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Brucellosis seroprevalence in captive scimitar-horned oryx (*Oryx dammah*) in the United Arab Emirates and associated risk factors

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ABSTRACT

Background: The scimitar-horned oryx (*Oryx dammah*) (SHO) is a large African antelope that became extinct in the wild just over two decades ago. Conservation of the species is of prime importance, but it might face pathogen stressors.

Methods and principal findings: *Brucella melitensis* biovar 1 was previously confirmed in a high-density captive population of SHO held in Abu-Dhabi emirate. The infection reached 67.0 % (95 % CI: 64.0–70.0) individual seroprevalence (n = 959) during testing performed between January 2013 and January 2015. A model based on a multivariable logistic regression analysis showed that the seroprevalence ranged from 51.2 (95 % CI: 39.6–62.7) to 86.9 % (95 % CI: 82.4–91.4) between six different enclosures, and probability of being seropositive was 1.83 (95 % CI: 1.32–2.55) higher in females than in males, 3.09 (95 % CI: 1.66–5.91) and 9.35 (95 % CI: 4.66–19.44) higher in subadults and adults than in juveniles, respectively. The three serological tests used in this study (Rose Bengal Test, lateral flow assay and in-house i-ELISA) had a perfect or near-perfect agreement (Cohen's Kappa coefficient > = 0.97). Recurrent high seroprevalence in time and congruence of results from three different serological tests point toward a persistent *B. melitensis* infection in a high-density captive SHO population.

Conclusion and significance

Testing strategy (Bengal Test, lateral flow assay or in-house i-ELISA) has no effect on the estimation of the brucellosis seroprevalence in SHO permitting the selection of a practical test. We call for an evidence-based control program, and *Brucella* vaccine efficacy and innocuity studies in this endangered species.

1. Introduction

With an estimated 5,000,000 to 12,000,000 true annual cases (Hull and Schumaker, 2018), human brucellosis or Malta fever is a zoonotic debilitating chronic bacterial disease caused by small non-encapsulated non-motile, facultative intracellular Gram-negative coccobacilli, that belong to the *Brucella* genus. It poses a serious public health hazard always associated with an animal reservoir.

The main cause of human brucellosis is *Brucella melitensis* (Young, 1995), which is also the main causative agent for brucellosis in goats and sheep.

There were on average 3.3 cases of human brucellosis/100,000 inhabitants diagnosed yearly between 2010 and 2015 in the Abu Dhabi Emirate (Al Shehhi et al., 2016).

The scimitar-horned oryx (SHO) (*Oryx dammah*) is a large desert antelope that once inhabited extensive areas of the Sahel from Mauritania to Egypt. It is now extinct in the wild because of intensive hunting, habitat loss, and competition with domestic livestock (IUCN, 2015). Global conservation efforts rely on captive stocks for possible reintroduction.

An outbreak of brucellosis due to *Brucella melitensis* biovar 1 has been confirmed in possibly the world's largest population of SHO, in the Emirate of Abu Dhabi (Lignereux et al., 2022).

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Reintroduction programs involve conducting a wildlife disease risk analysis (Jakob-Hoff et al., 2014) and preventing the introduction of exotic disease/pathogen into the host area is probably the most important responsibility of decision-makers (Kock et al., 2007).

As a prelude to control this outbreak, this study aimed at determining the associated risk factors, to concentrate the testing effort on certain enclosures or age groups. A test and isolation strategy aiming at removing infected SHO could be put in place afterwards.

2. Material and methods

2.1. Animals

Two animal holding facilities were sampled for brucellosis during this study.

The first animal holding facility (location: 24.219° N, 54.793° E) was described elsewhere (Lignereux et al., 2020, 2022). It was 6,000 m long and 800 m wide (Fig. 1) and was constituted of single fenced enclosures initially designed for livestock. It is unknown if this facility ever served its intended purpose, but it was devoid of livestock at the time of the study. A local farm compound – "izbas" (Al Shehhi et al., 2016) or "ezbas" (Chaber and Saegerman, 2017), was located 2500 m away from the nearest occupied enclosure. The entire facility was surrounded by a 50 m buffer zone. It received in late 2008 and without prior disease testing over 11,000 wild ungulates from Sir Bani Yas Island (SBYI) (location: 24.322° N, 52.598° E). A further 3000 gazelles were moved later from at least three other locations. The animals were kept on sandy ground and the manure was left to dry. Artificial shade structures were installed. Water and imported feed were provided daily.

