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Short communication

Disseminated *Rhodococcus equi* infection in dromedary camels (*Camelus dromedarius*)

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ABSTRACT

Rhodococcus (R). equi, a recognized pathogen in horses, is emerging as a human opportunistic pathogen, especially in immunocompromized people. It affects also New World camelids, but there are no reports of *R. equi* infection in Old World camelids yet.

Four cases of disseminated *R. equi* infection in adult breeding dromedaries occurred at one camel farm near Dubai within 16 months of each other. At necropsy the lungs were diffusely consolidated with large caseous areas. Histology revealed severe suppurative to necrotising pneumonia with multiple encapsulated abscesses. Immunohistochemistry enabled the detection of 15- to 17-kDa antigens (VapA) of *R. equi* in the lung sections. High numbers of *R. equi* were isolated from the lung lesions as well as from liver, spleen and mediastinal lymph nodes, indicative of septicaemia. The isolated strains were PCR-positive for the specific virulence plasmid (VapA-Gen) of *R. equi*, indicating virulent strains and containing an 85-kb type I plasmid.

This is the first report of disseminated *R. equi* infection in Old World camelids. Since adult camels in general do not suffer from bacterial caused pneumonia (except tuberculosis), this is a new emerging disease for camels.

Rhodococcus (R). equi, a recognized pathogen in horses (Ainsworth, 1999; Giguère and Prescott, 1997), is emerging as a human opportunistic pathogen, especially in immunocompromized hosts (Puthucheary et al., 2006). It affects also New World camelids (Leite et al., 1975; Hong and Donahue, 1995; Cuteri et al., 2001), but there are no reports of *R. equi* infection in Old World camelids yet. However, from March 2008 to July 2009 CVRL received carcasses of four female breeding dromedaries from one farm. Necropsy was immediately performed and samples were obtained for histopathology and bacteriology, using routine methods. Selected paraffin-embedded sections of the first two cases were used for immunohistochemistry with rabbit polyclonal and mouse monoclonal primary antibodies (MAb 10G5) against virulence-associated l5- to 17-kDa antigens (VapA) of virulent *R. equi* (Madarame et al., 1996). Ten samples from the soil of the camel-breeding farm were collected after the first two cases occurred and processed for bacteriology, using routine methods.

Two different PCR detection techniques were performed at IVD GmbH Hannover-Ahlem (Germany) on isolated *R. equi* strains from the first two cases: first, a chromosomal PCR, based on the isocitrate lyase-gene coding for the enzyme isocitrate lyase of *R. equi* (Venner et al., 2007). The second PCR was designed for a specific virulence plasmid (VapA-Gen) of *R.* equi (Venner et al., 2007). Plasmid profiles were established from two *R. equi* isolates according to the method previously described (Takai et al., 1995).

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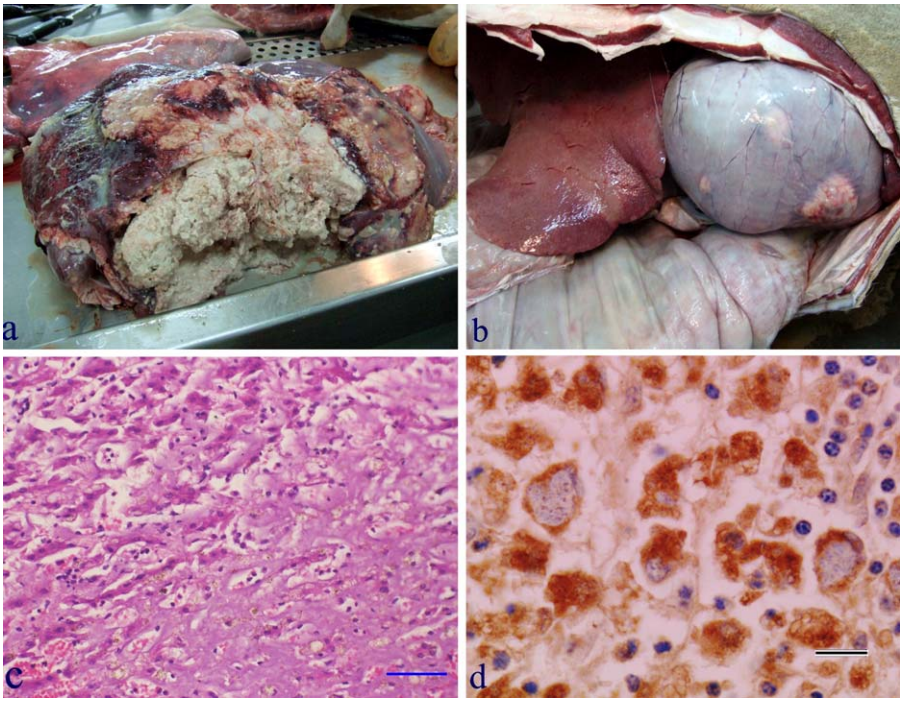

