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ORIGINAL PAPER

The Occurrence and Comparative Phenotypic Characteristics of *Staphylococcus* spp. from Healthy and Diseased, Household and Shelter Dogs, Based on Routine Biochemical Diagnostic Methods

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Abstract

To determine the staphylococcal colonization pattern in healthy and diseased dogs, living in two particular environments, a number of microbiological samples were taken. Overall, twenty dogs, either healthy or with infected skin lesions, were examined. In each case bacterial swabs were collected from the nasal mucosa, ear, perineum, lumbo-sacralis triangle, and from the infection sites if such were present. A total number of 104 isolates representing different staphylococcal species were isolated and identified using routine biochemical methods applied in diagnostic laboratories. Among 17 isolated staphylococcal species, *Staphylococcus intermedius* was the most common species isolated from both healthy or diseased dogs living either in animal shelter or household environments. The pattern of *Staphylococcus* sp. colonization differs considerably for animals living in the two tested habitats. In particular, *S. aureus* MRSA and MSSA isolates were detected only in infected skin lesion samples from animals that dwelled in the animal shelter. As could be expected, *S. intermedius* was found to be a predominant causative agent in canine skin infections. In our study, we demonstrated that *S. intermedius* in its carrier-state, inhabits mainly the mucosal membrane of the nasal vestibule. It was also found in the samples taken from the skin, the lumbo-sacralis triangle and perineum, but was rarely isolated from the ears.

Key words: *Staphylococcus* spp. from dogs, diagnostic, phenotyping, biochemical methods

Introduction

Staphylococcus species are considered to be one of the most widespread bacteria found in nature. Bacteria that constitute this genus, colonize a number of domestic animals, including dogs and cats. Usually they belong to the normal skin flora of these animals commonly present on skin and mucosal membranes (Stepanovic *et al.*, 2001; Hauschild and Wojcik, 2007). In 1976 Hajek described a new staphylococcal coagulase-positive species *Staphylococcus intermedius* which colonized predominantly the skin and the mucosal membrane of the nasal vestibule in dogs (Hajek, 1976). Later studies revealed that *S. intermedius* was the most frequent species isolated from healthy dogs. It comprised 40.3% of all isolated strains and was identified as a resident of the physiological bacterial flora of healthy dogs (Cox *et al.*, 1988; Król, 1998). Devriese and DePelsmaecker proved in their research that *S. intermedius* strains were predominantly isolated from nostrils and the rectum region of healthy dogs (Devriese and DePelsmaecker, 1987). These two ecological niches were proposed to be the source for colonization of other areas in healthy dogs by *S. intermedius* strains (Nagase *et al.*, 2002). Usually occurring in healthy individuals, under favorable conditions *S. intermedius* can cause a variety of infections in dogs and cats (Biberstein *et al.*, 1984, Kim *et al.*, 2005).

Dermatitis or inflammation of the skin is caused by the diverseness of allergens, irritation and infectious

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factors, or systematic diseases (Hendricks *et al.*, 2002; Kizerwetter-Świda *et al.*, 2009). Pyoderma is a type of dermatitis. It occurs fairly often among dogs. In pyoderma, either flesh or deep skin infections can be observed. Commonly in both cases, *S. intermedius* is the main causative agent. Infective skin lesions usually show up in warm, moist and wrinkled skin regions, where there appear to be propitious conditions for bacterial colonization and progression of pyoderma (Allaker *et al.*, 1993; Guardabassi *et al.*, 2004).

Notwithstanding the growing responsibility and microbiological awareness among dog owners and the increasing interest for the *S. intermedius* species among the veterinary society, this species still has not been adequately studied. Bearing this in mind, knowledge of the bacterial flora composition of the dogs skin would be extremely useful in the epidemiological study of a variety of a skin infections that occur in dogs.

The main goal of this project was to determine the similarity, disparity and potential relationship between the different colonization patterns of the staphylococcal species characteristic for healthy and diseased dogs, living either in a rescue shelter or households.

Commonly the diagnostics of bacterial strains isolated from dogs are based on phenotypic methods and these type of methods were considered in this study (Międzobrodzki *et al.*, 2008). Although based on the fact that some staphylococcal species (*S. intermedius* and *S. pseudintermedius*) can be distinguished almost only by genetic methods, the further introduction of genetic based techniques in routine veterinary diagnostics is essential (Bannoehr *et al.*, 2009; Devriese *et al.*, 2009).

