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Prevalence and Characteristics of *Streptococcus canis* Strains Isolated from Dogs and Cats

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Abstract

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To determine the prevalence of *Streptococcus canis* in dogs and cats, a total of 926 swabs were examined bacteriologically in the period from 2003 to 2005. Eighty-six isolates obtained from various anatomical locations were further characterized for their phenotypic properties. The most frequently isolated biotype produced phosphatase, leucine amidopeptidase, arginine dihydrolase, alpha-D- and beta-D-galactosidase and fermented lactose and ribose. Additional identification by species-specific amplification of the 16S-23S rRNA intergenic spacer region was consistent with *S. canis*. All isolates were susceptible to penicillin G and ampicillin. The least effective antimicrobial agent was found to be tetracycline (only 33.8% of susceptible strains).

Prevalence, S. canis, biochemical properties, 16S-23S rRNA intergenic spacer region

Streptococcus (*S.*) *canis* belongs to Lancefield serogroup G, which forms heterogeneous group of beta-hemolytic streptococci. Presently, three taxonomic types of this serological group can be distinguished, as follows: minute colony formers from humans (*S. anginosus* group), and large colony formers from humans (*S. dysgalactiae* ssp. *equisimilis*) and animals (*S. canis*).

The species *S. canis* was officially described by Devriese et al. (1986) and is considered to be part of healthy microbiota of skin and mucosa of animals, especially dogs and cats, and the udders of cows, although it may be responsible for opportunistic infections. In dogs, *S. canis* is isolated from a variety of diseases including skin infections, infections of urogenital and respiratory tracts, otitis externa, septicaemia, necrotizing fasciitis and streptococcal toxic shock syndrome (Bornand 1992; Miller et al. 1996; DeWinter et al. 1999). *S. canis* also causes various infections in cats, including arthritis, wound infections, septicaemia and streptococcal toxic shock syndrome, and it can cause mastitis in cows (Iglauer et al. 1991; Hassan et al. 2005). Very few human infections with *S. canis* have been documented; however, human infections with this species may be underestimated because many clinical isolates are reported to only as "group G *Streptococcus*". Syndromes that have been associated specifically with *S. canis* include septicaemia, meningitis and peritonitis (Bert and Lambert-Zechovsky 1997; Takeda et al. 2001; Whatmore et al. 2001).

In the present study, we isolated *S. canis* from specimens collected from healthy household pets and from pets with various opportunistic infections. These strains were characterized for their phenotypic properties and examined for their susceptibility to selected antimicrobial agents. Identification of all *S. canis* strains was confirmed by species-specific polymerase chain reaction (PCR).

Materials and Methods

Bacterial strains

Eighty six *S. canis* strains included in this study had been recovered from patients at the Veterinary Clinic of Pardubice during the period from 2003 to 2005. Samples were taken from 124 dogs and 21 cats with proceeding

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infection. In addition, 199 dogs and 50 cats without clinical signs of infection were included in the study. *S. canis* ATCC 43496, kindly provided by Dr. J. Motlová (National Institute of Public Health, Prague, CZ) was used as a positive control for PCR reaction. *Staphylococcus aureus* ATCC 25923 and *S. agalactiae* CCM 6187, from the Department of Microbiology culture collection, were used to perform CAMP reaction.

Isolation

Samples were cultured by using blood agar base supplemented with 5% sheep blood, Edward's modified medium supplemented with 7% sheep blood (both Oxoid, UK) and Slanetz and Bartley medium (HiMedia Laboratories, India). The plates were incubated at 37 °C for 24 h in normal atmosphere. Colonies resembling streptococci and surrounded by a zone of beta-haemolysis were selected for further investigation. Subsequently, all streptococcal strains were cultured by using Todd-Hewitt broth and blood agar base (both Oxoid, UK) supplemented with 5% sheep blood. The plates and tubes were incubated at 37 °C for 24 h in normal atmosphere. After the incubation period the strains were subjected to species identification and also evaluated for antimicrobial susceptibility.

