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GLANDERS IN A DROMEDARY CAMEL

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ABSTRACT

A natural infection of a glanders case in a dromedary camel (*Camelus dromedarius*) is reported here for the first time. This animal was most probably infected by glanderous horses which were housed nearby.

The camel showed the classical clinical signs of glanders with fever, emaciation, mucopurulent discharge from the nose, and glanderous lesions in the lung, choanae and nasal septae. The serum was positive in the CFT and cELISA.

B. mallei was isolated from the EDTA blood, from the choanal lesions as well as from nasal pus swabs after enrichment in guinea pig (Strauss reaction). All samples were positive for *B. mallei* with the PCR but negative for *B. pseudomallei*.

Key words: Dromedary camel, glanders

Glanders is a contagious, life-threatening disease of equids caused by the Gram-negative bacterium *Burkholderia (B.) mallei*. Glanders is a notifiable, OIE listed disease and is the subject of chapter 12.11. of the Terrestrial Animal Health Code (OIE, 2008). The infection is generally fatal. Susceptibility to glanders has been demonstrated in wild felines, bears, wolves, dogs and camels. Carnivores become infected by eating contaminated meat. Glanders remains endemic in a number of Asian, African and South American countries. It reappeared in Pakistan and Brazil in 2008 and 2009 and appeared for the first time in Kuwait in 2009 and in Bahrain in 2010 (Roberts *et al*,, 2010; Wernery, 2009).

Camels are also susceptible to *B. mallei* (Curasson, 1947). Curasson (1947) mentioned 2 Russian researchers Djounkovski and Petrowsky who inoculated glanderous material from horses intravenously into Russian camels. The animals developed characteristic nodules and ulcers in the nasal wall and in various organs 11 to 15 days p.i. Transmission by contact between camels and to horses and giraffes was also possible. Samartsev and Arbuzov (1940), however, considered this disease to be of no significance in camels. Mass malleinisation and clinical observation was carried out on 45,922 camels in Russia, but there was no evidence of the disease (Curasson, 1947). From 1966 to 1968 500,000 Mongolian camels were malleinised using the ophthalmic method. Only few reactors were found, but no clinical signs of glanders were observed (Splisteser, 2010, personnel com.).

Of great significance are the reports by Bergin and Torenbeek (1991) and Choy *et al* (2000) as well as Wernery *et al* (1997), Wernery and Kaaden (2002) and Neubauer *et al* (2007) who were the first to diagnose melioidosis in Australian and Emirati dromedaries. Clinical signs and pathological lesions of melioidosis which is caused by *B. pseudomallei* are very similar to glanders, and the organism can only be distinguished from *B. mallei* by molecular biological methods.

Present report is based on a spontaneous natural infection of glanders in a dromedary camel in Bahrain.

Materials and Methods

During investigations of a glanders outbreak in equids in Bahrain in 2010, also dromedaries were included in our investigations. On a small private farm where 2 glanders serological positive horses were detected, one camel showed severe mucopurulent discharge from both nostrils, fever, emaciation and fatigue. Nasal and eye swabs were collected and stored in Amies transport medium as well as 2 EDTA bloods were withdrawn. All samples were sent to CVRL in a cool container, and arrived at the laboratory within 24 hours. Several days later, the dromedary whose health had further deteriorated,

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was euthanised with T61 and a necropsy was performed. Before euthanisation blood was collected from the jugular vein for serological investigation. For microbiological and histological investigation, samples were taken from the lung and choanae, and these were dispatched to CVRL on dry ice after they were frozen at -20°C in Bahrain.

EDTA blood, nasal and eye swabs as well as lung and choanae specimen were examined for glanders bacteria using methods which have been described by Wittig *et al* (2006) in detail. In brief: 3 ml of EDTA blood was injected into 2 blood culture bottles, each (Oxoid, BCO102M) and incubated at 37°C for 11 days. Samples from blood culture bottles, different organs and swabs from different sites were streaked on the following agars:

- Conventional blood agar (1.5% agar) (Merck 1.10886.0500)
- Conventional blood agar with 3% glycerol (Glycerol-Panreac 131339.1212)
- Brilliant- green Phenol- red Lactose Sucrose Agar (BPLS) (Merck 1.07237.0500)
- Nutrient Agar (Oxoid CM0003)
- Stiff blood Agar (3% Agar) (Agar-Fluka 05040)
- Sabouraud's agar (Merck 1.07315.0500)

The agars were incubated at 37°C for 72 h except for Sabouraud's agar which was incubated at 30°C for 7 days and daily adspected. Suspicious *B. mallei* colonies were subcultured on normal blood agar plates which were again incubated at 37°C for 48 h. The pure colonies were Gram stained and tested for oxidase reaction (Bio Mérieux, 55635). Oxidase – positive colonies were then tested in the API 20 NE and API 20 E (Bio Mérieux) system, and their reactions were read after 24 and 48 hrs of incubation at 37°C.

