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Isolation of *Brucella melitensis* from an Arabian oryx *(Oryx leucoryx)*

S. OSTROWSKI, S. ANAJARIYYA, E. M. KAMP, E. BEDIN

THE Arabian oryx (*Oryx leucoryx*) became extinct in the wild in the early 1970s (Henderson 1974). The species was saved by a collaborative captive-breeding programme involving zoos in the USA (Dolan 1976). It is now the subject of reintroduction programmes in many of the countries where it previously naturally occurred. The National Wildlife Research Center (NWRC) was commissioned to reintroduce the oryx into the wild in suitable protected areas of Saudi Arabia (Ostrowski and others 1998).

In a recent review, Daszak and others (2000) underlined that the introduction of pathogens into previously unexposed wild populations can seriously challenge conservation efforts. In wildlife reintroduction operations, the risk of disease transmission is enhanced both for the reintroduced animals themselves and the native fauna (Woodford 1989). Thus, extreme care should be taken to reintroduce only animals determined to be free of pathogens. In addition, assessment of the susceptibility of the reintroduced species to communicable infectious diseases is an important factor to consider in the health management of such conservation projects.

The presence of brucellosis in free-ranging wild ruminant populations is a major health management problem in several countries because of the risk of transmission to livestock species (Thorne and others 1978, Meyer and Meagher 1995). It is therefore essential to record any cases of this disease identified during the course of a reintroduction programme where the reintroduced animals might be in direct or indirect contact with domestic livestock.

This short communication reports a case of brucellosis in a captive Arabian oryx whose progeny were part of a reintroduction project. This case is noteworthy as it affected a threatened species during a reintroduction programme and represents, to the authors' knowledge, the first documented case of brucellosis in antelopes belonging to the subfamily Hippotraginae.

In November 1999, an adult male Arabian oryx kept alone in an individual pen at the NWRC was presented with anorexia, poor body condition and a reluctance to walk. General examination and auscultation were unremarkable. The rectal temperature was within the normal range and routine haematological examination revealed no anomalies. Examination of the rumen revealed a discrete tympany and an abdominal compression which appeared mildly painful. A non-specific indigestion was suspected, possibly due to ingestion of excess fermentable feed. Symptomatic treatment was given using orally dosed propionic acid/calcium carbonate (Bykodigest; Schering-Plough) and 25 mg/kg long-acting oxytetracycline (Terramycin/LA; Pfizer), administered by deep intramuscular injection every two days for six days. The animal's appetite and general condition improved thereafter.

Six months later the animal was re-examined as it again presented with anorexia and progressive loss of body condition. Examination revealed enlarged, swollen testicles which appeared to be very painful. A speculative diagnosis of orchitis was made and the oryx was isolated for further investigations.

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S. Ostrowski, DVM, **S. Anajariyya,** DVM, MSc, **E. Bedin,** National Commision for Wildlife Conservation and Development, National Wildlife Research Center, PO Box 1086, Taif, Saudi Arabia
E. M. Kamp, Drs, Institute of Animal Science and Health (ID-Lelystad), Edelhertweg 15, 8219 PH Lelystad, The Netherlands

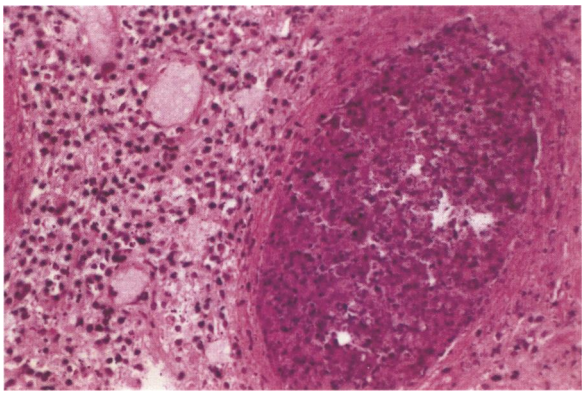

FIG 1: Photomicrograph of a testicle biopsy of the Arabian oryx. showing macrophagous cell infiltration of interstitial tissue, necrosis, and pyogenous inflammation of the seminiferous tube. Haematoxylin and eosin. x 400

Testicular biopsy revealed generalised necrosis and pyogenous inflammation of the seminiferous tubes. Although a thin crown of Sertoli cells was observed in affected tubes, there was no evidence of spermatogenesis. An infiltration of interstitial tissue, predominantly composed of mononucleate macrophagous cells was also observed. These lesions confirmed a severe pyogranulomatous and necrotic orchitis most probably of infectious origin (Fig 1). Brucellosis was suspected.