Phenotypic identification

The biotype of each *S. canis* strain was determined by the STREPTOtest 16 identification system using the Identification program TNW lite 6.0 (PLIVA-Lachema a.s., CZ). Lancefield typing was performed by latex agglutination using ITEST STREPTO GROUP (ITEST plus s.r.o., CZ). For the CAMP test, each isolate was streaked on 5% sheep blood agar plates at right angles to beta-haemolysin producing strain of *Staphylococcus aureus* and incubated overnight at 37 °C. *S. agalactiae* was included as a positive control. After incubation, plates were examined for synergistic haemolysis between the two organisms (Lammler et al. 1987).

Antimicrobial susceptibility testing

Following antimicrobial agents were used for disk diffusion testing: penicillin G, ampicillin, chloramphenicol, vancomycin, tetracycline, erythromycin and clindamycin (Oxoid, UK). In preparation for testing, the strains were grown overnight on 5% sheep blood agar plates at 37 °C. A bacterial suspension equal to a McFarland standard of 0.5 prepared in 0.85% saline was used to inoculate plates containing Mueller-Hinton agar (Oxoid, UK) supplemented with 5% sheep blood. The inhibitory zone diameters obtained around the antibiotic disks were measured after incubation for 24 h at 37 °C. Susceptibility, intermediate susceptibility and resistance to antibiotics were interpreted according to the recommendations of National Committee for Clinical Laboratory Standards (2001).

Species-specific PCR

PCR was performed with primer c-I 5´-TAAACCGAAAACGCTGTAAGTATTA-3´ and primer c-II 5´-ACCATTAGTTAGTGGGTTCCCCC-3´ as described previously by Hassan et al. (2003). The species-specific primers were targeted to 16S-23S rRNA intergenic spacer region and forming the amplicon with a size of 215 bp. The PCR reaction mixture (24 µl) contained 0.18 µl primer c-I (100 pmol·µl^{-1}) and 0.18 µl primer c-II (100 pmol·µl^{-1}), 2 µl dNTP (2.5 mM each, TAKARA BIO INC., Japan), 2.5 µl 10 × PCR buffer (TAKARA BIO INC.), 1.5 µl $MgCl_2$ (25 mM, TAKARA BIO INC.), 0.13 µl Taq DNA polymerase (5 U·µl^{-1}, TAKARA BIO INC.) and 17.52 µl distilled water. Finally one colony of tested strain was added to each reaction tube. The PCR assay was performed using Robocycler Gradient 96 (Stratagene, USA) as follows: initial denaturation at 94 °C for 4 min, 30 cycles of denaturation at 94 °C for 10 s, annealing at 61 °C for 30 s and extension at 72 °C for 10 s. Finally, a 10-min extension period at 72 °C was carried out. Positive and negative controls were included in each run of amplification. The presence of a specific PCR product was controlled by electrophoresis of 10 µl of the reaction product in a 1.5% agarose gel (SERVA Electrophoresis GmbH, Germany). The molecular weight of amplification product was determined using 100-bp DNA ladder (FERMENTAS INTERNATIONAL INC., Canada).

Results

Of the total of 394 animals included in this study, 58 (18%) dogs and 9 (12.7%) cats were positive for *S. canis*. The prevalence of *S. canis* samples taken from various anatomical locations of healthy animals and animals with proceeding infection (Tables 1 and 2) revealed a preponderance of recoveries from the rectum. The occurrence of *S. canis* in animals with proceeding infection is summarized in Fig. 1. Of 145 samples taken from local infections, 31 (21.4%) were positive for *S. canis*. This microorganism was most frequently isolated from animals with otitis externa. On the other hand, we did not find *S. canis* in specimens taken from animals with rhinitis.