A 10% tissue homogenate in NaCl from choanal lesion, lung tissue and nasal pus swab eluate was separately inoculated (1ml) intraperitoneally into three male adult guinea pigs for Strauss reaction. The animals were adspected every second day and euthanised after 14 days p.i.

Lung samples were also examined histologically using routine methods. The isolated strain and organ samples were examined with the PCR using the method described by Scholz *et al* (2006).

Serum was tested for antibodies with the OIE acknowledged Complement Fixation Test (CFT) and with the CVRL developed cELISA (Sprague *et al*, 2009).

Results

The dromedary which showed severe thick yellowish nasal discharge from both nostrils (Fig 1) was severely emaciated with no farcy lesions. During necropsy it revealed typical glanderous lesions in the lung, choanae and nasal septae. Golf ball size reddish grey nodules resembling tubercles with a central grey necrotic zone were detected in the lungs. In the choanae and nasal septae, stellate scars, ulcers and honeycomb necrotic patches covered with yellow pus (Fig 2) were seen. No glanderous lesions were observed in other organs.

The EDTA blood was incubated for 11 days in blood culture medium and pure colonies of *B. mallei* were isolated on 5 agars used except BPLS agar. These were visible after 48 h of incubation as small pale round colonies which grew bigger 24 h later. On blood agar with glycerol they appeared bigger after 48 h than on the other agars.

B. mallei was also isolated from the choanal pus but not from nasal and eye swabs and lung abscesses. All plates derived from these samples, revealed heavy growth of different bacterial species like *Corynebacteruim* spp., *Achromobacter denitrificans, Chryseobacterium indologenes, Streptococci* spp. Eye and nasal swabs showed also heavy growth of *Aspergillus fumigatus* on Sabouraud agar, where as choanal lesion and lung were negative for any fungal growth. Colonies from EDTA blood and choanal lesions were Gram negative but stained poorly (Fig 3). These were weak oxidase positive and showed positive reactions in Api 20 NE for reduction of nitrates(NO_3), arginine dihydrolase (ADH), assimilation of glucose (GLU), N – acetyl glucosamine NAG) as well as potassium gluconate (GNT). ADH changed colour only after 48 h. However, these tests could not identify *B. mallei* with certainty (Wittig *et al*, 2006). Two guinea pigs inoculated with nasal pus swabs and choanal lesions showed typical Strauss reaction (Fig 4) 12 days p.i. These were euthanised on day 14 and *B. mallei* was isolated in pure culture from their testicles from both animals. Strauss reaction was negative from lung tissue. Histological examination of the lung granulomas were characterised by central necroses surrounded by pyogranulomatous inflammation (Fig 5) which comprised of many neutrophils, some lymphocytes, macrophages, epithelial and few giant cells. No acid-fast rods or fungal hyphae were seen in special stained slides.

PCR showed positive reactions for *B. mallei* from nasal and eye swabs, choanal lesion, lung tissue and from the isolated strains but not for *B. pseudomallei*.

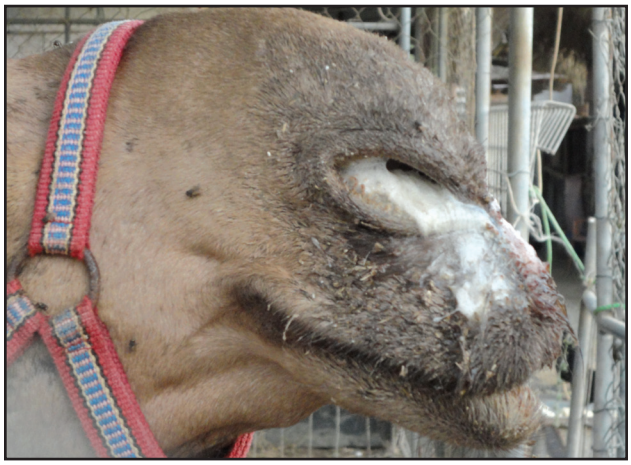

Fig 1. Severe mucopurulent discharge from both nostrils of a glanderous dromedary.

