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Short communication

Brucella melitensis biovar 1 isolation in a captive wildlife population in the United Arab Emirates. First isolation in the scimitar-horned Oryx (*Oryx dammah*)

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ABSTRACT

In 2013, *Brucella melitensis* biovar 1 was recovered from the stomach contents of a scimitar-horned Oryx - SHO (*Oryx dammah*) aborted foetus, and from the articular fluid of a sand gazelle (*Gazella marica*) in a captive wildlife collection near Abu Dhabi, United Arab Emirates. Other evidence of exposure to the pathogen was collected through serological testing (Rose Bengal test) and *B. melitensis*-specific PCR of samples from captive wildlife kept in six different enclosures. A Multiple Locus Variable Number of Tandem Repeats (VNTR) Analysis (MLVA) using 15 markers showed that the two strains isolated in animals kept in enclosures, located 1300 m apart from each other, shared an identical genotype. The phylogenetic analysis of MLVA-15 profiles retrieved from the public database suggested that these strains belong to the African clade, clustering regionally in the UAE, Oman and Qatar. This is the first confirmed case of *B. melitensis* in a SHO, an African antelope extinct in the wild and warrants further investigation.

1. Introduction

Human brucellosis or Malta fever poses a serious health hazard always associated to an animal reservoir. It is caused by small non-encapsulated non-motile, facultative intracellular Gram-negative coccobacilli, that belong to the *Brucella* genus. *Brucella melitensis,* the main causative agent for brucellosis in goat and sheep, is the main cause of human brucellosis (Young, 1995). Importantly, *Brucella melitensis* infections are also found in other farmed species, like camels (Gwida et al., 2012) and in wildlife (Dadar et al., 2021).

In the United Arab Emirates, brucellosis surveys in semi free ranging wildlife have been conducted in the past, without detecting anti-*Brucella* antibodies (Ofner et al., 2007). The disease has been documented in livestock (Mohammed et al., 2013) in a Nubian Ibex (*Capra ibex nubiana*) (Wazed Ali Mollah and McKinney, 2002), in a gazelle (Gyuranecz et al., 2016) and in humans; between 2010 and 2015, 3.3 cases of human brucellosis /100,000 inhabitants were diagnosed yearly in the Abu Dhabi emirate (Al Shehhi et al., 2016).

The Scimitar-horned Oryx (*Oryx dammah*) (SHO) is a large Sahelian antelope that is now extinct in the wild. Global conservation efforts rely heavily on captive stocks for possible re-introduction. Such programs involve conducting wildlife disease risk analysis (Jakob-Hoff et al., 2014).

The aim of this rapid communication is to describe a first confirmed case of *B. melitensis* in SHO.

2. Material and methods

The study site was a 5 km long animal holding facility constituted of more than 50 fenced enclosures (Lignereux et al., 2020) located (location: 24.219 ° N, 54.793 ° E) 45 km east to Abu Dhabi, the capital city of the UAE.

The study site was populated with six different species of wild ungulates after they were translocated from a private island (location: 24.322 ° N, 52.598 ° E) in 2008. In November 2012, there were 7931 indian blackbucks (*Antelope cervicapra*), 3894 SHO, 1300 sand gazelles