All 86 beta-haemolytic streptococcal isolates belonged to the Lancefield serogroup G and were classified as *S. canis*. According to the biochemical test results (listed in Table 3), the 86 *S. canis* isolates had 22 biotypes. The most common biotype (31 of 86 strains or 36%) produced phosphatase, leucine amidopeptidase, arginine dihydrolase, alpha-D- and beta-D-galactosidase and fermented lactose and ribose. All isolates produced arginine dihydrolase and failed to produce pyrrolidonylarylamidase. All isolates also failed to ferment sorbitol,

Table 1. Frequency of *Streptococcus canis* isolated from various anatomical sites of healthy dogs and cats

Anatomical sites	Dogs			Cats		
	Total of specimens (n = 539)	Number (%) of *S. canis* strains (n = 35)		Total of specimens (n = 169)	Number (%) of *S. canis* strains (n = 10)	
External ear canal	184	4	(2.2)	52	1	(1.9)
Nasal mucosa	10	0	(0)	1	0	(0)
Pharynx and oral cavity	84	7	(8.3)	38	4	(10.5)
Conjunctivae	12	0	(0)	16	1	(6.3)
Skin	44	3	(6.8)	25	0	(0)
Praeputium	48	6	(12.5)	0	0	(0)
Vaginal mucosa	30	2	(6.7)	3	0	(0)
Rectum	127	13	(10.2)	34	4	(11.8)

Table 2. Frequency of *Streptococcus canis* isolated from various anatomical sites of dogs and cats with proceeding infection

Anatomical sites	Dogs			Cats		
	Total of specimens (n = 176)	Number (%) of *S. canis* strains (n = 39)		Total of specimens (n = 42)	Number (%) of *S. canis* strains (n = 2)	
External ear canal	101	19	(18.8)	16	0	(0)
Nasal mucosa	1	1	(100)	1	0	(0)
Pharynx and oral cavity	18	3	(16.7)	7	1	(14.3)
Conjunctivae	1	0	(0)	1	0	(0)
Skin	21	3	(14.3)	8	0	(0)
Praeputium	8	1	(12.5)	1	0	(0)
Vaginal mucosa	6	3	(50.0)	1	0	(0)
Rectum	20	9	(45.0)	7	1	(14.3)

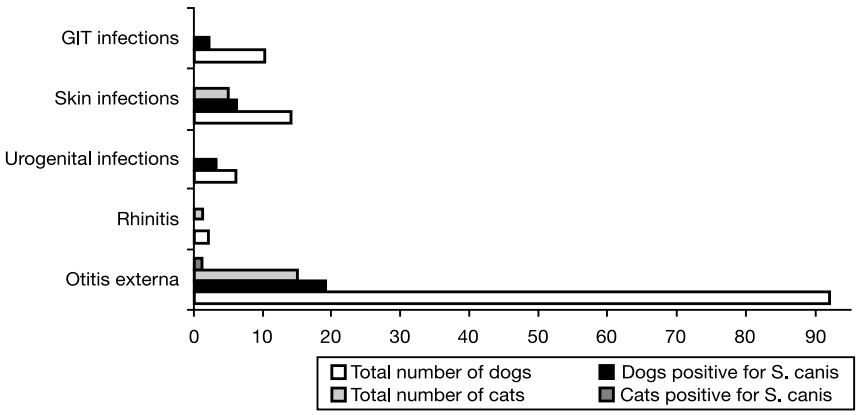

Figure 1. The occurrence of *S. canis* in dogs and cats with proceeding infection

inulin, raffinose and mellibiose. CAMP-like reaction was performed on sheep blood agar plates and could be observed for 58.1% (50 out of 86) of *S. canis* strains.