Experimental

Materials and Methods

Bacterial strain isolation. Bacterial samples isolated from 20 healthy and diseased dogs were examined. The examined group of dogs was heterogeneous. It consisted of 20 mongrels, both male and female that weighed from 5 to 45 kg. Among the 20 dogs, 12 had skin lesions and the remaining 8 had none. The dogs living in an animal shelter had been staying there for a few weeks up to 3 years. A similar time range was for the dogs living in household environments. Five out of 7 sheltered dogs, and 7 out of 13 household dogs demonstrated skin lesions. In the 8 remaining dogs, no skin lesions were observed, as shown in Figure 1.

Samples were taken from skin lesions found in sick dogs as well as from healthy dogs without skin changes. The samples were collected by a veterinarian. The material used for research was derived from skin lesions caused by flea allergy dermatitis. These lesions were caused by hypersensitivity, and may have had pus complications. The lesions were not only always exuding with pus or serum-pus, but also painful and strongly pruritic. However, there were no immunological changes, understood as autoaggression, such as lupus or pemphigus. There were no spontaneous bacterial infections either.

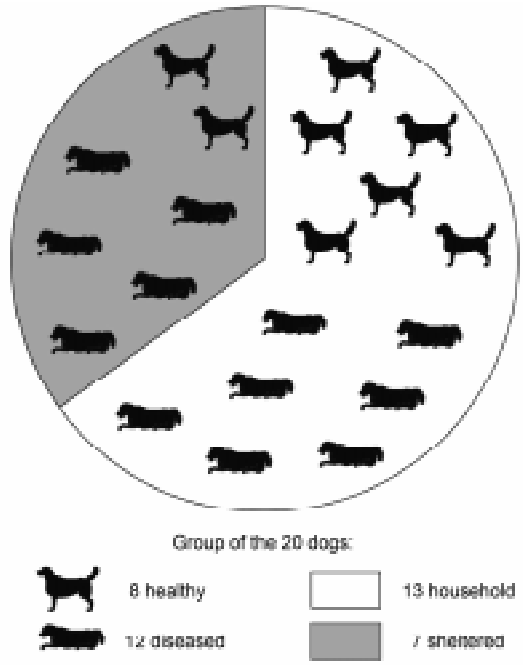

Fig. 1. Characeistics of all examined dogs

Swabs were taken from each dog from both the left and right ear, the nasal vestibule, and perineum, as well as from the back, and in the case of the diseased dogs, also from the infection site (Fig. 2). All the isolated samples/strains were described in the following manner: (i) classification of the etiological agent causing from skin infections; (ii) characteristic of the isolated bacterial strain by standard phenotypic methods;

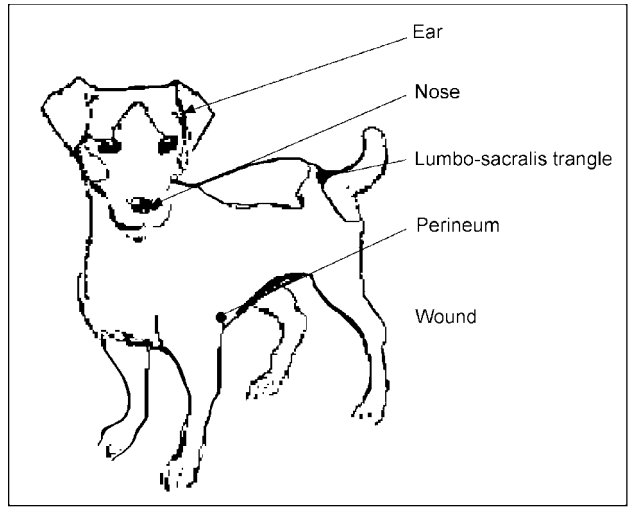

Fig. 2. Different reservoir sites for staphylococcal strains in dogs

(iii) correlation between a particular isolation site and a staphylococcal species. Microbiological samples isolated from dogs were inoculated onto Columbia blood (supplemented with 5% sheep blood), Baird-Parker and Candida ID agar within 2 hours of isolation. After an incubation period of 48 h at 35°C, all colonies were screened and staphylococcal-like colonies were isolated and plated on tryptic soy agar for 24 h at 35°C. Pure isolates were then used for future experiments. A total number of 104 isolates was received from 20 examined dogs.

