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Short communication

Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infections in cats

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ABSTRACT

Streptococcus equi subsp. *zooepidemicus* (*S. zooepidemicus*) is a commensal of the mucous membranes and skin of animals, notably equine, and is associated with various infections in animals and humans. Here, we describe an outbreak of respiratory disease in a cattery, which, to the best of our knowledge, is the first report of *S. zooepidemicus* infection in cats. Clinical disease was characterized firstly by abundant purulent nasal discharges and cough, progressing to sinusitis, dyspnea, symptoms of pneumonia and death. Pathological examination revealed different degrees of inflammation of the lower respiratory tract. *S. zooepidemicus* was the main bacteria isolated. Sequencing of the V2 fragment of the 16S gene revealed that the isolates were distributed in two previously described genogroups.
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1. Introduction

Streptococcus equi subspecies *zooepidemicus* (*S. zooepidemicus*), a Lancefield C beta-hemolytic streptococcus, is a commensal of the mucous membranes and skin of various animals species, notably equine, and has been associated with different infections in animals and humans (Quinn et al., 1994; Ruoff et al., 2003).

Feline respiratory disease is mostly multifactorial. Among the pathogens associated with respiratory infections in cats are feline herpes virus-1 (FHV-1), feline calicivirus (FCV), *Chlamydophila felis*, and different bacteria, such as *Mycoplasma felis* and *Mycoplasma gateae*, *Pasteurella multocida*, and *Streptococcus canis*. The role of bacteria in the disease process is usually considered to be secondary (Greene, 2006).

Here we describe an outbreak of respiratory disease in a cattery, which, to the best of our knowledge, is the first report of *S. zooepidemicus* infection outbreak in cats.

2. Materials and methods

2.1. Description of the cattery

Approximately 700 cats were raised freely in a cattery (cattery A) that comprised two 700 m^2, covered enclosures, which were previously used to raise poultry. Practically no compartmentalization was present in any of the enclosures except for a small separated area for kittens in one enclosure and one separated area for treatment of sick cats in the second enclosure. The original cats' population was formed by two main groups of animals: one formed of cats rescued during the evacuation of Israeli settlements in the Gaza strip in late 2005 and the other formed of cats raised in a shelter (cattery B) situated about 100 km to the north of cattery A and that was closed due to poor conditions. Small groups of cats were accepted to cattery A after its establishment. No history of vaccination was available for any of these populations. Prior to entry to the cattery, cats were castrated/neutered, but not vaccinated. The animals were feed with commercial cat food and received water *ad libitum*. Public access to the cattery was limited. A veterinarian visited the cattery approximately three times

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a week. Most of the sick cats were treated in the cattery, but a few were treated off-site by volunteers. In 2006, a few months after the establishment of cattery A, an outbreak of respiratory disease was reported. Nasal and pharyngeal swabs and bronchoalveolar lavage (BAL) from sick cats were sent for bacteriological culture in the Kimron Veterinary Institute (KVI). Following the first cases, dead cats started to be sent for *post-mortem* examination in the KVI and internal organs were sampled for histopathological and bacteriological exams.

2.2. Bacteriological culture and identification

Bacteriological culture was performed from BAL, nasal or pharyngeal swabs of sick cats and internal organs of dead cats (mostly lungs, also spleen, liver, kidney and intestine). Samples were inoculated onto nutrient agar, MacConkey agar and 5% sheep blood agar plates, incubated at 37 °C and examined after 24 and 48 h. An additional blood agar plate was incubated for 48 h in anaerobic conditions (Pack Anaero, Mitsubishi, USA). Bacteria that grew under aerobic conditions were identified by morphology and standard biochemical methods (Quinn et al., 1994). *S. zooepidemicus* final identification was confirmed with API Rapid ID32 Strep (bioMerieux, France). Anaerobic bacteria were identified with API ID32A (bioMerieux, France). BAL, swab and lung specimens were inoculated in Mycoplasma broth (Rosengarten et al., 1994) under 37 °C, 5% CO_2 for 48 h, and plated onto Mycoplasma agar (Rosengarten et al., 1994) afterwards. Mycoplasma agar plates were incubated in a moist chamber under the same conditions and observed for *Mycoplasma* colonies growth every 2 days for up to 10 days. Mycoplasma final identification was performed by immunofluorescence with antibodies for *M. felis*, *M. gateae*, *Mycoplasma arginini* and *Mycoplasma felifaucium* (Purdue University School of Veterinary Medicine, West Lafayette, IN). Slides for *Chlamydophila spp*. immunofluorescence stain (Chlamydia Cel LPS, Cellabs Pty. Ltd., Brookvale, NSW, Australia) were prepared from BAL, swab and lung samples.