The different species were kept separated and direct contact between enclosures was prevented by access corridors of at least 15 m wide. Some fences were in poor condition and animals could sometimes escape their enclosures. Most enclosures contained both sexes. There were 7931 Indian blackbucks (*Antelope cervicapra*), 3894 SHO, 1300 sand gazelles (*Gazella marica*), 258 mountain (*Gazella gazella*) and Indian (*Gazella bennetti*) gazelles and 11 Urial sheep (*Ovis orientalis*) in November 2012.

The SHO population was spread over 11 enclosures and had an important conservation value due to its unique, but low, genetic diversity with only seven haplotypes (Ogden et al., 2020). The enclosures with many animals in poor condition were deemed to be of lower interest for breeding and conservation purposes and were not tested.

This study focused on six pens (pens I to VI in Fig. 1) holding a total of 2537 SHO and spread across three testing campaigns, between January 2013 and January 2015.

A catching pen, alleyway and mobile chute system (Tamer®, Fauna Research, USA) were installed in each of the tested pens. The SHO were driven into the alleyway where they could be sorted: under the assumption that older animals would be more affected than younger animals, it was arbitrarily decided to put the testing effort on younger and better-looking individuals. Females were selected over males to enhance the breeding capacity. This process possibly led to a selection bias in this study.

The SHO were physically restrained in the chute for clinical examination, individual identification, sexing, ageing, and bleeding. Animals exhibiting only deciduous teeth were considered juveniles, those exhibiting one or two pairs of adult incisors, subadults and those exhibiting three or four pairs, adults. Subadults were estimated between 19 and 27 month-old (Lignereux et al., 2020).

The second animal facility was situated on SBYI. It was the source of the translocated population. There were about 1500 SHO among thousands of ruminants from different wildlife species on this island when this survey was done in April 2015.

2.2. Screening tests

Either the Rose Bengal test (RBT) or a lateral flow assay (LFA) was used to evaluate the exposure of each tested SHO to *Brucella* spp.