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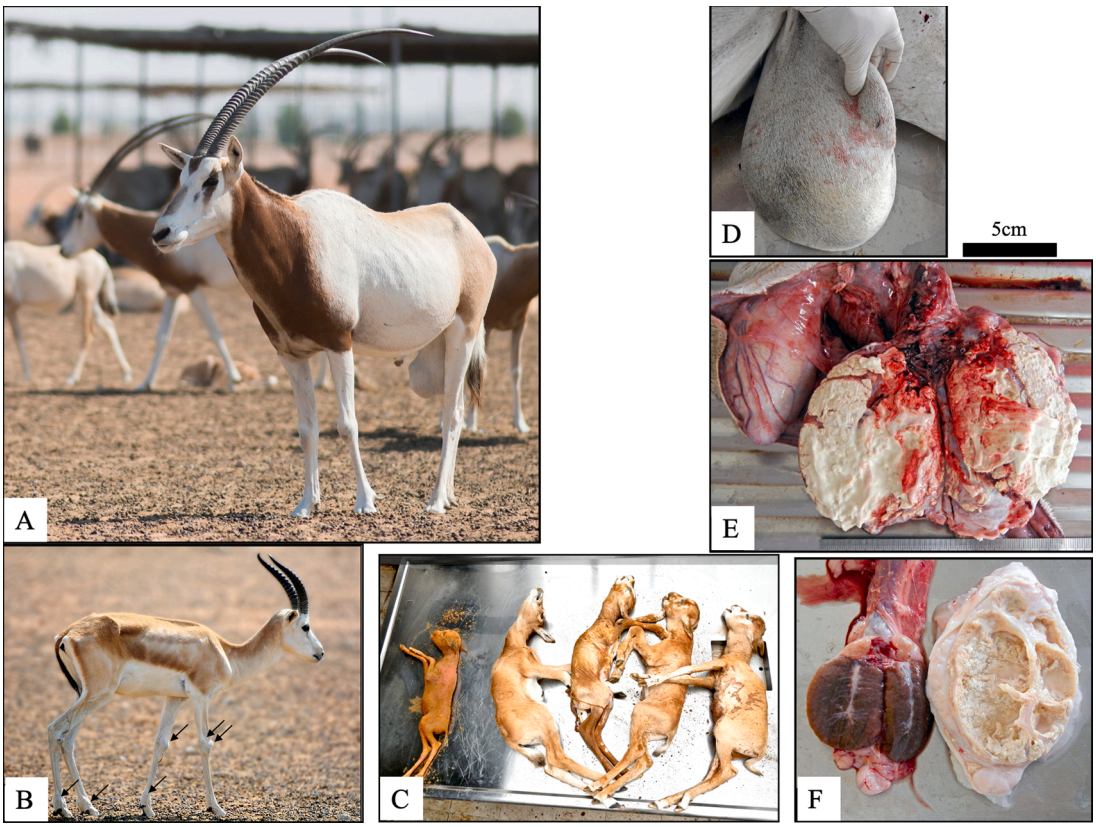

Fig. 1. Brucellosis gross lesions in the wildlife collection.
A: adult male scimitar-horned Oryx (SHO) presenting a bilateral testicular enlargement; B: sand gazelles with distended joints (arrows). Note the poor body condition of the affected gazelles; C: aborted and dead newborn SHO; Picture E: enlarged testicle of a Rose Bengal test positive SHO; F: same testicle after sagittal cut and content left in place. Note the size of the contralateral testicle in the upper left corner; G: the same testicle after removal of the caseous purulent material to visualise the thickened *septa testis*. The contralateral testicle is cut in half for comparison. The same scale is used for pictures D, E and F. The bar represents 5 cm.

(*Gazella marica*), 258 mountain (*Gazella gazella*) and Indian (*Gazella bennetti*) gazelles and 11 Urial sheep (*Ovis orientalis*). Species were separated from each other, and they were all breeding within species groups.

There was no history of testing or vaccination against brucellosis and the health status was unknown. Late 2012, brucellosis was suspected due to observation of enlarged testicles and hygromas, which are external clinical signs compatible with the disease (Garin-Bastuji et al., 1998). To confirm this, animals that presented those signs were restrained (Medetomidine, 0.6 mg and Ketamine, 100 mg per SHO, injected remotely and intramuscularly) and euthanized whenever their condition was detrimental to their welfare (mixture of Embutramide, Mebezonium and Tetracaine injected intravenously). Blood and testes from two males SHO, and blood and articular fluid from a male sand gazelle were collected and sent to a local veterinary laboratory to perform serological screening (RBT) and diagnostic (*Brucella melitensis*-specific PCR) tests for brucellosis. Histopathology was not performed. In addition, the RBT was also performed on blood collected from one aborted foetus and four dead newborn SHO.

