Outbreaks of Keratoconjunctivitis in a Camel Herd Caused by a Specific Biovar of *Moraxella canis*  

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Received 26 November 2009/Accepted 1 December 2009  

Two tributyrin hydrolysis-negative *Moraxella* isolates obtained in cases of keratoconjunctivitis in *Camelus dromedarius* in the Canary Islands showed highest degrees of 16S rRNA gene sequence similarity to *Moraxella canis*. A level of DNA relatedness to the *M. canis* type strain of 79% confirmed the identity of the isolates as a tributyrin hydrolysis-negative biovar of *M. canis*.

*Moraxella bovis* is the primary etiological agent of infectious keratoconjunctivitis (IKC), a highly contagious disease of cattle (9, 12). The main clinical signs include corneal ulceration and edema, ocular pain, photophobia, and lacrimation. Other bacterial species isolated from cattle with IKC are *M.ovi*, *M. bovoculi*, and *N. ovis* (1). Four cases of IKC due to *M. lacunata* in cameldids (6) and one case due to *M. lacunata* var. *liquefaciens* in llamas (2) have been described previously. Gionfriddo et al. (7) described direct corneal infection due to *Moraxella* spp. in llamas. We report two outbreaks of IKC in a herd of approximately 50 dromedary camels (*Camelus dromedarius*) in Lanzarote, Canary Islands, Spain, affecting both young and adult animals and males and females equally. Antimicrobial treatment (1 ml of 0.15-mg/ml amoxicillin by intraconjunctival injection) was successful, but a few months later, a second outbreak occurred. In both outbreaks, particular environmental conditions included severe windy weather which resulted in an atmosphere loaded with dust and dry manure particles. The clinical signs observed in the animals were severe conjunctivitis, corneal ulcerations, and edema, epiphora, and photophobia. No systemic clinical signs were observed in any camel. Samples from conjunctival exudates were taken for microbial culture. After culture on chocolate agar, *Moraxella*-like microorganisms were isolated. Antimicrobial susceptibility testing indicated that the organisms were susceptible to the following antibiotics: amoxicillin, penicillin, ampicillin, amoxicillin-clavulanic acid, cephalaxin, cefotiofur, gentamicin, neomycin, streptomycin, cloxacillin, doxycycline, apramycin, bacitracin, enrofloxacin, colistin sulfate, erythromycin, oxytetracycline, and tetracycline. The organisms were resistant to lincomycin, spectinomycin, spiramycin, and trimethoprim-sulfamethoxazole.

Autogenous vaccination was performed using the strain obtained during the second outbreak. Briefly, the strain was cultured on tryptic soy broth (Becton Dickinson, France) and incubated overnight at 37°C. The vaccine contained a minimum of 10⁹ *Moraxella* cells, killed by formalin (0.3%, vol/vol), per ml, with a commercial aluminum hydroxide gel (0.5 mg/ml) as an adjuvant. Animals were vaccinated intramuscularly with a 2-ml dose twice over a 30-day interval and treated with amoxicillin (0.15 mg/ml in 1 ml) once per week for 4 weeks by the intraconjunctival route. After treatment of the second outbreak, the ocular infections of the animals improved progressively and complete recovery was obtained within 3 to 4 weeks.