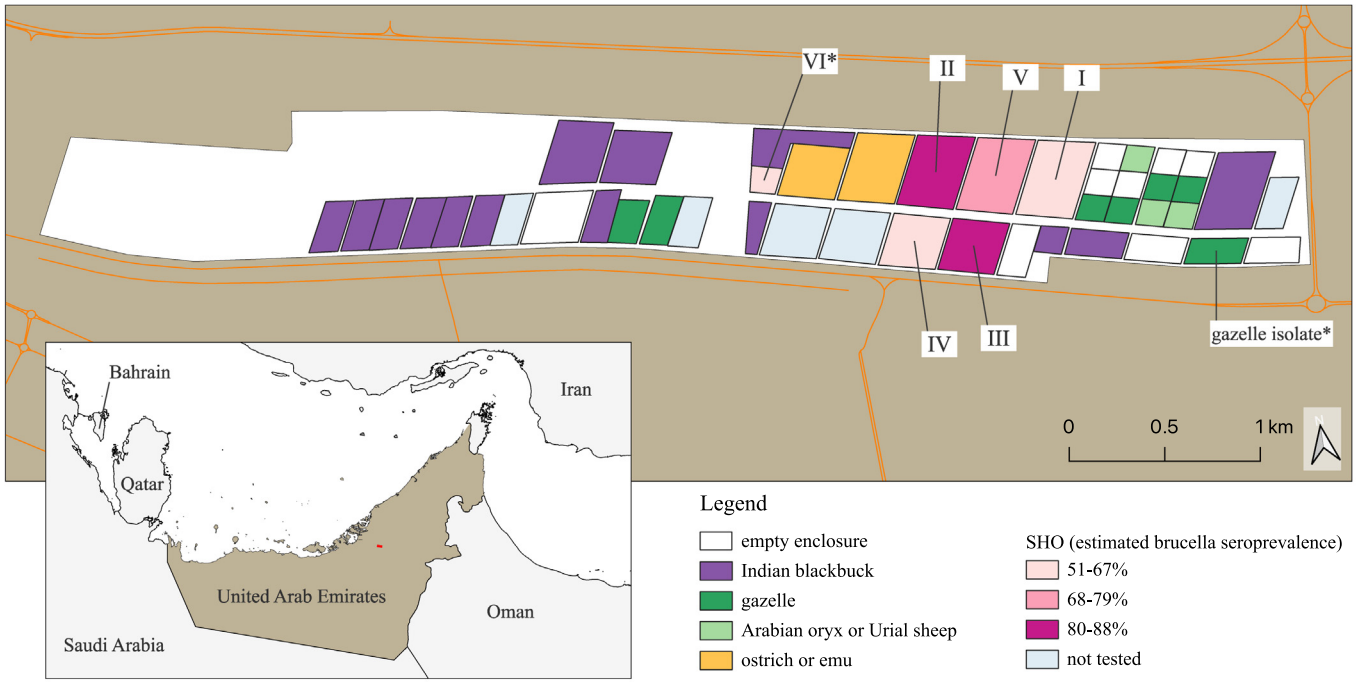

Fig. 1. Schematic representation of the field compound. The animal facility, represented in red, is located in the United Arab Emirates on the general map. On the simplified layout of the animal facility, the space devoid of animals is shown in white and the species are represented in different colours. The tested pens (I to VI) are shown and the estimated brucellosis seroprevalence in scimitar-horned oryx (SHO) is indicated with a pink gradient. *: enclosure where *Brucella melitensis* biovar 1 was isolated in 2013 (Lignereux et al., 2022). The map was made with QGIS 3.23 using a colourblind colour palette. The countries' shapefiles were uploaded from the GADM database (www.gadm.org). Note: the animal facility layout has been rotated as indicated by the north arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The RBT (Bengatest®, Synbiotics, France then Zoetis, USA) cost 0.25US$/test. RBT is a rapid buffered agglutination test, that requires fridge storage and little laboratory work. An inactivated, concentrated solution of *B. abortus* stained with rose Bengal is mixed on a clean single-use microscope slide with an equal volume of serum as described elsewhere (OIE - World Organisation for Animal Health, 2018) and read after four minutes of gentle shaking (see examples in Appendix 1, picture A).

The LFA (Anigen Rapid Bovine *Brucella* Ab Test Kit, RB2301DD, Bionote, South Korea) costs 3.8US$/test. LFA is a chromatographic immunoassay. It is a room temperature storable pen-side test, that can be performed on unclotted heparinized blood or serum.

An invisible band of *B. abortus* 1119-3 lipopolysaccharide (LPS) is deposited on a nitrocellulose membrane held in a plastic casing fitted with perforations to add the sample and visualize the result. A chromogenic reaction occurs within 20 min whenever anti-Brucella immunoglobulins are present, and their concentration dictates the strength of this reaction (see examples in Appendix 1, picture B). Slight reactions are considered positive. The test result was considered positive when the test band was seen by all persons from a panel of two or three to decrease interpretation subjectivity.

A third serological test, an in-house indirect Enzyme-Linked Immuno-Sorbent Assay (i-ELISA) was performed in parallel with RBT and LFA on a subset of serum samples to evaluate the agreement between tests.

This i-ELISA is accredited at the Belgian National Reference Centre (Sciensano, Belgium). It was described elsewhere (Rahman et al., 2012). Briefly, the 1/50 diluted serum samples were deposited in plates previously coated with smooth LPS from *B. abortus* strain Weybridge 99. The binding antibodies were detected with a protein G-horseradish peroxidase conjugate (Biorad, Belgium) and following the addition of O-phenylenediamine, the optical densities were measured at 490 nm and 620 nm with an iMark Microplate Absorbance Reader (Biorad, Belgium). The results were calculated based on the difference between the two measurements. Six dilutions (from 1/270 to 1/8640) of the OIE reference serum provided the standard curve and the cut-off was determined as the mean of 1/8640 dilutions of the standard curve.