Microscopic analysis. To confirm the purity identification of all the 104 isolates to Gram-positive cocci, Gram staining was performed (Beveridge, 2001).

Biochemical identification. Throughout the biochemical analysis, two reference strains *S. aureus* ATCC25933 and *S. epidermidis* ATCC 12228 were tested alongside the canine isolates. All isolates were evaluated for catalase activity and furazolidone resistance. Bacterial identification was performed by a coagulase tube test (Biomed, Poland). Detection of the clumping factor with rabbit plasma was performed by PASTOREX^{TM} STAPH-PLUS test (BIO-RAD). In order to confirm identification of staphylococcal species, ID32 STAPH (BioMerieux, Poland) analysis was done (Sasaki *et al.*, 2007a; Weese *et al.*, 2009).

Table I
Distribution of 104 strains of the *Staphylococcus* species in all examined dogs

Staphylococcus spp.	Living environment of examined dogs total number of bacterial strains (%)	
	7 sheltered dogs-45 strains	13 household-59 strains
S. intermedius	18 (40.0 %)	28 (47.4%)
S. aureus (MSSA)	3 (6.7%)	0
S. aureus (MRSA)	8 (17.8%)	0
S. warneri	0	4 (6.8%)
S. gallinarum	2 (4.4%)	0
S. hominis	0	3 (5.1%)
S. chromogenes	0	5 (8.5%)
S. epidermidis	0	1 (1.7%)
S. haemolyticus	1 (2.2%)	8 (13.5%)
S. lentus	3 (6.7%)	1 (1.7%)
S. saprophyticus	1 (2.2%)	0
S. equorum	3 (6.7%)	1 (1.7%)
S. xylosus	1 (2.2%)	5 (8.5%)
S. cohni	1 (2.2%)	1 (1.7%)
S. schleiferi	0	1 (1.7%)
S. simulans	3 (6.7%)	0
S. lugdunensis	0	1 (1.7%)
S. sciuri	1 (2.2%)	0
Total number of staphylococcal species	11	12

Results

A total of 104 staphylococcal strains were isolated from healthy and diseased dogs living in either animal shelter or households. All strains demonstrated typical colony growth on blood and Baird-Parker selective agar. Coagulase-positive staphylococci produced black, shiny, convex colonies with entire margins and clear zones with or without an opaque zone around the colonies.

Phenotypic characteristics of isolated strains. The free coagulase tube test revealed a positive reaction in 61 strains (59%), whereas no coagulation was observed in the remaining 43 (41%) strains. Although the production of the clumping factor was detected in 44 (42%) strains, 57 (55%) did not produce this factor, and for 3 (3%) strains the results were uncertain. The PASTOREX^{TM} STAPH-PLUS test showed that only 39 among all strains (37.5%) have the ability to autoagglutinate. In terms of the ID32 STAPH method, 104 strains were identifiable, Table I. *S. intermedius* (46 strains) and *S. aureus* (11 strains) were the only two coagulase-positive staphylococcal species isolated among all the tested canine strains. The remaining strains belong to the coagulase-negative group.

Domicile and *Staphylococcus* sp. variety. Table I presents the rate of occurrence of different staphylococcal species in swabs taken from the skin, ears, and mucosal membrane of the nasal vestibule, from dogs living in the animal shelter or households. A total of 17 different species belonging to the *Staphylococcus* genus were found. Animals living in the shelter carried 11 different species, whereas 12 species were isolated from dogs living in household conditions. Regardless of living environments, *S. intermedius* isolates were the most common. It accounts for 40% and 47.4% of all isolated strains, respectively for the animal shelter and household environment. Based on the obtained results the difference between staphylococcal profiles and the various environments that they colonize was noticeable. Dogs from the animal shelter were carriers for such staphylococcal species, like *S. gallinarum*, *S. saprophyticus*, and *S. simulans* that were not isolated from any animal living in the household environment. On the other hand, such species as *S. warneri*, *S. hominis*, and *S. chromogenes*, were isolated only from dogs living in the households. A fact worth noticing was that 11 *S. aureus* strains (MSSA and MRSA) were isolated only from samples obtained from the shelter.

Manifestation of particular staphylococcal species in samples isolated from the nasal vestibule are shown in Table II. In the group of 11 isolated species the most frequent, despite of habitat, was *S. intermedius*. Nevertheless, *S. intermedius* was detected more often in samples taken from dogs kept in households than in the animal shelter, 55.5% and 31.6% respectively.