Inoculated blood agar cultures were sterile after eight days of aerobic and microaerophilic incubation at 37°C.

Serological investigations were carried out. The qualitative card test (Brucelloslide-Test; bioMérieux) was positive; a *Brucella abortus* serum agglutination test standardised against the international standard serum of Weybridge (ID-Lelystad) was positive at 480 iu/ml; *B abortus* and *Brucella melitensis* complement fixation tests (ID-Lelystad) were positive at more than 200 iu/ml; and a *B abortus* indirect ELISA serological test (Bercovich and Taaijke 1990) was positive at 320 IU/ml. Retrospective card tests were also performed, showing that the animal had been positive in November 1999, but not in April 1999.

The oryx was euthanased six days later due to total anorexia, severe degradation of body condition and swelling of the carpal joints. A thorough postmortem examination was carried out. The testicles were enlarged and filled with purulent and necrotic material surrounded by a connective tissue capsule. Both carpal joints were enlarged; however, cut surfaces did not contain necrotic or purulent material. No other lesions were found.

TABLE 1: Results of bacteriology and biotyping carried out on an isolate from the testicle of an Arabian oryx

Test	Result
Bacteriology	
Morphological appearance	Gram-negative rod
Motility	Non-motile
Oxidase production	Yes
Catalase production	Yes
Urease production	Yes
Slide agglutination (anti-*Brucella abortus* serum)	Yes
Biotyping	
Requirement of carbon dioxide	No
Hydrogen sulphide production[a]	Yes
Urease activity[b]	Yes (half hour)
Growth after three days on tryptose agar	Yes
Growth after three days on tryptose agar + thionine	Yes
Growth after three days on basic fuchsin	Yes
Agglutination[c] with monospecific anti-A serum	Yes
Agglutination[c] with monospecific anti-M serum	No
Lysis by Tbilisi phage	No

[a] Lead-acetate strip method
[b] Christensen urea slopes method
[c] Isolate suspended in 0·5 per cent phenol-saline and heated for one hour at 60°C

Attempts to isolate the *Brucella* species were carried out at the Institute for Animal Science and Health at Lelystad. Spleen, testicle, penis, popliteal lymph node, jejunal lymph node, urine and blood samples were submerged in boiling water, cut into small pieces and homogenised in meat broth using a lab blender (Stomacher; UAC). Specimens were inoculated onto heart infusion agar supplemented with 5 per cent defibrinated sheep blood (HIS-agar) and on serum dextrose agar supplemented with antibiotics (SR209; Oxoid) (SDA + AB). In addition, 0·1 ml of the homogenate was inoculated for enrichment in serum dextrose broth with and without antibiotics. Every week for six weeks, a specimen of these broth cultures was inoculated onto HIS and SDA + AB. Plates and broth cultures were incubated at 37°C in an atmosphere of 5 per cent carbon dioxide.

All specimens were negative except for the testicle. Isolates from enriched broth with antibiotics were identified as *Brucella* species using morphological appearance, slide agglutination (Murex Biotech), motility, oxidase, catalase, and urease production (Table 1) (Alton and others 1988). No other pathogenic bacteria or protozoa were isolated. The isolate was identified as *B melitensis* biovar 2 using the following tests: carbon dioxide dependence, tested directly after primary isolation; hydrogen sulphide production; urease activity; growth in the presence of thionine (20 µg/ml) or basic fuchsin (20 µg/ml); agglutination test using A- and M- monospecific antisera obtained from the Veterinary Laboratories Agency, Weybridge, and lysis by Tbilisi phage (Table 1) (Alton and others 1988).