2.3. Statistical analysis

2.3.1. Serological tests comparison

In the absence of a "gold standard", i.e. actual bacteriological status of every single animal, the agreement between pairs of serological tests (RBT, LFA, and i-ELISA) was evaluated with the Kappa coefficient κ (Petrie and Watson, 2013).

2.3.2. Risk factors for exposure to brucellosis

Multiple logistic regression analysis (Prism 9, Graphpad, USA) was used to evaluate the effects of sex, age category, tested pen, testing protocol, and testing campaign on the initial individual serological outcome. The odds ratios (OR) were calculated from the estimated model parameters, and a Wald test assessed their significance through Z- and subsequent *P*-values.

The explanatory categories and the interactions that did not affect the outcome (odds ratios not significantly different from 1) were removed from the model and the regression analysis was re-run. The "margins" function in Stata (StataCorp, USA) was used to calculate the predicted seroprevalence associated with each risk factor. The pairwise comparison of the seroprevalences was performed after Bonferroni correction (Petrie and Watson, 2013).

For all statistical tests, a 95 % confidence interval was calculated using a binomial (Clopper-Pearson) exact method and all *P*-values inferior to 0.05 were considered significant.

3. Results

3.1. Initial individual seroprevalence

The first testing campaign spread from January to March 2013, 364 SHO were tested with RBT out of the 1399 SHO present in enclosures I, II, III, and IV (Fig. 1). A total of 424 SHO were tested with LFA during the second campaign from February to April 2014 out of the 726 SHO present in enclosure V. Finally, out of 412 SHO in enclosure VI, 62 subadults and 65 adults were tested with LFA, and 44 adults were tested with RBT during the third and last campaign from November 2014 to January 2015. Details can be seen in Table 1.

In total, 959 SHO were tested including 645 females and 314 males, 231 juveniles, 126 subadults and 602 adults. Also, 408 and 551 SHO were tested with RBT and LFA, respectively.

In addition, *Brucella melitensis* biovar 1 was isolated in 2013 (Lignereux et al., 2022) in enclosure VI – see Fig. 1.

Amongst the 959 SHO tested, 643 elicited a positive result, the overall observed seroprevalence was 67.0 % (95 % CI: 64.0–70.0).

The "testing protocol" (RBT *versus* LFA) did not significantly influence the serological outcome. With RBT as reference level, the odds ratio was 1.130 (95 % CI: 0.489–2.548; p = 0.77). The sampling protocol was therefore not included as an explanatory variable from the multiple logistic regression.

The two variables "testing campaign" and "tested pen" were not independent, and they could not be included together in the analysis, leading to two different scenarios: in the first one, the risk of being seropositive was higher during the second campaign (OR = 1.56 (95 % CI: 0.93–2.61)), and lower (OR = 0.64 (95 % CI: 0.34–1.05)) during the third campaign than it was during the first campaign.

The second scenario was conducted with the independent explanatory variables "sex", "age category" and "tested pen". The results (presented in Table 2) indicate that the likelihood of being seropositive was 1.83 times higher in females than it was in males, and the estimated seroprevalences were 60.0 % in males (95 % CI: 55.0–65.0) and 70.1 % in females (95 % CI: 67.5–73.9). It was also 3.09 and 9.35 times greater in sub-adults and adults than it was in juveniles, respectively, with an estimated seroprevalence of 34 % in juveniles (95 % CI: 23.3–45.0), 58.4 % in sub-adults (95 % CI: 48.8–68.2), and 79.4 % in adults (95 % CI: 75.3–83.4). All those differences were significant. The pen also influenced the seroprevalence: the estimated values ranged between 51.2 % (95 % CI: 39.6–62.7) in pen I and 86.9 % (95 % CI: 82.4–91.4) in pen III. The pairwise comparison with Bonferroni correction (Table 2) indicated that no significant difference existed between the higher seroprevalence in pens II and III, the intermediate seroprevalence in pens II and V, and the somewhat lower seroprevalence in pens I, IV, V and VI (Fig. 1).