2.3. Molecular typing

Total DNA was extracted directly from bacteria grown on blood agar using QIAGEN® DNeasy™ according to the manufacturer instructions. A 560 bp segment of the V2 fragment of the 16S rRNA gene was amplified as described before (Abdulmawjood and Lammler, 2000). The reaction was carried out using 100 ng of each primer V2-f (5′-GAGAGTTTGATCCTGGCTCAGCA-′3) and V2-r (5′-TTACCGCGGCTGCTGGCACGT-′3) in 25 μl containing 1 μl template DNA, 300 mM Tris–HCl pH 8.5, 75 mM $(NH_4)SO_4$, 17.5 mM $MgCl_2$, 10% DMSO, 100 μM Dxtp, and 5 units taq DNA polymerase (AmpliTap, Perkin–Elmer). PCR included a denaturation step at 95 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. PCR products were purified (Wizard PCR prep DNA purification system, Promega, Madison, WI, USA) and sequenced using an Applied Biosystems Automatic Sequencer with the two previous PCR primers. Sequences were assembled and aligned using CLUSTAL X and the neighbor-joining method implemented in MEGA 4.1. The robustness of branching patterns was tested by 1000 bootstrap pseudoreplications.

3. Results and discussion

From June 2006 to January 2008, a total of 78 dead cats (approximately 10% of the population in cattery A) were received for *post-mortem* examination in the KVI. Forty-nine cats showed signs of respiratory disease prior to death. Twenty-eight clinical specimens, including BAL, nasal and pharyngeal swabs from cats with clinical symptoms of respiratory disease were also received for bacteriological examination.

Clinical disease was characterized firstly by abundant purulent nasal discharge and cough, progressing then to sinusitis, dyspnea, symptoms of pneumonia and death.

Complete pathological examination revealed different degrees of inflammation of the lower respiratory tract in 39 cats. The major gross pathology findings were severe, acute and diffuse bronchopneumonia or bronchioalveolar pneumonia, either suppurative or necrosuppurative. Interstitial multifocal pyogranulomatous pneumonia was present in a few cases. Pleuritis was found in four cases with pyothorax present in one of these cases. Pyogranulomatous meningoencephalitis was found in four cases, two of them showing necrotizing lesions. In one case, necrosuppurative peritonitis was observed. The most common histopathological changes observed were a diffuse mixed infiltrate of neutrophils and histiocytes, with lymphocytes to a lesser extent, thickening of the inter-alveolar septa and multifocal bacterial colonies with cocoid forms. Pathological results were strongly consistent with bacterial infections. In ten cats with clinically suspected respiratory disease, no gross pathology changes could be found.

Bacteriology results are summarized in Table 1. *S. zooepidemicus* was the main bacterium isolated from clinical specimens and from dead cats with signs of respiratory disease. From dead animals, *S. zooepidemicus* was isolated from the lungs in all the cases, and also from the sinuses in a few (data not shown). *S. zooepidemicus* was isolated from the pleura in two out of four cases of pleuritis, from the brain in three out of the four cases of meningoencephalitis and from the peritoneum in one case of peritonitis. *S. zooepidemicus* was mostly isolated alone and was mostly dominant in mixed cultures. Interestingly, one strain of *S. zooepidemicus* (Ct28/07), isolated from the brain in a case of meningoencephalitis, grew only in an anaerobic atmosphere at first, requiring a 5% CO_2 atmosphere for growth thereafter. *S. zooepidemicus* was not isolated from any of the 29 dead cats that were received for *post-mortem* examination without clinical and pathological signs of respiratory disease, and only from two of ten cases in which respiratory disease was suspected prior to death, but no gross pathological signs were found in the *post-mortem* examination.

Feline respiratory disease is multifactorial and the role of bacteria is usually regarded to be secondary to viral infection (Greene, 2006) or stressful conditions. Because the vaccination status in cattery A was unknown, the possibility of *S. zooepidemicus* infection being secondary to viral infections could not be ruled out. Lesions suggesting

Table 1
Results of bacterial cultures of clinical samples of sick cats ($n = 28$) and *post-mortem* samples from cats with pathological signs of respiratory disease ($n = 39$) from cattery A.