Once the presence of *Brucella* was confirmed, another batch of fourteen samples consisting in different organs (SHO foetal spleen, lung, or stomach contents, or sand gazelle articular fluid) was collected randomly and aseptically in different enclosures in November and December 2013 from dead animals. They were sent under controlled temperature to Sciensano (ex. CODA-CERVA), Belgium for culture and biotyping using classical procedures described elsewhere (Alton et al., 1988).

The genotyping analysis was performed by Multiple Locus Variable Number of Tandem Repeats (VNTR) Analysis (MLVA) using 15 markers as described in (Le Flèche et al., 2006). Those markers were divided in three panels: 1, 2A and 2B, with eight, two and five microsatellites, respectively. Band sizes of tandem repeat units longer than 600pb were analysed on a 2% agarose gel. The remaining PCR products were analysed by capillary electrophoresis in a CEQ 8000 automatic DNA Analysis System (Beckman-Coulter) using a commercial kit (GenomeLab™ DTCS-Quick Start Kit, Beckman-Coulter) according to the manufacturer' instructions. Band size and peak numbers were converted to number of units using the *B. melitensis* 16 M reference strain (ATCC 23, 456) (Le Flèche et al., 2006).

Brucella melitensis alleles profiles were retrieved from the public database MLVA Bank (http://mLva.u-psud.fr/mLvav4/genotyping/index.php) as well as from the literature. The genotypes of all the *B. melitensis* strains isolated in Africa, the Middle East and Asia were included in our analysis (Garofolo et al., 2016; Georgi et al., 2017; Gopaul et al., 2014; Gyuranecz et al., 2016; Le Flèche et al., 2006; Menshawy et al., 2014; Mustafa et al., 2017; Osman et al., 2015; Schulze zur Wiesch et al., 2009; Tiller et al., 2009; Vergnaud et al., 2018). A total of 2729 profiles, including our profile, were then imported to Excel (Microsoft, USA) for sorting and cleaning. Loci with incomplete or missing information were not used. The columns presenting values for panel 1, 2A and 2B were copied 10, five and one time to increase their respective weight in the hierachical analysis performed with PHYLOViZ 2.0 software (Francisco et al., 2012). The phylogenetic tree was constructed using the Unweighted Pair-Group Method with Arithmetic mean (UPGMA) algorithm.

3. Results and discussion

Similarly to observations made during a brucellosis outbreak in Dorcas gazelles, (*Gazella dorcas*) (Wieckowski, 2017) and sable antelope

