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ENTEROCOCCI OF CANINE ORIGIN AND THEIR CHARACTERISTICS

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The aim of the present work is to study the prevalence of enterococci in the material isolated from healthy dogs and dogs with various infections and to determine properties of particular strains. Out of the total number of 1178 swab samples obtained from 444 dogs, 433 (36.7 %) of the specimens contained enterococci. The most frequently isolated species was Enterococcus (E.) faecalis. Out of other species E. faecium, E. durans, E. hirae, E. casseliflavus, E. mundtii, E. columbae, E. solitarius, E. rafinosus and E. gallinarum were identified. Determination of biochemical properties of individual strains was based on conventional tests and EN-COCCUS tests and results were analyzed using the Identification program TNW lite 6.0 and identification schemes described in literature.

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Introduction

The enterococci form a large group of gram-positive, usually catalase-negative cocci. The genus *Enterococcus* (E.), which included *E. faecalis* and *E. faecium*, was separated in 1984 from *Streptococcus* on the basis of DNA hybridization data by Schleifer and Kilpper-Bälz [1]. The number of species included in this genus varied from 16 to 19 according to the author [2–4]. Since that time, new species have been described and at the present the genus contains 25 species [5–9].

The enterococci are ubiquitous and can be found free-living in soil and water, on plants, in milk products, and as part of the normal flora of the gastrointestinal tract of warm-blooded animals. They can also be recovered from mucosa of urogenital and respiratory tracts [7,8,10–17].

Until the 1970s enterococci were considered to be microorganisms with low pathogenicity. During following years, enterococci were more often documented as the cause of various infections of humans and animals including nosocomial infections. Very serious and even life-threatening infections are caused by multidrug resistant strains [3,11,18–28].

E. faecalis is by far the most common isolated Enterococcus from the material recovered from human. It represents 80-90 % of isolated strains, more than E. faecium (8 - 16 %). Out of other species E. avium, E. gallinarum, E. durans, E. casseliflavus, E. hirae, E. mundtii and E. cecorum have been documented [8,13,24,29-36]. It is assumed that some enterococcal strains, especially those which are drug-resistant, can be of animal origin [12,23,37,38].

Rodrigues et al. [38] studied the prevalence of particular species in canine stool. Out of 85 isolated strains, 52 % were identified as E. faecium, 47 % as E. avium and 1 % as E. faecalis. According to Weisser [39] the predominant species were E. faecium and E. durans (18 %), only 16 % of isolates were identified as E. faecalis. Devriese et al. [37] found E. faecalis (45 %) and E. hirae (37 %) as the most frequently isolated species from rectal swabs.

Different species of enterococci are often associated with urogenital infections [40-43].

Simjee et al. [44] examined the distribution of enterococci among dogs with urinary infections. Out of 35 isolated strains, 37 % belonged to E. faecium, 31 % to E. gallinarum, 20 % to E. faecalis and 11 % to E. casseliflavus. Waever and Pillinger [45] proved the presence of E. faecalis together with other microorganisms in urine and urinary stones recovered from dogs diagnosed with urolithiasis.

Allen and Dagnall [46] analyzed bacteriological samples collected from genital tract of bitches. Enterococci were isolated from bitches during oestrus (20.3 %), from bitches with vaginal discharge (21.3 %) and from sterile bitches (22.7 %). Enterococci were identified in 19.6 % of prepucial swabs. Bjurström [47] obtained those organisms from 44.1 % of vaginal swabs of healthy bitches

and from 26.7 % of prepucial swabs of healthy dogs. Enterococci were identified only in 1.3 % of vaginal swabs recovered from bitches with vaginitis. Laznicka and Nesnalova [48] reported that enterococci are in 11.5 % causative agents of pyometritis. Hirsh and Wiger [49] cultivated those organisms from 5 % of bitches with pyometritis.

Enterococci were also isolated from oral cavity swabs. Lemperle and Exner [50] identified those organisms in 25 % of oral cavity samples. Isermann and Kaminski [51] and Isogai *et al.* [52] reported recovery of enterococci from the oral cavity of dogs with dental caries.

Devriese et al. [37] found E. faecalis (48 %) and E. faecium (20 %) as the most frequently isolated species from tonsil swabs. They cultivated also E. avium, E. avium-like, E. durans, E. rafinosus and E. cecorum. E. faecium strains recovered from dogs differed from those of human origin in utilization of sorbitol.

