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RESEARCH ARTICLE

Occurrence of *Pasteurellaceae* and *Neisseriaceae* bacteria in the pharyngeal and respiratory tract of dogs and cats – Short communication

BARBARA UJVÁRI[1], BETTINA ORBÁN[1], ZSUZSANNA INCZE[2], ROLAND PSÁDER[2] and TIBOR MAGYAR[1]*

[1] Institute for Veterinary Medical Research, Centre for Agricultural Research, P.O. Box 18, H-1581, Budapest, Hungary

[2] Small Animal Clinic, University of Veterinary Medicine, Budapest, Hungary

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ABSTRACT

The occurrence of members of the *Pasteurellaceae* and *Neisseriaceae* families was studied in dogs and cats. A total of 110 nasal and pharyngeal swab samples from 47 dogs and 8 cats were collected. Most of the strains were identified by 16S rDNA sequencing, except *Frederiksenia canicola* and *Pasteurella multocida* where species-specific polymerase chain reactions were applied. The most frequently isolated species was *F. canicola*, which occurred only in dogs, mainly in the pharyngeal cavity. The second commonest bacterium, *P. multocida* was found in both types of samples and in both hosts. Other species from the family *Pasteurellaceae*, such as *Haemophilus haemoglobinophilus*, *Pasteurella canis* and *P. dagmatis*, were detected only in dogs. All isolated species belonging to the family *Neisseriaceae*, mainly representing *Neisseria weaveri*, were found only in the pharyngeal cavity. *Neisseria weaveri* and *N. zoodegmatis* could be detected in both hosts. *Neisseria dumasiana* and *N. canis* were isolated from dogs, while *N. shayeganii* only from a cat. For phylogenetic analysis, *rpoB* gene sequencing was performed, where the strains were on monophyletic branches and clearly separated from each other. In this study, recently described species such as *F. canicola*, *N. shayeganii* and *N. dumasiana* were detected that had never been isolated in Hungary before.

KEYWORDS

Frederiksenia, *Pasteurellaceae*, phylogeny, *Neisseriaceae*

Representatives of multiple species belonging to the *Pasteurellaceae* and *Neisseriaceae* families have been found on the mucosal surfaces of the upper respiratory tract of vertebrates as opportunistic pathogens (Kuhnert et al., 2012; Hurst, 2018). Moreover, our knowledge is constantly expanding by new bacterial species such as *Frederiksenia canicola* (Korczak et al., 2014), *Neisseria shayeganii*, *N. wadsworthii* (Wolfgang et al., 2011) and *N. dumasiana* (Wroblewski et al., 2017).

Currently *F. canicola* is the only member of the *Frederiksenia* genus in the *Pasteurellaceae* family. Originally it was isolated mainly from the oral cavity of dogs and from dog bite wounds of humans, but also from cat, lion, hedgehog and banded mongoose (Korczak et al., 2014). Recent studies have proved that Tasmanian devils (*Sarcophilus harrisii*) and other marsupial species may also be infected by *F. canicola* (Brix et al., 2015; Hansen et al., 2017).

Animals carrying *Neisseria* might look clinically healthy, yet certain species of that genus can induce disease (Hurst, 2018). Infection by *N. animalis* and *N. zoodegmatis* has been reported in dogs, cats and large felines (Lloyd and Allen, 1980; McParland et al., 1982; Corboz et al., 1993; Cantas et al., 2011). In humans, *N. weaveri* causes skin and soft tissue infections starting from a dog bite wound that may evolve to septicaemia, abscess formation, septic arthritis, tenosynovitis and osteomyelitis (Shinha, 2018). In another case, *N. weaveri* was isolated from the bronchial lavage of a human patient having lower respiratory tract infection (Panagea et al., 2002).

*Corresponding author. Tel.: +36 1 467 4092
E-mail: magyar.tibor@agrar.mta.hu

The aim of the present study was to survey the occurrence of bacterial species belonging to the *Pasteurellaceae* and *Neisseriaceae* families in the nasal and pharyngeal cavity of dogs and cats in Hungary.

