the isolated *Tetratrichomonas* species had any gynaecological relevance and may cause abortions like *Trichomonas foetus* in cows.

However, when the prepuces of both positive camel bulls flushed with oxidised water repeatedly investigated samples were negative for flagellates after that treatment.

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References

Felleisen RS (1997). Comparative sequence analysis of 5.8S rRNA genes and internal transcribed spacer (ITS) regions of trichomonadid protozoa. Parasitology 115, 111-119.

Felleisen RS, Lambelet N, Bachmann P, Nicolet J, Müller N and Gottstein B (1998). Detection of *Tritrichomonas foetus* by PCR and DNA enzyme immunoassay based on rRNA gene unit sequences. Journal of Clinical Microbiology 36:513-519.

Henning K and Sager H (2007). The diagnosis of the trichomonad epidemic in the cow. Tieraerztliche Umschau 62:A48-A50.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton, B, Meintjes P and Drummond A (2012). Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647-1649.

Merck Veterinary Manual (2016). Trichomoniasis. 11th Ed. Merck, USA. pp 1384-1385.

Merkt H, Moussa B, El-Naggar MA and Rath D (1987). Reproduction in camels: a review. FAO Animal Production Health Paper, Rome.

Tenter AM (2006). Protozooeninfektionen der Wiederkäuer. In Schnieder, T. (Ed.): Veterinaermedizinische Parasitologie. 6th Ed. Parey in MVS Medizinverlage, Stuttgart. pp 119-160.

Tibary A and Anouassi A (1997). Theriogenology in *Camelidae*: anatomy, physiology, pathology and artificial breeding. Abu Dhabi Printing and Publishing Co., Mina, Abu Dhabi, UAE.

Tibary A, Fite C, Anounassi A and Sghiri A (2006). Infectious causes of reproductive loss in camelids. Theriogenology 66:633-647.

Wernery U (1991). The barren camel with endometritis – Isolation of *Trichomonas fetus* and different bacteria. Journal of Veterinary Medicine Series B – Zentralblatt fur Veterinarmedizin Reihe B – Infectious Diseases and Veterinary Public Health 38(7):523-528.

Wernery U, Kinne J and Schuster RK (2014). Camelid Infectious Disorders. OIE Book. pp 149-161.