Fig. 1. *Rhodococcus equi* infection in dromedary camels: Massive caseous pneumonia of the first dromedary (a) and several caseous necrosis in the wall of compartment 3 and hepatomegaly of the second dromedary (b); amyloidosis of hepatic Disse' space compressing the hepatocellular plates (c) (H&E, scale bar: 100 μm, original magnification: 220×) and swollen macrophages with both positive granular bacteria against l5–17-kd antigens of virulent *R. equi* and basophilic dot- or rod-shaped microorganisms negative against l5- to 17-kDa antigens of virulent *R. equi* (d) (Immunohistochemistry with Mayer's haematoxylin counterstain, scale bar: 200 μm, original magnification: 800×).

Massive pneumonia involving 60–70% of the lungs was seen at necropsy in all cases (Fig. 1a). Beside large caseous areas small, tan nodules were distributed throughout the rest of the lung. Mediastinal lymph nodes and liver were enlarged. Furthermore, several caseous necrosis (3–5 cm in diameter) extending through the entire wall of the forestomach compartment 3 (Fig. 1b) were observed in the second case. Histological examination of the lung revealed marked, multifocal, obliterative inflammation comprised of large numbers of lymphocytes, plasmacytes and neutrophils with central necrosis. No fungal hyphae or acid-fast rods were detected in special stained slides, but Gram-positive coccal bacteria were observed in the debris. Histology of mediastinal lymph nodes revealed suppurative inflammation with numerous macrophages, containing coccal bacteria. Marked and severe diffuse hepatic amyloidosis with deposits in the Disse' space, compressing the hepatocellular plates (Fig. 1c) was revealed in the first and second case, respectively, but not in the other 2 cases. Immunohistochemistry enabled the detection of 15- to 17-kDa antigens (VapA) in the lung sections of the first two cases. These antigens were frequently observed in the abscesses and to a lesser extent in the purulent debris of the alveoli and bronchi (Fig. 1d).

High numbers of *R. equi* were isolated from lung lesions, liver, spleen and mediastinal lymph nodes from all four cases as well as from the gastric abscesses in the second case. Additionally, *Corynebacterium pseudotuberculosis* (medium numbers) was isolated from the lung in the first case, *Streptococcus* spp. (high numbers) from the liver of the second case and *Staphylococcus aureus* from lung (low numbers), liver, udder and lymph nodes (medium numbers) in the last case. No *R. equi* were isolated from the soil samples collected at the camel-breeding farm.

Chromosomal PCR was positive of the isolated strains of the first two animals, confirming *R. equi.* Both strains were also positive for the virulence plasmid (VapA-Gen) of *R. equi*, indicating virulent strains. Plasmid profiles established from isolates of the last two camels revealed an 85-kb type I virulence plasmid.

The macroscopic findings in all dromedaries were similar to the caseous pneumonia described in a 2-year-old male llama caused by *R. equi* (Hong and Donahue, 1995). Accordingly, severe pyogranulative bronchopneumonia with nodules containing caseous yellowish pus is typical in affected foals (Caswell and Williams, 2007). Half of the foals with lung lesions develop also intestinal lesions, most commonly in the large intestine (Caswell and Williams, 2007). They start as focal mucosal ulcers and later form foci of caseous necrosis extending through the entire wall (Mariotti et al., 2000) similar to lesions in the wall of compartment 3 of the second dromedary. The survival rate in foals is significantly lower among foals with extrapulmonary disorders (EPDs) than among foals without EPDs (Reuss et al., 2009). In this study EPDs were found in 111 out of 150 foals with *R. equi* infections (74%), but many EPDs were only recognized after death. Only one of the four dromedaries developed EPDs. However, *R. equi* was isolated from several organs of all dromedaries, hence