Serological evidence of brucellosis, occasionally supported by bacterial isolations, has been reported by a number of authors in a variety of antelope species (Davis 1990). However, a detailed assessment of clinical disease has rarely been made on these animals (Sachs and Staak 1966, Madsen and Anderson 1995). In Saudi Arabia, captive mountain gazelles *(Gazella gazella)* without clinical symptoms, and one individual with testicular abscesses, were incidentally found to be seropositive to *B abortus* and *B melitensis*. However, no *Brucella* organisms were isolated after prolonged incubation (B. Flavell, M. Osama Badri, unpublished data). Greth and others (1992a) have documented a positive serology (complement fixation test) in one of 78 tested captive Arabian oryx at the NWRC. However, no isolation was carried out and the animal was seronegative three years later (S. Ostrowski, unpublished data). To the authors' knowledge, no clinical cases of brucellosis have been reported in the Hippotraginae subfamily and the isolation of a *Brucella* species from an Arabian oryx constitutes the first confirmed case in this bovid subfamily. Although it was unlikely that Hippotraginae were not susceptible to this disease which affects ruminants in general, the paucity of cases may possibly indicate a lower susceptibility or a difficulty in detection.

Disease management during the reintroduction of the Arabian oryx includes preventive measures such as vaccination, and the provision of clinically healthy animals free from exotic pathogens for release. Although the reported case did not concern an animal to be reintroduced, the long and nearly subclinical progress of the disease in the infected animal, only confirmed by a late isolation of the *Brucella* agent, was of great concern. Therefore, during 2000, the 245 oryx kept at the NWRC were all serologically tested for brucellosis (card agglutination test). In addition, sera of all the individuals to be reintroduced, and of individuals raised in the proximity of the infected male and of individuals reintroduced since 1995 (retrospective study on sera sampled on the day of reintroduction) were also tested using *B abortus* and *B melitensis* complement fixation tests. All tests were negative.

In Saudi Arabia, brucellosis occurs in sheep, goats, camels and cattle (Radwan and others 1983), with *B melitensis* being the major cause of ovine, caprine and human brucellosis

(Alton 1990, Scrimgeour 1995). Ovine and caprine brucellosis eradication campaigns are in progress, but a relatively high seroprevalence (4·5 per cent) is still present around the Arabian oryx breeding centre (S. Anajariyyah, unpublished data). Although the NWRC is surrounded by a sanitary buffer zone, and the risk of direct transmission from infected livestock to captive oryx is minimal, cases of infectious diseases related to the presence of domestic livestock have already been documented (Greth and others 1992a, b). The main reservoirs for *B melitensis* in Saudi Arabia seem to be sheep and goats (Radwan and others 1983); however, it is also possible that wildlife could act as reservoirs or disseminators under favourable circumstances. Because climatic factors present in this area (desiccation and exposure to sunlight) work against the survival of *Brucella* organisms (Crawford and Hidalgo 1977), it is speculated that the brown-necked raven *(Corvus ruficollis)* might have disseminated the infectious agent to the captive oryx, possibly picking up *Brucella* organisms by feeding on placentas or aborted fetuses of recently calved livestock and contaminating drinking troughs in the oryx breeding unit. However, the role of this species as local reservoir or disseminator to oryx has yet to be determined.

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ABSTRACT

Cardiopulmonary effects of prolonged anaesthesia via propofol-medetomidine infusion in ponies

TEN healthy ponies underwent anaesthesia and the cardiopulmonary effects of total intravenous (IV) anaesthesia with propofol and medetomidine and of atipamezole on recovery were measured. Sedation was induced by IV medetomidine (7 µg/kg of bodyweight). Anaesthesia was induced by IV propofol (2 mg/kg) and maintained for four hours with infusions of medetomidine (3·5 µg/kg per hour) and propofol (0·07 to 0·11 mg/kg/minute). Spontaneous respiration was supplemented with oxygen. During anaesthesia mean cardiac index and heart rate increased significantly until 150 minutes, then decreased. Mean arterial pressure and systemic vascular resistance index increased significantly between 150 minutes and four hours. Recoveries were without complications within 28 minutes (five ponies received atipamezole at 60 µg/kg during recovery) or 39 minutes (five ponies not given atipamezole). The technique, in which cardiovascular function is comparable to or better than under inhalation anaesthesia, may prove suitable for horses in which prompt recovery is essential. Oxygen supplementation may be needed in some animals in which severe hypoxia may develop.

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