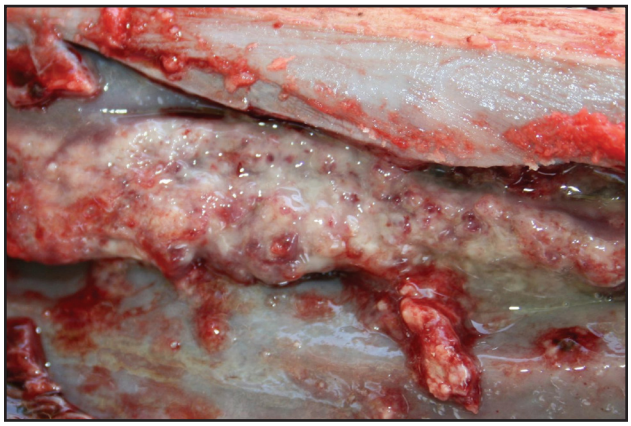

Fig 2. Glanderous lesions in the choanae of a dromedary.

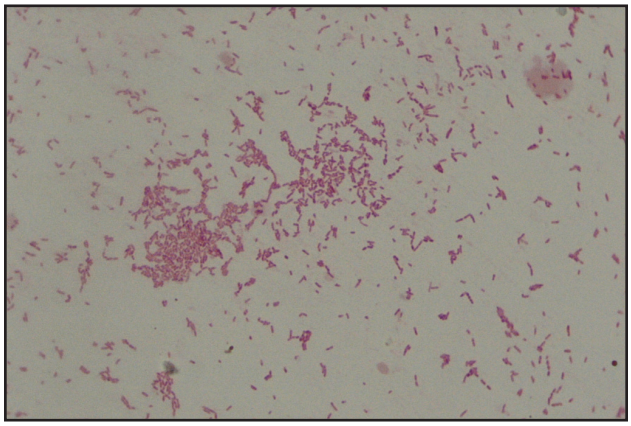

Fig 3. *B. mallei* strain isolated from the dromedary EDTA blood is poorly Gram stained.

Serum which was tested with the CFT and cELISA was positive. The CFT titre was 1:10 ++++ and the cELISA showed a percentage inhibition of 57%.

Discussion

Old World Camelids (OWC), the dromedary and the bactrian camel are susceptible to glanders and

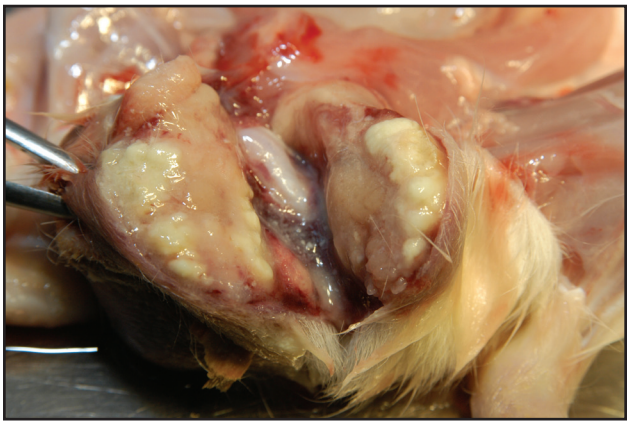

Fig 4. Straus reaction: orchitis with abscessation.

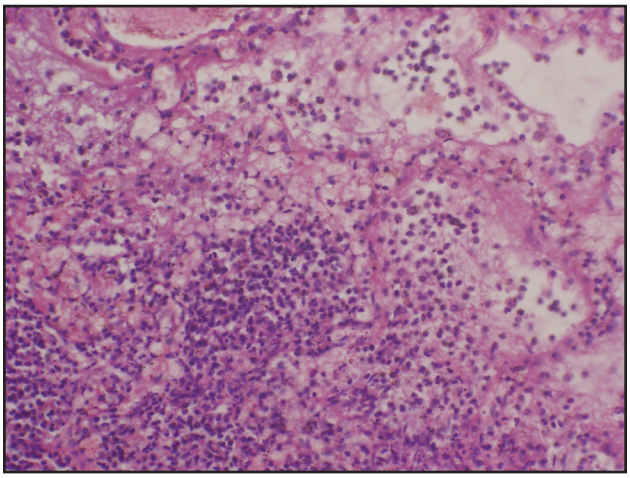

Fig 5. Pyogranulomatous inflammation of the lung with "pseudo tubercles."