Abramson et al. [53] examined samples collected from nasal cavity of 37 dogs. The authors stated in their study that enterococci and staphylococci predominate in the anaerobic and aerobic microflora. Angus et al. [54] proved the presence of enterococci in tracheal specimens recovered from dogs with respiratory tract infections. Out of 44 tracheal cultures, 12.1 % contained enterococci.

In other studies enterococci are mentioned in association with external ocular diseases, otitis externa and mastitis [55–57].

The purpose of the work reported here is to study the prevalence of enterococci in material isolated from healthy dogs and dogs with various infections and to determine properties of particular species.

Experimental

The isolates used in this study were recovered from dogs-patients at the Veterinary clinic in Pardubice. Swabs were taken from various anatomical sites such as rectum, prepuce, vagina, oral and nasal cavity, conjunctiva, meatus acusticus externus and skin.

Specimens were cultivated on Blood Agar Base No. 2 (OXOID) with 5 % defibrinated sheep blood, on Edwards Medium (OXOID) with 5 % defibrinated sheep blood and on selective agar for isolation of fecal streptococci (IMUNA). The plates were incubated at 37 °C for 24 hours. After the evaluation of colonial morphology, the representative colonies were purified on sheep blood agar and tested for hemolytic activity and pigment production.

Catalase production was determined with the use of 3 % hydrogen peroxide. The production of pyrrolidonylarylamidase was proved by PYR test (ITEST). Determination of group antigen was performed using SLIDEX STREPTO D test (BioMERIEUX). Proteolytic activity was tested on Soft Agar Gelatin Medium

(HiMedia) and the reaction was visualized with 3 % solution of HgCl₂. The aesculin hydrolysis was examined on Bile Esculin Azid Agar (HiMEDIA), on which enterococci grew in black colonies surrounded by dark coloured medium. Motility was determined in 0.4 % Brain Heart Infusion Agar (OXOID). Motile strains formed filamentous projections around the point of their inoculation.

Suspected enterococcal strains were cultivated on Brain Heart Infusion Agar plates (OXOID) with 6.5 % concentration of NaCl or pH adjusted to 9.6 to confirm their growth. We also tested the growth of strains on Blood Agar at 45 °C and thermoresistance of 60 °C for 30 minutes.

The determination of biochemical properties was performed using commercially produced EN-COCCUS test (LACHEMA) and conventional tests prepared in laboratory according Motlova [4]. The final results were analyzed on PC using the Identification program TNW lite 6.0 from the microbiological informative system INFOS (CCM Brno, distribution PLIVA – LACHEMA a.s. Brno).

Results and Discussion

Enterococci, especially *E. faecalis* and *E. faecium*, are considered to be dangerous pathogens, in regard to their growing importance in aetiology of different infectious diseases known above all in human population. Most of published studies focus on enterococci isolated from human sources, significantly less reports analyse the presence of these microorganisms in animals. Therefore the aim of our work is to study the prevalence of enterococci in clinical material recovered from dogs and to determine their properties.

A total number of 1178 swabs was studied. They were obtained from 444 dogs in the period from 1999 to 2004. The distribution of enterococci in samples from various anatomical locations of healthy dogs is summarized in Table I. It shows the preponderance of recoveries from rectum (80.9%) and vagina (35.5%). Out of total number of samples, 189 (16%) were recovered from local infections. The occurrence of enterococci in sites with proceeding infection is summarized in Table II. Enterococci were cultivated from pathological processes localized in skin (79.2 %), oral cavity (73.3 %), rectum (66.7 %) and from purulent matter taken from anal glands (75%). Representation of individual species and their prevalence in various materials are shown in Table III and Table IV. The results document that the most frequently isolated species was E. faecalis. Out of 420 strains isolated from samples which were recovered from locations without clinical appearance of the disease, it was identified in 50 % of cases. Out of 83 strains recovered from clinical changes, it was identified in 63.9 % of cases. The prevalence of E. faecium among healthy dogs and dogs with infections was 21.9 % and 13.3 %, respectively. Our results are consistent with the findings about

prevalence of *E. faecalis* and *E. faecium* in material of human origin, however they do not reach the same frequency of occurrence as stated by Moellering [11], Aguirre and Collins [30], Wilson *et al.* [13], Hsueh *et al.* [35], Motlova [8] and Pappas *et al.* [36].