Table II
Staphylococcal strain distribution in the nasal mucosa

Staphylococcus spp.	Living environment of examined dogs total number of bacterial strains (%)	
	7 sheltered dogs-19 strains	13 household-18 strains
S. intermedius	6 (31.6%)	10 (55.5%)
S. aureus	6 (31.6%)	0
S. lentus	2 (10.5%)	1 (5.6%)
S. equorum	2 (10.5%)	0
S. haemolyticus	1 (5.3%)	2 (11.1%)
S. sciuri	1 (5.3%)	0
S. cohni	1 (5.3%)	0
S. lugdunensis	0	1 (5.6%)
S. chromogenes	0	1 (5.6%)
S. xylosus	0	1 (5.6%)
S. warneri	0	2 (11.1%)
Total number of staphylococcal species	7	7

Table III
Staphylococcal strain distribution in the perineum region

Staphylococcus spp.	Living environment of examined dogs total number of bacterial strains (%)	
	7 sheltered dogs-19 strains	13 household-12 strains
S. intermedius	4 (33.3%)	4 (33.3%)
S. aureus	2 (16.7%)	0
S. gallinarum	2 (16.7%)	0
S. saprophyticus	1 (8.3%)	0
S. equorum	1 (8.3%)	0
S. simulans	1 (8.3%)	0
S. lentus	1 (8.3%)	0
S. hominis	0	2 (16.7%)
S. warneri	0	2 (16.7%)
S. chromogenes	0	1 (8.3%)
S. haemolyticus	0	2 (16.7%)
S. epidermidis	0	1 (8.3%)
Total number of staphylococcal species	7	6

S. aureus strains were isolated mainly from the mucosal membrane of the nasal vestibule of dogs living in the shelter in the range of 31.6%. Adjacent to coagulase-positive species, coagulase-negative staphylococcal species were also present in the biological material. While, *S. equorum*, *S. sciuri* or *S. cohni* were found only in dogs from the shelter, *S. lugdunensis*, *S. chromogenes*, *S. xylosus* or *S. warneri* were presented in the samples taken from the animals living at home. This should be treated as a trend because the number of examined dogs was relatively low.

S. intermedius was also the most prevalent species among isolates from the perineum region, Table III. Furthermore, it was the only staphylococcal species shared among household and shelter dogs. The following species were detected in swabs taken from the perineum region of the sheltered animals: *S. aureus*, *S. saprophyticus*, *S. gallinarum*, *S. equorum*, *S. simulans*, and *S. lentus*. Subsequently, domestic dogs carried *S. hominis*, *S. warnerii*, *S. chromogenes*, *S. haemolyticus*, and *S. epidermidis* in the material from the same isolation region.

Table IV
Staphylococcal strain distribution in the lumbo-sacralis triangle region

Staphylococcus spp.	Living environment of examined dogs total number of bacterial strains (%)	
	7 sheltered dogs-8 strains	13 household-12 strains
S. intermedius	6 (75.0%)	2 (16.7%)
S. aureus	2 (25.0%)	0
S. xylosus	0	3 (25.0%)
S. haemolyticus	0	2 (16.7%)
S. cohni	0	1 (8.3%)
S. schleiferi	0	1 (8.3%)
S. hominis	0	1 (8.3%)
S. equorum	0	1 (8.3%)
S. chromogenes	0	1 (8.3%)
Total number of staphylococcal species	2	8

From the lumbo-sacralis triangle region shown in Figure 2, 9 species of *Staphylococcus* were distinguished, Table IV. In comparison to the samples taken from shelter animals, the dogs living at home were characterized by a broad range of staphylococcal species, 2 and 8 species respectively. As mentioned above only two species were isolated from the lumbo-sacralis triangle samples from sheltered dogs: *S. intermedius* (75%) and *S. aureus* (25%).

The above data demonstrated that *S. intermedius* in carrier-state, predominantly colonized nares of tested dogs. Over 50% of all animals, regardless of their living environment, sick or healthy carried strains of this species in the nasal vestibule. The next preferred region was the lumbo-sacralis triangle and perineum. *S. intermedius*, however was isolated more often from the sheltered dogs (75%) then from animals living in households (16.7%), Table IV. In both healthy and diseased dogs, the patterns of colonization were similar, as it is shown in Figure 3A and Figure 3B.