3.2. Serological tests agreement

A subset of 67 SHO sera samples, consisting of the 62 samples collected in November 2014 from subadult SHO in pen VI, and five other samples also randomly chosen, underwent RBT, LFA and i-ELISA tests. Twenty-two and 44 samples were classified as positive and negative by all three tests, respectively. (Table 3).

The i-ELISA and LFA were in perfect agreement (κ coefficient = 1; 95 % CI: 0.76–1.24). RBT failed to detect a sample that was found seropositive by both the LFA and the i-ELISA leading to a near-perfect agreement (κ coefficient = 0.97; 95 % CI: 0.73–1.21).

3.3. Seroprevalence on SBYI

Out of the 50 adult females tested on SBYI, one elicited a positive reaction with LFA.

Table 1
Number of tested scimitar-horned oryx and their distribution according to sex, age category and enclosures.

Date	Tested pen	Testing protocol	Male	Female	Juvenile	Subadult	Adult	Negative	Positive	n tested	Observed sero-prevalence (in %)	95 % CI*
2/01/2013 to 22/01/2013	I	RBT	66	68	105	18	11	103	31	134	23.1	16.3–31.2
28/01/2013 to 29/01/2013	II	RBT	22	20	37	5	0	19	23	42	54.8	38.7–70.2
12/02/2013 to 25/02/2013	III	RBT	54	62	78	35	3	32	84	116	72.4	63.3–80.3
27/02/2013 to 19/03/2013	IV	RBT	39	33	10	4	58	30	42	72	58.3	46.1–69.9
10/02/2014 to 24/04/2014	V	LFA	89	335	1	2	421	68	356	424	84.0	80.1–87.3
18/11/2014 to 06/01/2015	VI	LFA	33	94	0	62	65	51	76	127	59.8	50.8–68.4
18/11/2014 to 06/01/2015	VI	RBT	11	33	0	0	44	13	31	44	70.5	54.8–83.2
TOTAL			314	645	231	126	602	316	643	959	67.0	64.0–70.0

Legend: RBT, Rose Bengal Test; LFA, lateral flow assay.
* 95 % confidence interval (binomial exact).

Table 2
Calculation of the odds ratio and the estimated seroprevalence for each risk factor (n = 959).

		Odds ratio				Estimated seroprevalence		
Risk factor	Variable	Odds ratio	95 % CI*	|Z|	*P*-value	Estimated sero-prevalence (in %)	95 % CI*	Bonferroni groups
	intercept	0.14	0.08–0.23	7,516	<0.0001			
age category	juvenile	1 (reference)				34.2	23.4–45.0	
	subadult	3.09	1.66–5.91	3,493	0.0005	58.5	48.8–68.2	
	adult	9.35	4.66–19.44	6,152	<0.0001	79.4	75.3–83.4	
sex	male	1 (reference)				60.0	55.0–65.0	
	female	1.83	1.32–2.55	3,588	0.0003	70.7	67.5–73.9	
tested pen	pen I	1 (reference)				51.2	39.6–62.7	A
	pen II	5.75	2.69–12.6	4,454	<0.0001	80.6	71.8–89.3	B C
	pen III	10.16	5.60–19.00	7,456	<0.0001	86.9	82.4–91.4	C
	pen IV	1.17	0.52–2.61	0.391	0.6959	54.4	44.2–64.5	A
	pen V	2.57	1.21–5.38	2,478	0.0132	68.7	62.2–75.1	A B
	pen VI	1.27	0.63–2.54	0.661	0.5088	55.8	49.0–62.6	A

* 95% confidence interval (binomial exact).

Table 3
Serological tests agreement (n = 67).

RBT	LFA	i-ELISA	Number of animals
+	+	+	**22**
+	+	–	0
+	–	+	0
–	+	+	**1**
–	+	–	0
–	–	+	0
–	–	–	**44**
Total			67

Legend: RBT, Rose Bengal Test; LFA, lateral flow assay; and i-ELISA, indirect enzyme-linked immunosorbent assay.