A total of 110 samples were collected at the Small Animal Clinic of the University of Veterinary Medicine, Budapest, Hungary. The subjects (47 dogs and 8 cats) were presented for endoscopic examination for different reasons, and were selected randomly, regardless of breed, age, sex, clinical signs, nutritional practices and diseases. Each animal was sampled only once. Swab samples were taken from both the nasal and pharyngeal cavities, and were streaked onto Columbia agar containing 5% sheep blood. Plates were incubated aerobically at 37 °C for 24 h in an atmosphere of 5% CO_2. Small-to medium-sized, greyish colonies with an appearance typical of *Pasteurellaceae* or *Neisseriaceae* were selected for further characterisation. Only isolates confirmed to belong to these two bacterial families were included in the study.

For the identification of *F. canicola* and *Pasteurella multocida,* species-specific polymerase chain reactions (PCRs) were used as described by others (Townsend et al., 1998; Korczak et al., 2014). Other isolates were identified by 16S rDNA sequencing with universal primers 27F (5′- AGAGTTTGATCMTGGCTCAG -3′) and 1512R (5′- ACGGITACCTTGTTACGACTT -3′) as described previously (Angen et al., 1998; Korczak et al., 2014). Phylogenetic analysis was performed on (499-nt-long) alignments of partial sequences of the *rpoB* gene (Korczak et al., 2014). Sequencing of PCR products was performed by Macrogen Europe (Amsterdam, The Netherlands). The provenance of the newly gained sequences was confirmed by the use of the BLASTn program. Nucleotide sequences were aligned and compared using Geneious Prime software (version 2019.2.1; http://www.geneious.com). Nucleotide sequence data were analysed using MEGA7 software (Kumar et al., 2016). The GenBank accession numbers for sequences obtained in this study are MT438763–MT438818.

A total of ten bacterial species were found, five from each of the families *Pasteurellaceae* and *Neisseriaceae* (Table 1). In samples from the nasal cavity, both the incidence and the diversity of bacterial species were lower than in those collected from the pharyngeal area.

Table 2 shows the individual results of bacterial isolation. In two canine cases, *F. canicola* was recovered from both the nasal and pharyngeal cavities, while in one case two different species could be isolated from the nasal and pharyngeal cavities of a dog (*P. multocida* and *Haemophilus haemoglobinophilus*, respectively). Otherwise, isolation was only successful from either the nasal or the pharyngeal cavity. In dogs, 14 out of the 47 (29.8%) animals harboured more than one bacterium species, while this ratio was 1 out of 8 (12.5%) among the cats.

Frederiksenia canicola was found only in samples from dogs, and it was the most frequently isolated species (40.4%, $n = 19$). It was present in both types of samples, but it was more prevalent in the pharyngeal than in the nasal cavity. *Pasteurella multocida* was also isolated rather

Table 1. Incidence of bacterial species identified in the study

Bacterial species	Dog ($n = 47$)			Cat ($n = 8$)			Total
	Nasal swab	Pharyngeal swab	Total cases (%)	Nasal swab	Pharyngeal swab	Total cases (%)	
Frederiksenia canicola	3	18	19 (40.4)	0	0	0	21
Pasteurella multocida	2	4	6 (12.8)	0	2	2 (25.0)	8
Neisseria weaveri	0	7	7 (14.9)	0	1	1 (12.5)	8
Haemophilus haemoglobinophilus	0	7	7 (14.9)	0	0	0	7
Pasteurella canis	0	5	5 (10.6)	0	0	0	5
Neisseria dumasiana	0	5	5 (10.6)	0	0	0	5
Neisseria zoodegmatis	0	1	1 (2.1)	0	1	1 (12.5)	2
Pasteurella dagmatis	1	0	1 (2.1)	0	0	0	1
Neisseria shayeganii	0	0	0	0	1	1 (12.5)	1
Neisseria canis	0	1	1 (2.1)	0	0	0	1