The etiological factor for skin lesions of canine origin. The rate of occurrence of different staphylococcal species, isolated from 12 dogs with infection sites are listed in Figure 4. The most common species found in 7 out of 12 examined animals (58.3%) was *S. intermedius*. In 5 out of 7 dogs living in households it was the only staphylococcal species isolated from the skin lesion. Moreover, 7 additional *Staphylococcus* spp. were isolated, including: methicillin-resistant

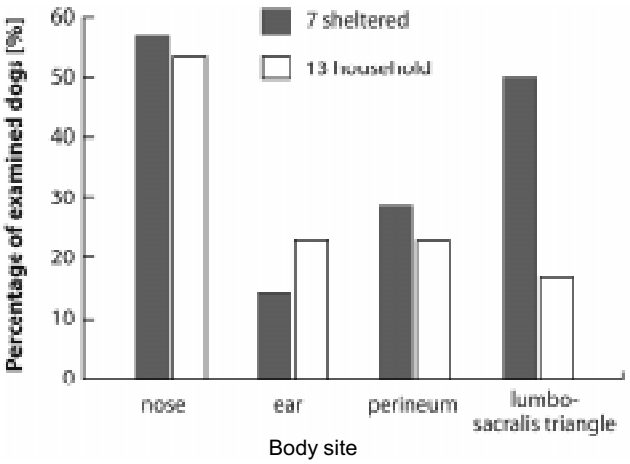

Fig. 3A. Distribuion of *S. intermedius* in the carrier-state at different body sites in sheltered and household dogs

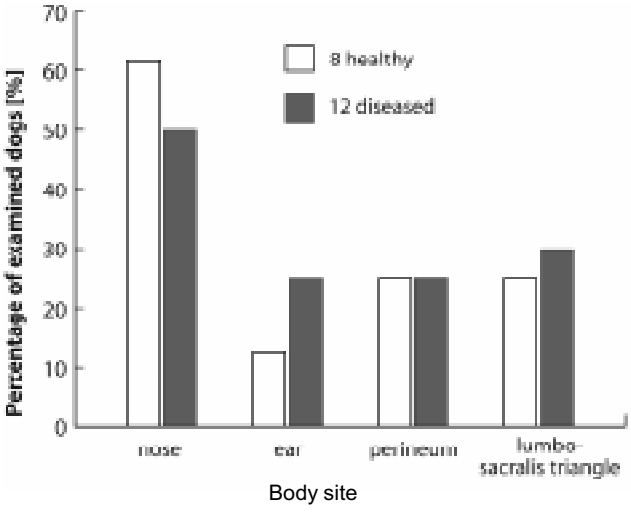

Fig. 3B. Distribuion of *S. intermedius* in the carrier-state at different body sites in healthy and diseased dogs

S. aureus (MRSA), *S. haemolyticus*, *S. chromogenes*, *S. simulans*, *S. equorum*, *S. xylosus*, and *S. cohni* which appeared to be single cases. Staphylococcal species distribution in diseased dogs was also analyzed based on habitat, Figure 4. Among the 8 species mentioned earlier, 6 originated from animals living in the shelter and three from dogs living at home. Evidently, *S. intermedius* was the dominant species in each habitat, nevertheless it was isolated more repeatedly from animals living in households (71.4% of all animals). A greater variety of staphylococcal species were found in skin lesion samples taken from animals living in the shelter, where *S. intermedius* occurred in 40% of these animals. Among these species *S. aureus* (MRSA), *S. simulans, S. equorum*, *S. xylosus*, and *S. cohni* were identified. In skin infections of dogs living in household conditions, beside *S. intermedius* strains, only *S. haemolyticus*, *S. chromogenes* were found in skin infections.

Discussion

Normal animal skin is colonized by numerous bacterial species, which contribute to the physiological skin flora. *Staphylococcus* species are not out numbered among this flora. The most typical staphylococcal species isolated from canine skins are *S. intermedius*, *S. xylosus*, *S. sciuri*, *S. capitis*, *S. chromogenes* and *S. lentus*, by Nagase *et al.* (Nagase *et al.*, 2002). At the same time other studies show a slightly different profile of isolated strains from dog skin at carrier-state: *S. intermedius*, *S. aureus*, *S. simulans*, *S. heamolyticus*, and *S. saprophyticus* (Hauschild and Wójcik, 2007). So far not many studies have been done to determine the staphylococcal colonization pattern based on a specific part of the body or living habitat conditions of the animals from which swabs were taken.