Table I Frequency of enterococci among specimens collected from various anatomical sites of healthy dogs

Site of isolation	No. of collected specimens	No. (%) of positive specimens	No. of isolated strains
Rectum	277	224 (80.9)	261
Prepuce	147	34 (23.1)	43
Vagina	76	27 (35.5)	27
Oral cavity	223	33 (14.8)	39
Nasal cavity	14	0	0
Conjunctiva	18	2 (11.1)	3
Meatus acusticus externus	148	23 (15.5)	24
Skin	86	12 (13.9)	23
Total	989	355 (35.9)	420

Table II Frequency of enterococci among specimens recovered from pathological processes of dogs

Site of isolation	No. of collected specimens	No. (%) of positive specimens	No. of isolated strains
Rectum	21	14 (66.7)	16
Anal glands	8	6 (75.0)	6
Prepuce	42	12 (28.6)	14
Vagina	15	4 (26.7)	4
Uterus	3	1	1
Oral cavity	15	11 (73.3)	11
Tonsils	4	3	3
Nasal cavity	7	2 (28.6)	3
Sputum	3	2	2
Conjunctiva	6	1 (16.7)	1
Otitis externa	41	3 (7.3)	3
Skin	24	19 (79.2)	19
Total	189	78 (41.3)	83

E. faecalis was found to be predominant species among the rectal swabs collected from healthy dogs (50.6%) and dogs with local infections (43.7%). Our results do not correspond to those reported by Weisser [39] and Rodrigues et al. [38]. Out of other species E. faecalis, E. durans, E. hirae, E. casseliflavus and E. mundtii were identified. On the other hand, Devriese et al. [37] found that E. faecalis and E. hirae were the most frequently isolated species from rectal swabs. Out of 8 samples recovered from purulent matter taken from anal glands, 5 containance.

Table III Prevalence of enterococcal species in various anatomical sites of healthy dogs (n = 420 strains)

	No. (%) of		Source					
Species	isolated strains	Rectum	Prepuce	Vagina	Oral cavity			
E. faecalis	210 (50.0)	132	17	19	18			
E. faecium	92 (21.9)	65	12	2	5			
E. casseliflavus	11 (2.6)	5	2	2	0			
E. hirae	13 (3.1)	7	1	0	2			
E. cecorum	3 (0.7)	0	1	0	1			
E. columbae	4 (0.9)	1	1	0	1			
E. solitarius	4 (0.9)	0	0	0	2			
E. mundtii	9 (2.1)	4	0	2	2			
E. rafinosus	3 (0.7)	1	1	0	1			
E. gallinarum	2 (0.5)	1	1	0	0			
E. durans	23 (5.5)	16	3	0	2			
E. species	46 (10.9)	29	4	2	5			

	No. (%) of _		Source					
Species	isolated strains	V _{skin}	Nasal cavity	Conjunctiva	Meatus acusticus externus			
E. faecalis	210 (50.0)	13	0	3	8			
E. faecium	92 (21.9)	4	0	0	4			
E. casseliflavus	11 (2.6)	0	0	0	2			
E. hirae	13 (3.1)	2	0	0	1			
E. cecorum	3 (0.7)	1	0	0	0			
E. columbae	4 (0.9)	0	0	0	1			
E. solitarius	4 (0,9)	1	0	0	1			
E. mundtii	9 (2.1)	0	0	0	1			
E. rafinosus	3 (0.7)	0	0	0	0			
E. gallinarum	2 (0.5)	0	0	0	0			
E. durans	23 (5.5)	1	0	0	1			
E. species	46 (10.9)	1	0	0	5			

ned E. faecalis. In addition to this, 4 specimens included Escherichia coli and 1 sample E. faecium.

Out of 147 prepucial isolates collected from healthy dogs, 34 (23.1 %) were identified as enterococci. Out of 43 isolated strains, 39.5 % belonged to *E. faecalis* and 27.9 % to *E. faecium*. From other species *E. casseliflavus* and *E. durans* were cultivated. Enterococci were present in 28.6 % (12 cases) of dogs with balanoposthitis. In this clinical material *E. faecalis* (63.6 %) also predominated. Out of 76 vaginal swabs from healthy bitches, enterococci were recovered in 27 cases (35.5 %). The most frequently isolated species from samples recovered from bitches was also *E. faecalis* (70.3 %). In vaginal swabs obtained from bitches with vaginitis enterococci were identified in 26.7 % of the collected specimens. These

Table IV Prevalence of enterococcal species in specimens recovered from pathological processes of dogs (n = 83 strains)