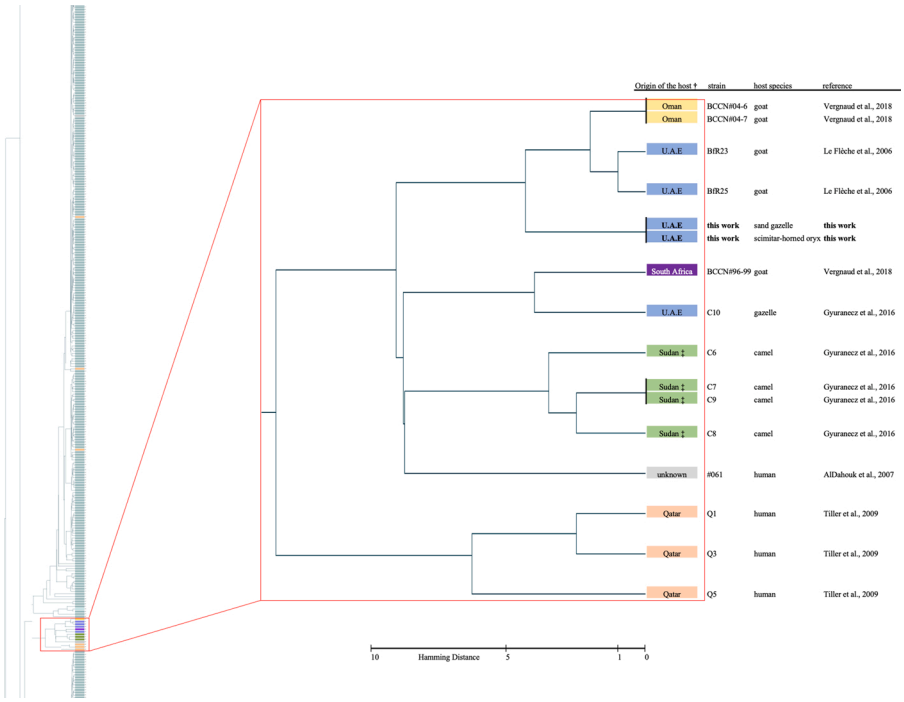

Fig. 2. Cluster analysis based on UPGMA phylogenetic tree inferred from 2729 *Brucella melitensis*. Legend: On the left, a detail of the overall tree showing the cluster (red box) to which our two isolates belong. On the right, this cluster (with country of origin, host, reference). A different colour represents each country of origin. †: Origin of the host as mentioned in the referenced paper. ‡: The host originated from Sudan but brucellosis was diagnosed in the U.A.E.

(*Hippotragus niger*) (Glover et al., 2020), hygromas, i.e. distension of the tibio-tarsal and metacarpo/tarso-phalangeal joints were observed in sand gazelles (Fig. 1-**B**). The presence of hygroma motivated the sampling of one adult male which elicited a positive RBT on serum and a positive *B.melitensis*-specific PCR result on articular fluid collected from the hygroma. Reluctance to move and loss of body condition possibly contributing to death were observed in gazelles.

On November 13, 2013, the carpometacarpal joint synovial fluid collected on another sand gazelle provided a positive culture (with only one colony seen) of *B. melitensis* biovar 1, followed two weeks later by another positive culture performed on the stomach contents of an aborted SHO and in an enclosure located 1300 m away from the gazelle.

In SHO, the clinical picture contrasted from what was observed in gazelles, and was more comparable to lesions described in sheep (Garin-Bastuji et al., 1998), goat (Poester et al., 2013) or alpine ibex (Mick et al., 2014). The uni- or bilateral testicular enlargement triggered the decision to sample the two SHO which appeared otherwise in excellent body condition (Fig. 1-**A**). The affected testicle(s) had undergone important gross lesions with distension due to a caseous purulent material replacing the seminal tissue and separated by thickened septa (Fig. 1-**E and F**). Positive *B.melitensis*-specific PCR results were obtained from the testicular tissues of both animals.

Out of the one aborted SHO foetus and the four dead newborns collected on a single day, the foetus and one newborn were positive to the RBT. None of the four newborns had a stomach content compatible with colostral intake, limiting the transfer of maternal immunity.

The isolation of *B.melitensis* from the stomach content of an aborted foetus (Fig. 1-**C**) reveals the transplacental transmission of the pathogen already reported in sheep (Grilló et al., 1997).

Females SHO did not exhibit obvious external signs and we find no particular external signs were observed in Indian blackbucks from both sexes.

The two isolates showed an identical MLVA-15 profile (Appendix) not described in the MLVA databank. This illustrates the clonal expansion of the bacteria usually observed during brucellosis outbreaks (Dorneles et al., 2014) and suggests a unique origin. They clustered with the genotype of strains previously isolated from the region and belonging to the African cluster (Fig. 2).

The phylogenetically most closely related strains were isolated from goats sampled in the UAE (Le Flèche et al., 2006) and Oman (Vergnaud et al., 2018) and another closely related strain was isolated from a sand gazelle in Abu Dhabi (Gyuranecz et al., 2016). Four strains recovered previously in the UAE from "Sudanese" camels (Gyuranecz et al., 2016) clustered with the strains isolated during this study. Together these strains constitute a cluster coherent with geographical data and limited to Oman, UAE and Qatar.

This study documents the presence of *Brucella melitensis* in at least two species and six enclosures in this wildlife collection. We suggest integrating brucellosis in wildlife disease risk assessment associated with possible reintroduction programs of those species. An effort to limit the risk of transmission to humans and the spread to neighbouring livestock farms should be initiated. Our study highlights the gap in our knowledge in brucellosis and its pathobiology in different wildlife of high conservation value.

Ethics statement

The animal collection health management provided the data presented in this study and this work was not performed primarily for research purposes.

Declaration of Competing Interest

The authors declared that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2022.109360.

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