4. Discussion

4.1. Screening tests and tests agreement

The disease has never been documented before in the SHO. The screening tests based on *B. abortus* antigens such as the ones used in this study cross-react with anti-*B. melitensis* immunoglobulins (Díaz-Aparicio et al., 1994; Blasco et al., 1994; OIE - World Organisation for Animal Health, 2018). However, brucellosis serological tests might have limited sensitivity (Se), with falsely negative reactions common in vertically or pseudo-vertically infected sexually immature females (Saegerman et al., 2010). For instance, RBT sensitivity (Se) in goats and sheep was 80.2 % and 82.8 % respectively (Rahman et al., 2013). On the other hand, false-positive reactions due to other Gram-negative bacteria have occurred, limiting the specificity (Sp) (Weynants et al., 1996; Saegerman et al., 2004). In *Brucella*-free goats, RBT Sp was 100 % (Blasco et al., 1994).

The results of both the multivariable logistic regression and Kappa coefficient tend to indicate that RBT and LFA are somewhat interchangeable and provide results that are not significantly different.

However, as indicated by the pairwise comparison on 67 serum samples, LFA detected one more positive sample than RBT which might translate into a slightly higher sensitivity of the LFA. The LFA was more expensive than RBT but carried the advantages of a pen-side test: in our experience, it provided a quicker result with positive results usually obtained within three to five minutes and while the animal was still being handled, allowing for immediate segregation of seropositive SHO.

Those practical and important observations might be of prime interest in further steps of a brucellosis control program in wild or wild-captive species. Importantly, LFA is not currently recommended test by the OIE (OIE - World Organisation for Animal Health, 2018).

The three serological tests results matched very well, even perfectly for the LFA and i-ELISA, but in the absence of the actual brucellosis sta-

tus for each individual, it is difficult to further evaluate the tests' parameters, notwithstanding that *B. melitensis* biovar 1 has been isolated from SHO in this setting (Lignereux et al., 2022).

The RBT detects both IgG and IgM, which can be detected first in seroconverting animals, while the protein G used in the i-ELISA will specifically bound IgG. A different testing panel with animals of different age or sex distribution might nevertheless provide a different agreement between tests and could be interesting to investigate further.

4.2. Seroprevalence and risk factors

Brucellosis is a debilitating disease. Favouring animals in good apparent condition over debilitated individuals for testing purposes might have introduced a possible selection bias likely to underestimate the brucellosis seroprevalence.

From our results, both sexes, all age categories and all tested enclosures were affected by brucellosis.

The 67.0 % individual seroprevalence observed is higher than the highest prevalence reported in wild animals in the literature. For instance, 36 % of Alpine ibex (*Capra ibex*) (ANSES, 2015) and 56 % of male bison (*Bison bison*) (Meyer and Meagher, 1995) were seropositive. A substantial difference is that, in this study, the SHO were not living in their natural environment. This high level of prevalence might stem from a recent introduction of the pathogen in a population previously naïve and could evolve towards *equilibrium* at a lower seroprevalence once the disease becomes enzootic and herd immunity is acquired. Nevertheless, such elevated prevalence suggests a high transmissibility of the pathogen, possibly due to a favourable combination of individual factors such as low genetic diversity (Biebach and Keller, 2010), species-specific characteristics (such as host susceptibility and behaviour) and husbandry practices (high animal density, absence of cleansing and animal waste removal including foetal membranes).

The effect of sex on *Brucella* seroprevalence has been observed on multiple occasions: in cattle (Awah-Ndukum et al., 2018; Assenga et al., 2015; Muma et al., 2006; Mai et al., 2012), goats (Solorio-Rivera et al., 2007) or bison (Meyer and Meagher, 1995). In agreement with other studies (ANSES, 2015; Tadesse, 2016), our results suggest that females were more affected than males, but the cause remains unknown. Perhaps it could be related to the longer lifespan of females or/and the dominance of certain males or/and due to the design of the survey with a prior selection of mostly females, younger and better-looking individuals.

As reported in other domestic species like cattle and small ruminants (Boukary et al., 2013), all studied age categories were exposed to brucellosis, and the level of exposure increased with age, possibly due to a repeated risk of becoming infected over time.