Source _	Species						
	E. faecalis	E. faecium	E. hirae	E. mundtii	E. species		
Rectum	7	3	0	0	6		
Anal glands	5	1	0	0	0		
Prepuce	7	4	0	1	2		
Vagina	4	0	0	0	0		
Uterus	1	0	0	0	0		
Oral cavity	7	0	0	1	3		
Tonsils	2	0	0	0	1.		
Nasal cavity	2	0	0	0	1		
Sputum	2	0	0	0	0		
Conjunctiva	1	0	0	0	0		
Otitis externa	1	0	0	0	2		
Skin	14	3	1	0	1		
No. (%) of isolated strains	53 (63.9)	11 (13.3)	I (1.2)	2 (2.4)	16 (19.3)		

results remain consistent with findings published by Bjurström [47].

Out of 15 samples collected from oral cavity of dogs with inflammatory disease of gums and dental caries, enterococci were cultivated in 11 cases (73.3 %). *E. faecalis* was found to be predominant species among these specimens (63.6 %). Similar results were reported by Isermann and Kaminski [51].

Enterococci were most frequently recovered from inflammatory processes localized on skin (79.2 %). Among these samples *E. faecalis* predominated (73.7 %), *E. faecium* was cultivated less frequently (15.8 %). Organisms were often isolated together with *Staphylococcus intermedius* and aerobic spore forming bacteria.

503 strains recovered from clinical material were identified as enterococci according to their serological and biochemical properties (Table V and VI). 1.5 % of *E. faecalis* strains was beta-hemolytic on sheep blood agar. According to Motlova [4] the number of beta-hemolytic isolates is even higher (6 %). Facklam *et al.* [58], on the other hand, described *E. faecalis* as non-beta-hemolytic species. Unlike these authors, we did not find beta-hemolysis performed by *E. durans*.

Even though enterococci are characterized as catalase-negative microorganisms, some strains are known to produce pseudo-catalase [1,3,4]. Pseudocatalase positive reaction was produced by 132 (26.2%) of 503 strains tested. The pseudo-catalase test was positive for majority of *E. solitarius* strains. Similar results for *E. solitarius* were reported by Schleifer and Kippler-Bälz [1].

Most enterococcal strains produce the group D antigen. Our study did not prove the presence of this antigen among some *E. faecalis*, *E. faecium*, *E. hirae*,

Table V Properties of enterococcal strains isolated from dogs (n = 503 strains)

Caraina	No of stroins			Hemolysis	
Species	No. of strains	a	lpha	beta	gamma
E. faecalis	263	7.6+		1.5	90.9
E. faecium	103	;	32.5	0	16.5
E. casseliflavus	11		75	0	25
E. hirae	14	4	46,2	0	53.8
E. cecorum	3		0	100	0
E. columbae	4		25	0	75
E. solitarius	4		0	0	100
E. mundtii	11		54.5	0	45.5
E. rafinosus	3		100	0	0
E. gallinarum	2		100	0 0	
E. durans	23	39.1		9.1 0	
E. species	62		54.8	0	45.2
Species	Pseudo- catalase	PYR ⁺⁺	Group D antigen	Protease	Motility
E. faecalis	46	100	97.7	63.9	3
E. faecium	5.8	100	98	8.7	3.8
E. casseliflavus	0	100	100	0	90.9
E. hirae	0	100	64.3	0	0
E. cecorum	0	0	0	0	0
E. columbae	0	. 0	25	75	0
E. solitarius	100	100	100	0	0
E. mundtii	0	100	81.1	0	0
E. rafinosus	0	100	100	0	0
E. gallinarum	0	100	100	0	0
E. durans	4.3	100	91.3	8.7	0
E. species	11.3	100	93.6	30.6	3.2

numbers are percentages

E. columbae, E. mundtii and E. durans strains. Our results correspond to the findings of Motlova [4] and Franz et al. [5].

Protease activity was noted with 39.6 % of isolated strains. The protease was produced most frequently by E. faecalis (63.9 %) and E. columbae (75 %). These findings are consistent with data reported by Koch et al. [28], who found 22-66 % of isolates to be positive.

Motility was shown only by 90.9% of *E. casseliflavus*. Our observations differ from the findings published by Motlova [4], who reported 100% of motile isolates. The results found for *E. faecium* remain consistent with this author.