melioidosis (pseudoglanders) (Wernery and Kaaden, 2002). Dromedaries have been artificially infected with *B. mallei* but this is the first report of a natural infection in a dromedary.

B. mallei was isolated in pure culture from EDTA blood indicating a septicaemia, the bacteria was also directly isolated from the pus which had accumulated in the choanae, but not from nasal and eye swabs and not from the lung abscesses. A positive culture from the nasal swabs was only possible through enrichment of a guinea pig, but was unsuccessful with lung tissue. Direct isolation from the nasal swabs was negative most probably due to the heavy overgrowth of different bacteria and *Aspergillus*. In a previous outbreak in horses it has been shown that it can be very difficult to isolate the pathogen due to overgrowth by other bacteria species or fungus when plates have to be incubated over 3 days and due to the fact that in general, the number of *B. mallei* microorganisms in clinical samples is very low (Wittig *et al*,, 2006; Wernery, 2009). Therefore,

an enrichment in male guinea pigs should always be tried. However, this test is not pathognomic because other pathogens can also cause peritonitis and/or orchitis. Therefore, *B. mallei* must be re-isolated from testicles. Although the camel revealed typical glanderous lung lesions, the direct and the guinea pig inoculation failed to produce a positive result from the lung. One explanation could be that the samples were frozen at -20°C for more than 20 days. Freezing clinical samples at -20°C over a longer period of time seems to destroy this pathogen.

All samples were positive in the PCR. This included the strains isolated, nasal and eye swabs, lung abscess and lesions from the choanae. As *B. mallei* was not isolated directly from swabs because of heavy contamination with fungal and bacteria colonies and not at all from the lung abscesses due to the freezing destruction, the PCR was positive indicating an important advantage towards other diagnostic methods. The PCR not only detects the DNA of non-viable organisms but also can process samples within 24 to 48 hours, where as culture may take more than one week.

Clinical signs as well as gross pathological and microscopical lesions are very similar to changes seen in equids. These changes are dominated by severe mucopurulent nasal discharge, nodules and ulcers with pus in the choanae and nasal septae and granulomas in the lung which resemble tubercle lesions. As they undergo the same evolution as tubercle lesions, they are named ''pseudo tubercles''.

Before the dromedary was euthanised, blood was withdrawn and tested with the CFT and cELISA. Both tests were positive. The CFT, which is the only serological test acknowledged by the OIE, is an accurate test that has been used for glanders diagnosis for many years (Neubauer *et al*, 2005). It is reported to have a sensitivity of 90-95%. However, it has been shown that around 1% of the tested equine sera show false positive reactions (cross reaction), which causes uncertainty in times of ever increasing horse transport (Wernery *et al*, 2004). Therefore other serological tests have been developed, but none of these tests can differentiate between *B. mallei* and *B. pseudomallei* infection although a cELISA with a specifity of 100% for serodiagnosis of human melioidosis has recently been developed using a monoclonal antibody to a specific epitope on the lipopolysachharide (LPS) of *B. pseudomallei* (Thepthai *et al,* 2005). Our CVRL in house cELISA for *B. mallei* which is not yet acknowledged by the OIE also uses an anti – LPS monoclonal antibody (Mab 3D 11) is currently being validated. It has the advantage that, so far, no cross reactions have been observed, and in few cases has identified in the Bahrain equine glanders outbreak reactors earlier than the CFT. Both tests are also applicable in camels.

Curasson (1947) has already reported a transmission of glanders between camels to horses and giraffes.

In the Bahraini outbreak, the disease was first detected in horses. In the premises where the positive dromedary was detected, two out of 7 horses were serologically positive and showed clinical signs of glanders and were euthanised. Six dromedaries were kept a couple of meters away from the sick horses in a separate enclosure, 3 died showing clinical signs of glanders, but were not necropsied, except one, which is reported here.

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