Because the two predictors ("testing campaign" and "tested pen") were dependent, the analysis was performed using one predictor or the other at a time. The risk of being seropositive fluctuated with the testing campaign: it was lower during the third campaign when it was expected to increase due to brucellosis biology. On the other hand, the analysis based on the tested enclosures provided a more likely scenario: it appeared that the highest level was found in two geographically close enclosures (Fig. 1, pen II and pen III) and that there may have been a centrifugal gradient of seroprevalence, with intermediate (pen V and pen VI) and lower (pen I and pen IV) levels observed further from the first two enclosures. All enclosures shared the same husbandry conditions, had similar animal composition and density, and the animals were of the same origin. No selection or testing was done before this study. It is important to note that not all enclosures containing *Brucella*-susceptible species were tested and the parameters of brucellosis transmission amongst other species – mainly the Indian blackbucks and the gazelles – remain unknown.

Nevertheless, the serological results suggest that animals from all tested pens were exposed. In this view and unless proven otherwise, it would be appropriate to consider all enclosures of this animal facility as infected. Whole-genome sequencing has provided fundamental insights for examining transmission dynamics of *B. abortus* in bison and elk (*Cervus canadensis*) in the Greater Yellowstone Ecosystem. Such genomic approaches, relying on the analysis of *B. abortus* strains isolated from different wildlife species, obtained during previous and contemporary outbreaks allowed specific epidemiological reconstructions of "who-infected-whom" (Kamath et al., 2016). In the studied population, *Brucella* was isolated only twice: from a SHO in an enclosure with the lowest seroprevalence (Fig. 1 pen VI) and a gazelle located 1300 m far away (Lignereux et al., 2022). In this context, a balanced sampling (i.e. proportional to disease prevalence) is important to make sound transmission inferences for infectious diseases in wildlife (Kamath et al., 2016).

A limited serological investigation in SBYI wildlife showed only one seropositive individual out of 50 in 2015. Yet, in the absence of an epidemiological inquiry and additional testing, it is not possible to conclude whether the single seropositive SHO found on the island was truly or falsely seropositive. We suggest further investigation of brucellosis on SBYI, including culture, molecular typing and phylogenetic analysis for comparison with known genotyping profiles (Kamath et al., 2016; Lignereux et al., 2022).

4.3. Limitations of the study

Our study was limited by the absence of a gold standard and the lack of systematic use of diagnostic tests (*Brucella*-specific PCR and culture/isolation/typing).

5. Conclusion

Constructing *B. melitensis* phylogenies and transmission events based on the analysis of *B. melitensis* isolated from wildlife and livestock (sheep, goat cattle, and camels) in the region will substantiate the claim that brucellosis is likely to have been introduced in the SHO after they have been translocated to the fenced facility. The SHO raised in high-density captive conditions might constitute a maintenance host for *B. melitensis*. However, what would happen under natural circumstances remains unknown.

Our results prompt the implementation of measures to control the infection in this threatened species. In our study, LFA showed a nearly 100 % agreement with the RBT and 100 % agreement with the i-ELISA. This suggests that LFA could be considered for recommendation by the OIE, after further validation.