The species identification was based especially on determination of biochemical properties of individual strains identified with conventional tests and EN-COCCUS tests produced by the company Pliva-Lachema. The results were analyzed using the Identification program TNW lite 6.0 and identification schemes

th pyrrolidinylarylamide

Table VI Growth properties of enterococci isolated from dogs in various conditions (n = 503 strains)

Ct.	N6-4! :	Growth properties in				
Species	No. of strains	6.5 % NaCl	pH = 9.6	40 % bile	45 °C	
E. faecalis	263	100+	100	100	100	
E. faecium	103	100	100	100	100	
E. casseliflavus	11	100	100	100	1 0 0	
E. hirae	14	100	100	100	100	
E. cecorum	3	0	100	100	100	
E. columbae	4	25	75	100	75	
E. solitarius	4	100	100	100	100	
E. mundtii	11	100	100	100	100	
E. rafinosus	3	100	100	100	100	
E. gallinarum	2	100	100	100	100	
E. durans	23	91.3	100 -	100	91.3	
E. species	62	95.2	100	98.4	100	
	Aesculin	Thermoresis	tant strains			
Species	hydrolysis	(60 °C, 3	30 min)	_		
E. faecalis	100	97	.7			
E. faecium	100	94	.2			
E. casseliflavus	100	72	.2			
E. hirae	100	10	0			
E. cecorum	100	0				
E. columbae	100	7:	5			
E. solitarius	100	10	0			
E. mundtii	100	90	.9			
E. rafinosus	100	66	.7			
E. gallinarum	100	0				
E. durans	100	91	.3			
E. species	100	88	.7			

^{*} numbers are percentages

described in literature [2,4,6,8,29,58–60]. Out of 503 enterococcal strains, 441 (87.6 %) were classified into individual species.

The biochemical characteristics are summarized in Table VII. Our results correspond with the findings of Devriese *et al.* [37] in that some strains of *E. faecium* of canine origin are able to utilize sorbitol.

Results of our study revealed that *E. faecalis* (52.3 %) and *E. faecium* (20.5 %) were the most frequently isolated species. *E. durans* (4.6 %), *E. hirae* (2.8 %), *E. casseliflavus* (2.2 %), *E. mundtii* (2.2 %), *E. columbae* (0.8 %), *E. solitarius* (0.8 %), *E. raffinosus* (0.6 %) and *E. gallinarium* (0.4 %) were cultivated less frequently. 12.4 % of strains tested were classified only as *Enterococcus* sp., because their properties did not correspond to any known species.

In our tests, the most commonly isolated enterococci from dogs as well as from humans are *E. faecalis* and *E. faecium*.

Table VII Biochemical properties of enterococci isolated from dogs determined by EN-COCCUS test and conventional tests (n = 503 strains)

	No. of				EN-COC	CUS test [↔]		<u>.</u>	
Species strains		ARG	SOE	ARA	MAN	SOR	MLB	RAF	MLZ
E. faecalis	263	100 ⁺	0	0	100	100	0	0	94.2
E, faecium	103	100	0	100	100	52.4	91.2	23.3	1.9
E. casseliflavus	11	54.5	0	100	100	72.7	100	72.7	54.5
E. hirae	14	100	0	0	0	0	100	100	0
E. cecorum	3	0	0	0	66.7	66.7	100	100	66.7
E. columbae	4	0	0	75	100	75	100	100	0
E. solitarius	4	100	0	0	100	100	0	0	100
E, mundtii	11	100	0	100	100	100	100	81.8	0
E. rafinosus	3	0	100	100	100	100	100	100	100
E. gallinarum	2	100	0	100	100	0	100	100	0
E. durans	23	100	.0	0	0	. 0	82.6	. 0	. 0

a !	Conventional tests***						
Species	ARA	MAN	SOR	RAF	LAC		
E. faecalis	0	100	100	1.1	100		
E. faecium	100	96.1	73.8	7.7	100		
E. casseliflavus	100	100	54.5	100	100		
E. hirae	7.1	0	7.1	85.7	100		
E. cecorum	0	100	66.7	100	100		
E. columbae	75	100	75	100	100		
E, solitarius	0	100	100	0	0		
E. mundtii	100	100	100	72.7	100		
E. rafinosus	100	100	100	100	100		
E. gallinarum	100	100	0	100	100		
E. durans	4.3	4.3	0	0	100		

⁺ numbers are percentages

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ARG – arginine; SOE – sorbose; ARA – arabinose; MAN – mannitol; SOR – sorbitol; MLB – melibiose; RAF – raffinose; MLZ – melezitose

^{***} ARA - arabinose; MAN - mannitol; SOR - sorbitol; RAF - raffinose; LAC - lactose

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