Table 2. Bacterial species isolated from each host

	ID		Fc	Hh	Pm	Pc	Pd	Nw	Nd	Ns	Nz	Nc
D	1	p	+									
D	2	p	+	+								
D	3	p		+								
D	4	p	+									
D	5	p	+									
D	6	p				+						
D	7	p	+									
D	8	p	+			+						
D	9	n	+									
D	9	p	+									
D	10	p	+		+							
D	11	p	+					+*				
D	12	p	+									
D	13	p				+						
D	14	n			+							
D	15	n	+				+					
D	16	n			+							
D	16	p		+								
D	17	p		+								
D	18	p			+							
D	19	p	+		+			+*				
D	20	p	+		+							
D	21	p	+									
D	22	p	+					+	+			
D	23	p		+					+			
D	24	p	+					+	+			
D	25	p							+			
D	26	p		+								
D	27	n	+									
D	27	p	+					+				
D	28	p	+									
D	29	p									+	
D	30	p				+		+	+			
D	31	p	+	+		+		+				+
C	1	p			+							
C	2	p			+					+	+	
C	3	p						+*				

Fc – *Frederiksenia canicola*, Hh – *Haemophilus haemoglobinophilus*, Pm – *Pasteurella multocida*, Pc – *Pasteurella canis*, Pd – *Pasteurella dagmatis*, Nw – *Neisseria weaveri*, Nd – *Neisseria dumasiana*, NS – *Neisseria shayeganii*, NZ – *Neisseria zoodegmatis*, NC – *Neisseria canis*, D – dog, C – cat, p – pharyngeal swab, n – nasal swab, *not shown on the phylogenetic tree (Fig. 1).

frequently, in 12.8% of dogs and 25.0% of cats. This species occurred equally in the pharyngeal and nasal areas. *Haemophilus haemoglobinophilus* was detected only in canine pharyngeal samples, from seven cases (14.9%). *Pasteurella canis* and *P. dagmatis* were isolated only from dogs in some cases (Table 1). Interestingly, these findings are in agreement with the results of Brix et al. (2015) and Hansen et al. (2017), who found similar prevalences of *Pasteurellaceae* species in the oral cavity of Tasmanian devils and other marsupial species, i.e. in very different hosts at a distinct geographical location.

A few years ago, *P. dagmatis*-like organisms were described in Hungary (Sellyei et al., 2010) from the oral cavity of cats. The 16S rRNA gene sequences of these strains were different from those of the strain found in the present study. The 1,308 bp sequences of the *P. dagmatis*-like isolates revealed only 97.48–97.86% identity to the *P. dagmatis* isolate of this study, while the latter isolate showed 100% 16S rRNA gene sequence identity to the *P. dagmatis* NCTC 11617 reference strain.

Neisseria species were also identified in the samples quite often. The following five species were found: *N. weaveri*, *N. dumasiana*, *N. zoodegmatis*, *N. shayeganii* and *N. canis*. Interestingly, they were isolated only from the pharyngeal cavity. *Neisseria weaveri* and *N. zoodegmatis* were detected in both host species; *N. dumasiana* and *N. canis* were isolated only from dogs, while the *N. shayeganii* isolate originated from a cat. The *Neisseria* species recovered in this study (Table 1) showed a resemblance to those isolated the most commonly from dog bite wounds (*N. weaveri*, *N. zoodegmatis*, *N. animolaris*, *N. subflava*) (Shinha, 2018). Due to the small number of samples from cats, no far-reaching conclusions can be drawn from our results. However, it could be concluded that, in accordance with the findings of