In the recent study we took an effort to characterize the contribution of the different staphylococcal species in the colonization of dogs living in two different environments: the animal shelter and households. Our data confirm the general trend that *S. intermedius* strains are isolated at a higher rate from dogs that inhabit households, whereas *S. aureus* is isolated from dogs living at the shelter. These differences could be explained by distinctness in animal exposition to other

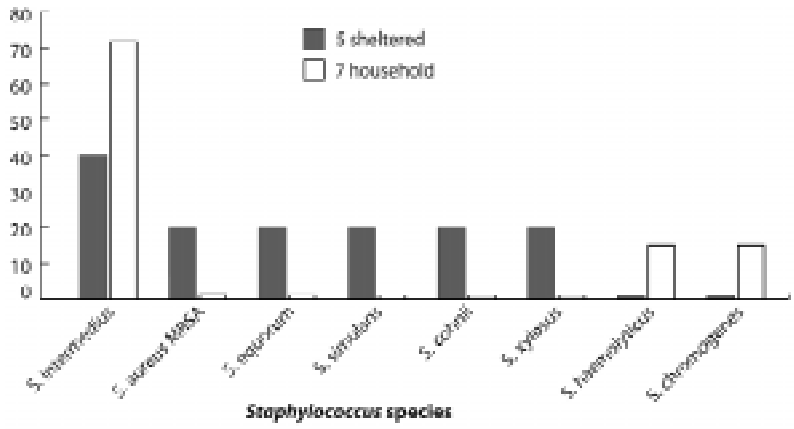

Fig. 4. Distribution of *Staphylococcus species* found in infected skin lisions of 12 examined dogs

animals and people (Talan *et al.*, 1989; Król, 1998). *S. aureus* strains isolated during our research, seem to be an excellent example. The animals from the shelter living in small closed areas and are in constant contact with other animals and moreover with the personnel, who are one of the main carriers of *S. aureus* (Kloos and Musselwhite, 1975). Humans are colonized by MSSA or MRSA by a carriage rate of 20–40% (Shopsin *et al.*, 2000). These numbers grow even higher in the case of elderly persons or hospital personnel, where they can reach up to 90% (Szewczyk, 2005). Throughout our study, *S. aureus* strains were isolated only from dogs living in the animal shelter and comprise 24.5% of all strains isolated in these animals. Among this group we isolated both MSSA and MRSA strains.

On the other hand our data confirm, that *S. intermedius* is present in carrier-state in dogs. Isolates of this species were the most common, regardless of habitat and dog health conditions. The most favorable place for bacterial colonization was the mucosal membrane of nostrils. It was a reservoir for nearly 55% of all *S. intermedius* isolates, followed by the lumbo-sacralis triangle region (almost 30%).

Dermatitis is the most common type of infectious disease found in dogs (Ackerman, 1994). Dermatoses have a variety of etiological factors, where some of them are caused by particular bacterial strains. 90% of pyoderma in dogs is caused by *S. intermedius* strains infection (Craig, 2003), while *S. aureus* is one of the causative agents in skin lesions of non-pyodermal origin (Biberstein *et al.*, 1984). Our data show that *S. intermedius* species seem to be the only species presented in microbiological samples from infected skin lesions of dogs living in both animal shelter and household environments. It was isolated from almost 60% of all diseased animals. Five out of seven of these dogs had only *S. intermedius* strains presented in the infected skin lesion. In the remaining two cases, *S. intermedius* strains were accompanied by other coagulase-negative strains.

The achieved data enriches our knowledge of the colonization of dogs by not only staphylococcal coagulase-postive strains like *S. aureus* or *S. intermedius* but also by coagulase-negative strains. The presented results are also important for understanding the epidemiology of infectious animal diseases, in which bacteria from the staphylococcal genus play a crucial role. In the last few years, a new *Staphylococcus* species – *S. pseudintermedius* was separated from the group previously considered to be *S. intermedius* species (Devriese *et al.*, 2005; Władyka *et al.*, 2008). *S. pseudintermedius* can be easily misclassified as *S. intermedius* by routine biochemical methods used in standard diagnostics. Furthermore, it seems that all the isolates classified in our and other projects by routine biochemical identification tests seem to be *S. pseudintermedius.* Therefore, the final microbiological identification must require the use of genetic methods (Sasaki *et al.*, 2007a; Bannoehr *et al.*, 2009).