Sample	
Lungs (pneumonic)	*Streptococcus zooepidemicus* (12)
	S. zooepidemicus + *Pasteurella multocida* (5)
	S. zooepidemicus + *M. gateae* (2)
	S. zooepidemicus + *Streptococcus canis* (1)
	S. zooepidemicus + *P. multocida* + *Chlamydophila spp.* (1)
	S. zooepidemicus + *P. multocida* + *M. felis* (1)
	S. zooepidemicus + *P. multocida* + *S. canis* (1)
	P. multocida (2)
	P. multocida + *S. canis* (1)
	Chlamydophila spp. (2)
	Prevotella spp. + *Fusobacterium mortiferum* (1)
	Escherichia coli (1)
	Contaminated (1)
	No growth (8)
Brain	*S. zooepidemicus* (3)
	No growth (1)
Peritoneum	*S. zooepidemicus* (1)
BAL, nasal and throat swabs	*S. zooepidemicus* (8)
	P. multocida (2)
	S. zooepidemicus + *P. multocida* (1)
	S. zooepidemicus + *P. multocida* + *M. felis* (1)
	M. felis (2)
	M. felis + *M. gateae* (2)
	Other[a] (4)
	Contaminated (5)
	No growth (3)

[a] Miscellaneous bacteria isolated: *Escherichia coli*, *Aeromonas hydrophila*, *Staphylococcus pseudintermedius*, *Mycoplasma spp.*, one case each.

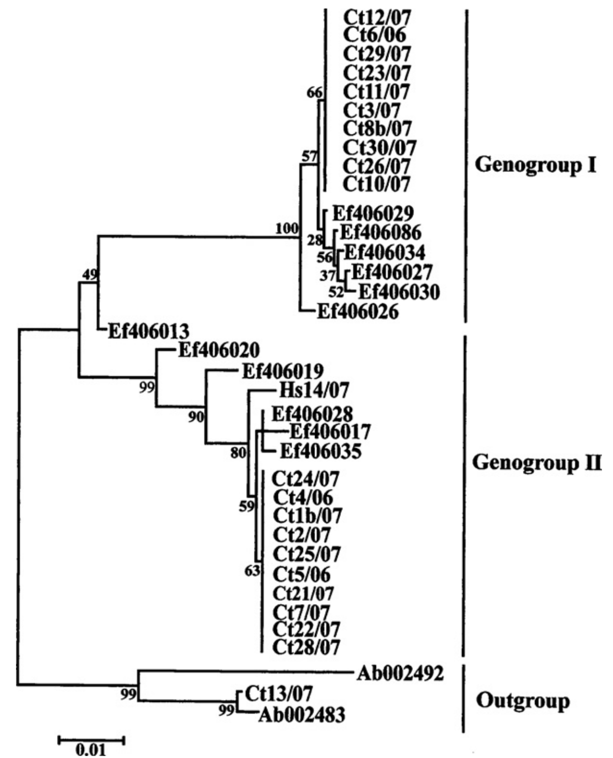

Fig. 1. Phylogenetic tree of 22 feline *S. zooepidemicus* isolates and reference strains. The optimal tree was inferred by using the neighbor-joining method. The percentage of successful bootstrap replicates ($n = 1000$) is indicated at nodes. Evolutionary distances were computed with the Kimura 2-parameter method. Branch lengths' are proportional to the number of nucleotide changes. Scale bar shows number of base substitution per site. Ct corresponds to feline isolates, Hs corresponds to equine ones. Ct 13/07 is a *S. canis* isolate. Genbank accession numbers of reference strains: *S. zooepidemicus* Bd 147/04 (ef406029), *S. equi* subsp. *equi* (ef 406086), *S. zooepidemicus* ATCC43079 (ef406034), *S. zooepidemicus* Bd 2/04 (ef406027), *S. zooepidemicus* Bd 16498/05 (ef406030), *S. zooepidemicus* Bd 23752/03 (ef406026), *S. zooepidemicus* Bd 15630/02 (ef406013), *S. zooepidemicus* Bd 1976/03 (ef406020), *S. zooepidemicus* Bd 1902/03 (ef406019), *S. zooepidemicus* Bd 707/03 (ef406017), *S. equi* subsp. *ruminatorum* CCUG 47520 (ef406035), *S. zooepidemicus* Bd 81/04 (ef406028), and out group *S. dysgalactiae* (ab002492), *S. canis* ATCC 43498 DSM 201715 (ab002483).