The results of antimicrobial susceptibility tests (Table 4) showed that all isolates were sensitive to penicillin G and ampicillin. The least effective antimicrobial agent was found

Table 3. Biochemical properties of *S. canis* strains isolated from dogs and cats

Test	Number (%) of positive *S. canis* strains (n = 86)	
Pyrrolidonyle arylamidase	0	(0)
Voges-Proskauer test	2	(2.3)
Hippurate	1	(1.2)
Phosphatase	76	(88.4)
Leucine amidopeptidase	83	(96.5)
beta-D-Glucuronidase	10	(11.6)
alpha-D-Galactosidase	83	(96.5)
Esculin	14	(16.3)
Arginine	86	(100)
Urease	2	(2.3)
Mannitol	1	(1.2)
Sorbitol	0	(0)
Trehalose	13	(15.1)
Lactose	65	(75.6)
Raffinose	0	(0)
Inulin	0	(0)
Mellibiose	0	(0)
Ribose	67	(77.9)
beta-D-Galactosidase	80	(93.0)
CAMP-like reaction	50	(58.1)

to be tetracycline (only 33.8% of susceptible strains). The respective rates of resistance to vancomycin, chloramphenicol, erythromycin and clindamycin were 10.5%, 7%, 3.5% and 2.3%, respectively.

Using species-specific oligonucleotide primers, all 86 *S. canis* strains were detected yielding an amplicon with a size of 215 bp. *S. dysgalactiae* ssp. *equisimilis*, also belonging to the Lancefield serogroup G, and all the other control strains of various species and serogroups were negative throughout.

Discussion

A total number of 86 of *S. canis* strains was isolated from 926 swabs obtained from 323 dogs and 71 cats in the period from 2003 to 2005. The distribution of *S. canis* in samples from various anatomical locations shows the preponderance of recoveries from the rectum (14.9% in dogs and 12.2% in cats) and the genital tract - prepuce (12.5% in dogs) and vagina (13.9% in dogs). Our results are consistent with the findings of Moyaert et al. (2006) about the prevalence of *S. canis* in rectal swabs. On the other hand, our findings concerning the isolation rate of this bacterium from vagina appeared to be lower than other reports (Clemetson and Ward 1990; Gunzel-Apel et al. 1999; Siemieniuch et al. 2005). In these reports, the prevalence of *S. canis* in vaginal swabs was found to be 52%, 40% and 37.5%, respectively. The differences in the occurrence of *S. canis* might be due to the differences in epidemiological conditions between countries. Concerning the distribution of *S. canis* in samples taken from sites with proceeding infections, it was cultivated from pathological processes localized in the genital tract, skin, and gastrointestinal tract and from otitis externa. It is not possible to estimate the contribution of *S. canis* to genital and gastrointestinal tract infections and skin infections due to the small number of samples in our study. As for specimens taken from animals with otitis externa, our results are consistent with observations of Bornand (1992) and Bensignor et al. (2000). However, considering the fact that *S. canis* was found almost only in mixed cultures, it most likely represents a secondary invading species. Within the

Table 4. Percentage of antibiotic susceptibility results for *S. canis* isolated from dogs and cats

Antimicrobial agent	Disk content	*S. canis* (n = 86 strains)		
		Susceptible	Intermediate	Resistant
Penicillin G	10 U	100	0	0
Ampicillin	10 µg	100	0	0
Vancomycin	30 µg	89.5	0	10.5
Clindamycin	2 µg	90.7	7.0	2.3
Chloramphenicol	30 µg	86.0	7.0	7.0
Erythromycin	15 µg	87.2	9.3	3.5
Tetracycline	30 µg	33.8	36.4	29.8

group of animals with rhinitis we did not find any sample positive for *S. canis*. This is in contrast to the findings of Knotek et al. (2001), who found 7.5% of samples positive for *S. canis*.

All beta-haemolytic streptococci of the Lancefield serological group G isolated in this study belong to the species *S. canis*, as defined by Devriese et al. (1986). No significant differences in biochemical properties between *S. canis* strains isolated from healthy animals and from animals with proceeding infection were observed. There was one prevalent biotype among the isolated strains which produced phosphatase, leucine amidopeptidase, arginine dihydrolase, alpha-D- and beta-D-galactosidase and fermented lactose and ribose. Results from the biochemical tests that are typically variable (including pyrrolidonylarylamidase, alpha-D- and beta-D-galactosidase, beta-D-glucuronidase, and acidification of lactose and trehalose) were generally within the range of proportions reported for *S. canis* by other researchers (Clark et al. 1984; Devriese et al. 1986; Efstratiou et al. 1994; Vieira and Castro 1994; Soedarmanto and Lammler 1996). Only 16.3% of *S. canis* strains were positive for esculin hydrolysis, which is in contrast to the results of Devriese et al. (1986) and Vieira and Castro (1994), who found 100% and 90% of strains positive, respectively. On the other hand, Efstratiou et al. (1994) described only 40% of strains as esculin positive and all positive reactions were reported as delayed, which might be responsible for some of the discrepancy. According to Hassan et al. (2005) all *S. canis* isolates appeared to be esculin negative on primary culture; whereas, the isolates were uniformly positive after they were subcultured. This could be another potential source of variation.