Conjunctival secretions were collected with sterile swabs and sent to the microbiology laboratory within 4 h after collection. The strains grew well on blood agar, chocolate agar, and brain heart infusion agar at 37°C within 24 h. Strains did not grow on MacConkey agar, as was the case for the six tested *M. canis* strains, in opposition to the original description (8). No hemolysis was observed. After Gram staining, Gram-negative diplococci were observed, and the bacterial cells were approximately 2.5 to 3 μm in diameter. The Gram-negative diplococci isolated were catalase and oxidase positive and, upon testing with the API NH system (bioMérieux, Marcy-l’Etoile, France), yielded code 53013, which does not result in identification. Strains did not produce acid from glucose, fructose, maltose, and saccharose and did not produce indole. Ornithine decarboxylase, urease, beta-galactosidase, pyrrolidonyl aminopeptidase, trypsin, and proline aminopeptidase activities were absent. The strains produced gamma-glutamyltransferase (GGT) and weakly produced alkaline phosphatase. Both camel strains were GGT positive, whereas all tested *M. canis* strains were strongly GGT positive and the *M. catarrhalis* strains were GGT negative. Both camel strains were negative for tributyrin hydrolysis, for acid production from ethylene glycol, and for growth on acetate, whereas the tested *M. canis* strains were positive for these three characteristics and the *M. catarrhalis* strains were positive for tributyrin hydrolysis but negative for acid production from ethylene glycol and for growth on ace-
One camel strain (ULPGC 53013) and one *M. canis* strain (U34) were nitrate reductase negative. Identification of the bacteria by standard methods was not possible, and final identification as *M. canis* was achieved using molecular methods. The tRNA intergenic length polymorphism (tDNA-PCR) patterns (3) of the two available camel isolates were compared to those of other *Moraxella* species and of eight *M. canis* strains, including the *M. canis* type strain. The patterns of the camel strains were highly similar to those of the *M. canis* strains and clearly different from those of *M. cuniculi* and *M. catarrhalis*. Briefly, all *M. canis* strains tested and the two camel strains had the following tRNA intergenic spacers in common: 58.6 (standard deviation [SD], 0.2), 65.9 (0.2), 72 (0.2), 81.8 (0.3), 83.6 (0.2), 119.5 (0.0), 166.3 (0.2), 192.6 (0.4), and 215.7 (0.4) bp. The two camel strains had an additional spacer of 63.6 bp (SD, 0.1 bp), not observed in the tDNA-PCR patterns of the eight other *M. canis* strains.

Sequence analysis of the 16S rRNA genes was carried out as described previously (13). A phylogenetic tree was constructed with a bacterial software module (SmartGene, Zug, Switzerland). Figure 1 presents a 16S rRNA gene-based phylogenetic tree comprising most species of the genus *Moraxella* and including the two available camel strains, which clustered most closely with the *M. canis* type strain, with sequence similarities of 99.7% (for camel strain ULGC 53131) and 98.4% (for camel strain ULPGC 53013).

DNA relatedness was determined by the staff of the LMG culture collection (Ghent, Belgium). Total DNA was prepared according to a modification of the method described by Wilson (14), while hybridizations were performed at 37°C according to a modification of the method described by Ezaki et al. (5). DNA hybridization with the type strain of *M. canis*, LMG 11194, revealed a level of DNA-DNA relatedness of 79%, indicating that both camel strains were genuine *M. canis* strains, despite the biochemical differences and the minor differences in tDNA-PCR patterns and 16S rRNA gene sequences.

To our knowledge, cases of infectious bovine keratoconjunctivitis caused by *M. canis* have not yet been reported, and this account of two outbreaks of ocular infection in a camel herd in Lanzarote is the first description of infection with this organism in animals. *M. canis* was shown previously to be a commensal of the oral cavities of dogs and cats (8). Some previously published case reports describe infections with *M. canis* in humans, i.e., a dog bite-associated infection (8), isolation of *M. canis* from a lymph node of a debilitated alcoholic patient (13) and from a foot ulcer of a diabetic patient (4), and a case of polyarticular septic arthritis with multiple myelomas in a 55-year-old patient (11). *M. canis* is a species with a high degree of intraspecific genotypic and phenotypic variability, as exemplified by the findings of this study and by the original description (8). Possibly this variability is explained by the apparently broad host range of this species, which now has been isolated from humans, dogs, cats, and camels.

After treatment with antibiotics and autovaccination, the ocular infections of the animals improved progressively and complete recovery was obtained within 3 to 4 weeks. The usefulness of autovaccines has not been clearly established, but some reports (10) suggest that administration of an autovaccine leads to the activation of immunologic effector mechanisms which contribute to the recovery of diseased animals.

**Nucleotide sequence accession numbers.** 16S rRNA gene sequences determined in this study have been deposited in GenBank under accession numbers FM244731 (for strain ULPGC 53013) and FM244730 (for strain ULPGC 53131).
Pieter Deschaght is indebted to the IWT for Ph.D. research grant IWT-SB/71184.

REFERENCES