The number of dogs used in the project was twenty which was not very high. However they were used to show whether the similarity in lesions is because of same etiological factors in diseased dogs (twelve) and to recognize the staphylococcus species in healthy dogs at carrier state (eight). The staphylococcal species isolated from healthy and diseased dogs were compared to understand the colonization although disease was not studied. The main question was, do the carriage or the life conditions (house/shelter) effect the occurrence of disease?

The less number of dogs taken in the project resulted from trouble in selection of animals. There were often abrasions or wounds present on the skin. Only the dogs with skin lesions were taken for the project. The idea was that the similarity in skin lesions is caused by staphylococci, the carriage in same area and the localization of dogs in environment. Thus the work was to be done with high precision. Dogs were taken from one acute shelter hostel and the skin lesions were clinically evaluated. All the samples were taken by same veterinary doctor.

Analysis shown in the paper is an introduction on the general elaboration of carriage phenomenon in dogs living under different environments (house/shelter) and of isolated strains from skin lesions in part of dogs in the population. Thus showing the relation between diseased to healthy dogs and house to shelter conditions.

A total of one hundred four bacterial strains were isolated from twenty dogs. Higher species heterogeneity was observed among bacteria in dogs from shelter and that this difference did not affect pathology, which is mainly caused by *S. intermedius*, independent of the living environment of dogs. So the etiological factor can be easily observed among the tested dogs and the number of dogs used were enough to give the phenomenon. Authors were interested neither in the numbers of dogs nor in possessed isolates, but in observation of differences in colonization and etiological factors according to dogs' life environment.

In conclusion, we described the isolation of various staphylococcal strains which were constituents of the normal biocenosis of the skin of dogs. We also reported staphylococci as etiological factors of wound infections in household or rescue shelter dogs, healthy or diseased. Our observations enriched by recent reports by other authors expand the knowledge to be more precise which brings focus to ecological phenomena and epidemiological pathways. A carriage phenomenon and transient colonization by such strains in dogs and/or their owners facilitate the transmission of staphylococci between humans and domestic animals

(Simoons-Smit *et al.*, 2000; Nagase *et al.*, 2002) with a particular share of staphylococci from poultry (Wieliczko *et al.*, 2002). As it is reported the genomic similarity of strains isolated in veterinary pathology is particularly specific (Cuteri *et al.*, 2004, Jakubczak *et al.*, 2007). The dissemination of methicillin-resistant *S. pseudintermedius* in various animals and the confirmed presence of their resistance genes places them as potentially serious pathogens (Ruscher *et al.*, 2009). They have to be precisely analysed by a number of validated and recommended methods (Cuteri *et al.*, 2004; Małachowa *et al.*, 2005). Recently elaborated molecular methods should be introduced to restriction analysis of the chromosomal DNA with the final aim of increasing the effectiveness in discriminating closely related strains (Krawczyk *et al.*, 2007; Black *et al.*, 2009). A need of such an investigation is additionally enhanced by single reports on human colonization by *S. intermedius* (Talan *et al.*, 1989; Mahoudeau *et al.*, 1997; Kikuchi *et al.*, 2004) as well as recently reported infections caused by *S. pseudintermedius* (Van Hoovels *et al.*, 2006; Sasaki *et al.*, 2007b). Thus the elaboration of new procedures and diagnostic schemes for both veterinary and human bacteriology are an emerging challenge.

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Literature

Ackerman L. 1994. Guide to Skin and Haircoat Problems in Dogs. Alpine Publications, Loveland CO, pp. 7–19.

Allaker R.P., N. Garrett, L. Kent, W.C. Noble and D.H. Lloyd. 1993. Characterization of *Staphylococcus intermedius* isolates from canine pyoderma and from healthy carriers by SDS-Page of exoproteins, immunoblotting and restriction endonuclease digest analysis. *J. Med. Microbiol.* 39: 429–433.

Bannoehr J, Franco A, Iurescia M, Battisti A, Fitzgerald JR. 2009. Molecular diagnostic identification of *Staphylococcus pseudintermedius*. *J. Clin. Microbiol*. 47: 469–71.

Beveridge T.J. 2001. Use of the gram stain in microbiology. *Biotech. Histochem.* 76: 111–118.