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 declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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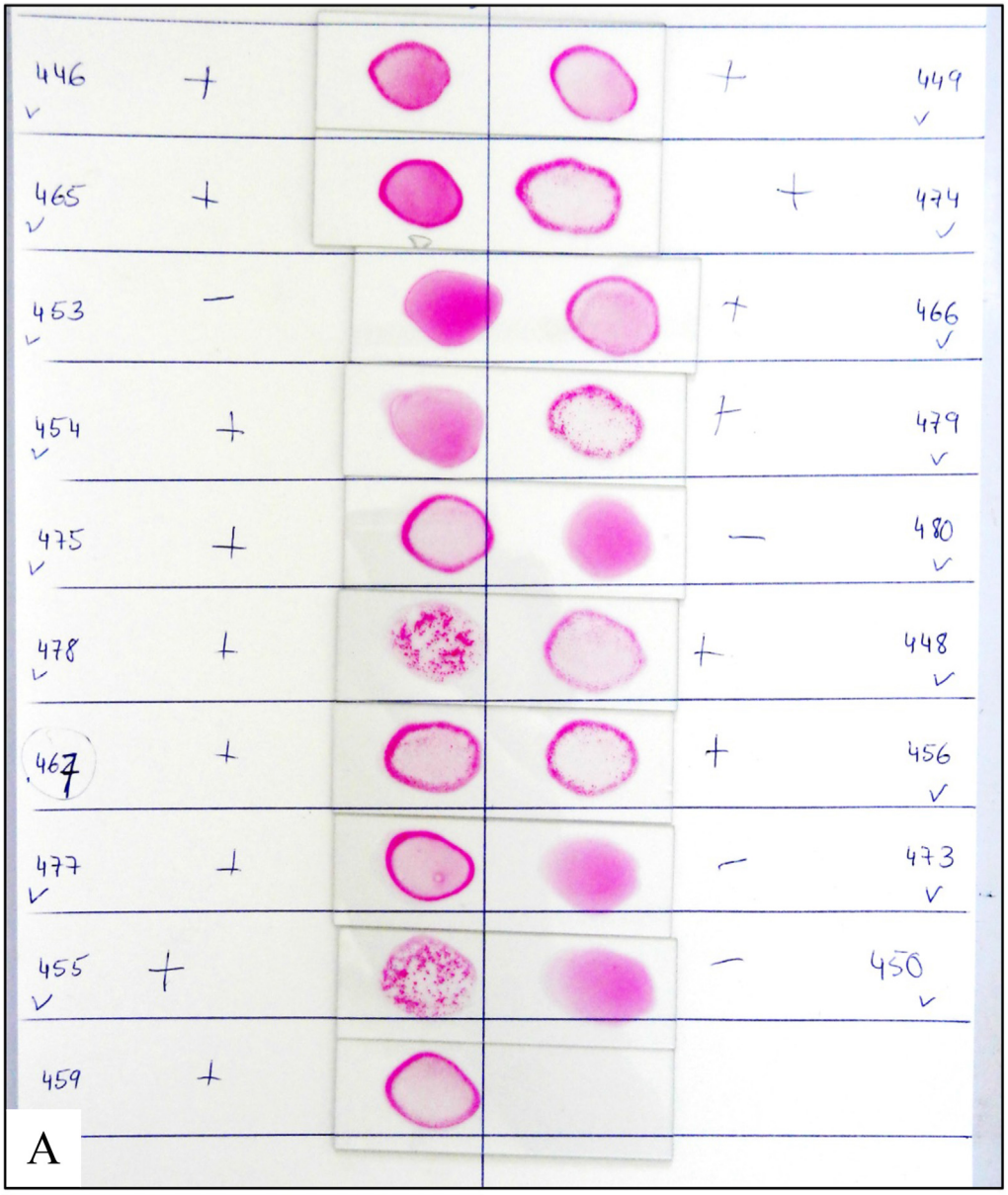
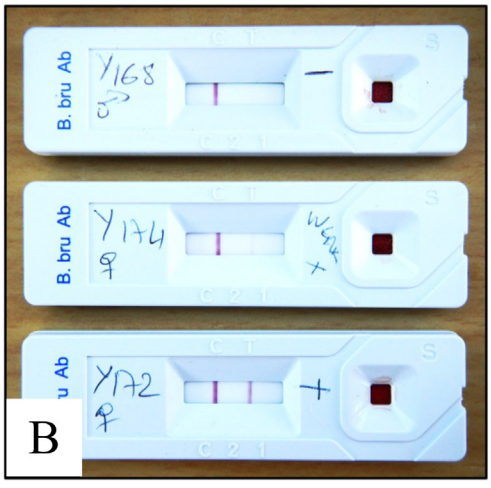
Appendix 1. Examples of brucellosis serological tests performed in scimitar-horned oryx

Picture A: examples of Rose Bengal Test. Picture B: examples of Bionote Anigen Rapid Bovine *Brucella* Ab Lateral Flow Assay (LFA).





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