Another phenotypic characteristic of *S. canis* investigated was the CAMP-like reaction which was positive for 58.1% of strains. Our results are lower than the observations of Lammler et al. (1987), Vieira and Castro (1994) and Facklam (2002), who reported CAMP-like reaction in 74.1%, 100% and 95% of strains, respectively. On the contrary, other studies have reported the absence of CAMP-like reactions (Clark et al. 1984; Devriese et al. 1986).

The antibiotic susceptibility patterns of *S. canis* isolates found in our study are generally in agreement with the findings of other authors (Wu et al. 1997; Kataja et al. 1998; Hassan et al. 2005; Moyaert et al. 2006). In addition, our rates of resistance to erythromycin and tetracycline are much lower than those reported by Wu et al. (1997), who found 38.2% of strains resistant to erythromycin and 73.5% of strains resistant to tetracycline. After tetracycline, the second least effective antimicrobial agent was found to be vancomycin. In this study, only 89.5% of *S. canis* strains were susceptible to vancomycin. Our results are lower than the observations of Wu et al. (1997), who reported 100% of strains susceptible to vancomycin. On the other hand, Zaoutis et al. (1999) described tolerance to vancomycin in 66.7% of isolates. The significance of *in vitro* vancomycin resistance is uncertain, since our results may not reflect clinical efficacy. However, due to increasing resistance of bacterial isolates to antimicrobial agents, this study once more emphasizes the importance of susceptibility testing in order to establish correct therapeutic protocol.

For further characterization, the species-specific oligonucleotide primers were successfully used in PCR for the 16S-23S rDNA intergenic spacer region. The PCR methodology used in our study was based on the investigations of Hassan et al. (2003), who studied *S. canis* genes encoding 16S rRNA and 16S-23S rDNA intergenic spacer region. The PCR method presented in this study allowed a rapid and reliable identification of *S. canis* and might help to improve the diagnosis of this bacterial species in animal and human infections.

In conclusion, the prevalence of *S. canis* among household pets was found to be 17% with the preponderance of recoveries from the rectum and genital tract. Although this microorganism is commonly isolated from the skin and urogenital tract of animals, it can still be responsible for various, mostly acute infections. Even if the biochemical characteristics are suitable to confirm the preliminary identification of *S. canis* isolates,

additional molecular biological techniques may help with the unambiguous identification of *S. canis* isolates from clinical samples.

Výskyt a vlastnosti kmenů *Streptococcus canis* izolovaných ze psů a koček

Pro určení rozšíření druhu *Streptococcus canis* u psů a koček bylo bakteriologicky vyšetřeno celkem 926 vzorků odebraných v letech 2003 až 2005. U 86 izolovaných kmenů byly dále zjišťovány jejich fenotypové vlastnosti. Nejčastěji byl izolován biotyp, pro který byla charakteristická produkce fosfatázy, leucin-aminopeptidázy, arginin dihydrolázy, alfa-D- a beta-D-galaktosidázy a tvorba kyselin z laktózy a ribózy. Další průkaz založený na amplifikaci druhově specifické oblasti 16S-23S rRNA potvrdil identifikaci *S. canis*. Všechny izolované kmeny byly citlivé na penicilin G a ampicilin. Jako nejméně účinný se jevil tetracyklin (jen 33,8 % citlivých kmenů).

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