feline infectious peritonitis were found in a few cases during the pathological examination, but the presence of feline coronavirus was discarded by immunohistochemical stains (data not shown). Lesions resembling feline distemper were also observed in a few cats, but most of these were not included in this report due either to lack of respiratory symptoms or *S. zooepidemicus* isolation. The prevalence of FHV-1 and FCV was not assessed, but we assumed that these viruses were most probably present in the cattery, as suggested by typical clinical signs of infection with these viruses in some cats. Infections by FHV-1 and FCV are limited mostly to the upper respiratory tract and although they may predispose to secondary infections they do not directly cause pneumonia (Greene, 2006). Stress caused by the transfer and merging of different populations of cats in a single enclosure may have greatly predisposed to the respiratory infections reported here.

Adequate hygienic conditions and ventilation were present in cattery A and the enclosures were apparently not over crowded. Clinically ill cats were treated with antibiotics, but not always fully separated from the other animals. In addition, *S. zooepidemicus* was isolated also from cats showing minor signs of respiratory disease (data not shown), which probably shed the organism long before being detected and treated. Thus, *S. zooepidemicus* may have become persistent in the cattery in spite of sufficient hygienic practices and treatment.

Twenty-two isolates of *S. zooepidemicus* were subjected to molecular typing. Analysis of the V2 fragment of the 16S gene sequences and comparison to reference strains and published sequences revealed two genogroups described before (Baverud et al., 2007) (Fig. 1). Israeli cat isolates belonging to the same genogroup showed 100% homology. Cat isolates in genogroup II showed a two nucleotide difference compared to *S. zooepidemicus* strain no. 14, which was isolated from a case of metritis in a mare. *S. zooepidemicus* isolates belonging to genogroup I were closely related to *Streptococcus equi* subsp. *equi* reference strains EF 406086, similarly to previous work (Baverud et al., 2007). No correlation was found between genogroup

and time of isolation during the outbreak. The earliest typed strains isolated Ct4/06 and Ct5/06 were clustered in genogroup II, but Ct6/06, which was isolated within 2 months from the above strains, was clustered in genogroup I. Since some strains isolated early in the outbreak were not typed, it is unknown which genogroup appeared first in the outbreak. In addition, no correlation between genogroup and virulence (as determined by source, dead cat or clinical specimen) could be found.

One strain of *S. zooepidemicus* (Ct29/07) included in the molecular analysis was isolated from a fibrin purulent infiltrate in the brainstem region of the skull of a dead cat that was raised close to the place where cattery B was located. The owner of this cat volunteered in cattery B, but it is unknown if this cat was previously raised in that cattery. Cattery B was closed due to poor conditions and all the animals were transferred to cattery A at the time of its establishment. This population constituted most of the cats in cattery A at that time. Close to the place where that cat was raised there was a petting farm, with goats, sheep and other farm animals. It is possible that *S. zooepidemicus* has been introduced to cattery A by the population of cats brought from cattery B and that those cats were somehow infected either by direct contact with animals in the petting farm or through stray cats in the same region. Strain Ct29/07 was included in genogroup I, whereas early strains in the outbreak were included in both genogroups. By the time this communication has been written, only one other case of *S. zooepidemicus* outside the aforementioned catteries A and B was found. In this case, *S. zooepidemicus* was isolated from a nasal swab from a house cat (Ct30/07) and it was apparently not linked to the outbreak described here.

Outbreaks of *S. zooepidemicus* infections have been reported in different species, such as clinical mastitis in sheep (Las Heras et al., 2002), hemorrhagic pneumonia in dogs (Garnett et al., 1982; Kim et al., 2007; Pesavento et al., 2008) and human dairy-born septicemia (Edwards et al., 1988; Kuusi et al., 2006). The direct transmission of *S. zooepidemicus* from horses to a human has been suggested (Rose et al., 1980) and the zoonotic potential of this pathogen should not be over-looked. Based on the bacteriological and consistent pathological results described above, we concluded that *S. zooepidemicus* was the main etiological agent in this outbreak of feline respiratory disease. To the best of our knowledge, this is the first report of an outbreak of *S. zooepidemicus* infections in cats.

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