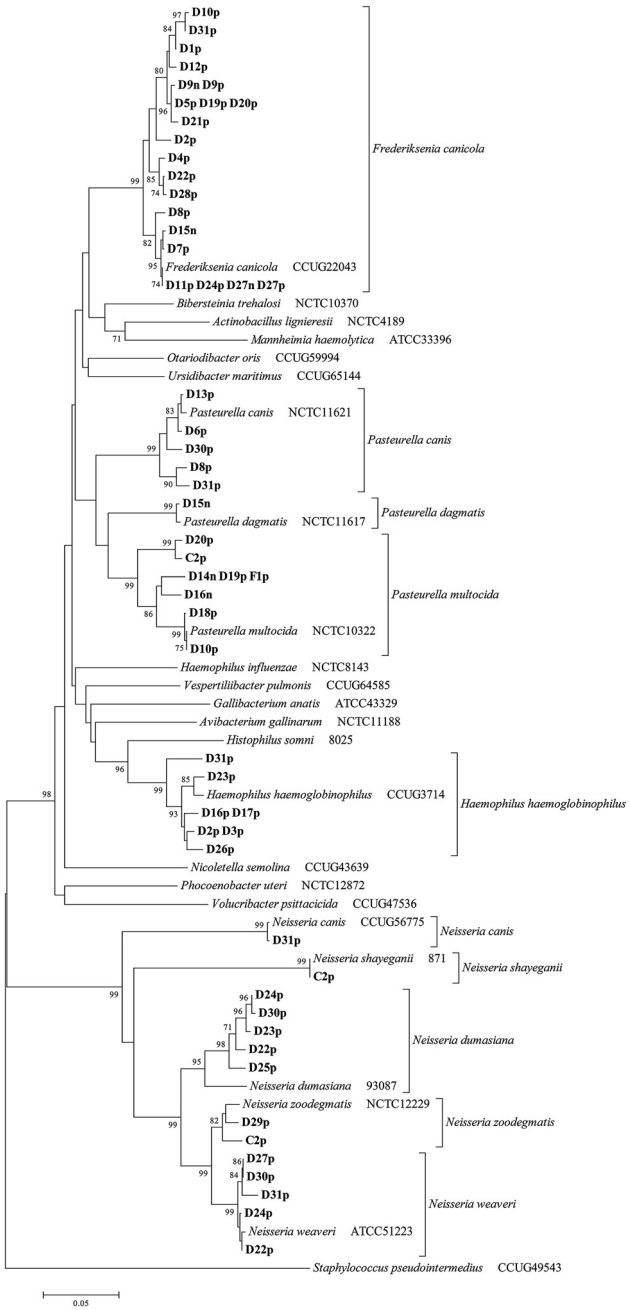

Fig. 1. rpoB gene based phylogenetic tree of isolates belonging to the *Pasteurellaceae* and *Neisseriaceae* families. The evolutionary history was inferred using the Neighbour-Joining method. Evolutionary analysis were conducted in MEGA7 (Kumar et al., 2016). D – dog, C – cat, p – pharyngeal swab, n – nasal swab

Sturgeon et al. (2014), members of the *Neisseriaceae* family can frequently be found in cats, which also poses a potential zoonotic risk.

Analysis of the partial *rpoB* sequences proven to have a greater resolution than the 16S rDNA allows accurate identification among the species of *Pasteurellaceae* and *Neisseriaceae*. The method described by Korczak et al. (2004) was successfully applied to all strains isolated in this study. On the *rpoB*-derived phylogenetic tree the *Pasteurella*, *Haemophilus*, *Frederiksenia* and *Neisseria* strains isolated in this study belonged to monophyletic branches according to their bacterial family, genus and species, and clearly separated from each other (Fig. 1).

In this study, we found several bacterial species that were described in the last decade but had not previously been identified in Hungary, including *F. canicola* (Korczak et al., 2014), *N. shayeganii* (Wolfgang et al., 2011) and *N. dumasiana* (Wroblewski et al., 2017). The results of this study indicate that routine diagnostic laboratories should also take into account the potential presence of these newly described species during the bacteriological examination of nasal or pharyngeal samples from dogs and cats. Our

findings also suggest that these species may occur in bite wounds inflicted on humans by dogs or cats.

Further research should be done to investigate the clinical significance, antibiotic resistance and human health implications of these bacterial species to allow their better recognition. This seems to be of particular interest for *F. canicola*, a previously undetected yet highly prevalent bacterial species.

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