Biberstein E.L., S.S. Jang and D.C. Hirsh. 1984. Species distribution of coagulase-positive staphylococci in animals. *J. Clin. Microbiol*. 19: 610–615.

Black C.C., S.M. Solyman, L.C. Eberlein, D.A. Bemis, A.M. Woron and S.A. Kania. 2009. Identification of predominant multilocus type, pulsed-field gel electrophoresis cluster, and novel staphylococcal chromosomal cassette in clinical isolates of *mecA*-containing, methicillin-resistant *Staphylococcus pseudintermedius*. *Vet. Microbiol.* 139: 333–338.

Cox H.U., J.D. Hoskins, S.S. Newman, C.S. Foi, G.H. Turnwald and A.F. Roy. 1988. Temporal study of staphylococcal species on healthy dogs. *Am. J. Vet. Res*. 49: 747–751.

Craig M. 2003. Diagnosis and Management of Pyoderma in the Dog. *In Practice* 25: 418–425.

Cuteri V., M.L. Marenzoni, R. Mazzolla, N. Tosti, L. Merletti, S. Arcioni and C. Valente. 2004. *Staphylococcus aureus*: study of genomic similirity of strains isolated in veterinary pathology using amplified fragment length polymorphism (AFLP). *Comp. Immunol. Microbiol. Infect. Dis.* 27: 247–253.

Devriese L.A. and K. DePelsmaecker. 1987**.** The anal region as a main carrier site of *Staphylococcus intermedius* and *Streptococcus canis* in dogs. *Vet. Rec.* 121: 302–303.

Devriese L.A., K. Hermans, M. Baele and F. Haesebrouck. 2009. *Staphylococcus pseudintermedius* versus *Staphylococcus intermedius*. *Vet. Microbiol.* 133: 206–207.

Devriese L.A., M. Vancanney, M. Baele, M. Vaneechoutte, E. De Graef, C. Snauwaert, I. Cleenwerck, P. Dawyndt, J. Swings, A. Decostere and F. Haesebrouck. 2005**.** *Staphylococcus pseudintermedius* sp. Nov., a coagulase-positive species from animals. *Int. J. Syst. Evol. Microbiol.* 55: 1569–1573.

Guardabassi L., M.E. Loeber and A. Jacobson. 2004**.** Transmission of multiple antimicrobial-resistant *Staphylococcus intermedius* between dogs affected by deep pyoderma and their owners. *Vet. Microbiol.* 98: 23–27.

Hajek V. 1976**.** *Staphylococcus intermedius*, a new species isolated from animals. *Int. J. Syst. Bacteriol.* 26: 401–408.

Hauschild T. and A. Wójcik. 2007**.** Species distribution and properties of staphylococci from canine dermatitis. *Res. Vet. Sci.* 82: 1–6.

Hendricks A., H.J. Schuberth, K. Schueler and D. Lloyd. 2002**.** Frequency of superantigen-producing *Staphylococcus intermedius* isolates from canine pyoderma and proliferation-inducing potential of superantigens in dogs. *Res. Vet. Sci.* 73: 273–7.

Jakubczak A., P. Szweda, K. Łukaszewska and J. Kur. 2007**.** Molecular typing of *Staphylococcus aureus* isolated from cows with mastitis in the east of Poland on the basis of polymorphism of genes coding protein A and coagulase. *Pol. J. Vet. Sci.*10: 199–205.

Kikuchi K., T. Karasawa, C. Piao, I. Itoda, H. Hidai, H. Yamaura, K. Totsuka, T. Morikawa and M.Takayama. 2004**.** Molecular confirmation of transmission route of *Staphylococcus intermedius* in mastoid cavity infection from dog saliva. *J. Infect. Chemother.* 10: 46–48.

Kim T.J., Y.R. Na and J.I. Lee. 2005**.** Investigation into the basis of chloramphenicol and tetracycline resistance in *Staphylococcus intermedius* isolates from cases of pyoderma in dogs. *J. Vet. Med. B. Infect. Dis. Vet. Public. Health* 52: 119–124.

Kizerwetter-Świda M., D. Chrobak, M. Rzewuska and M. Binek. 2009**.** Antibiotic resistance patterns and occurence of *mecA* gene in *Staphylococcus intermedius* strains of canine origin. *Pol. J. Vet. Sci.* 12: 9–13.

Kloos W.E. and M.S. Musselwhite. 1975**.** Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl. Environ. Microbiol*. 30: 381–395.

Krawczyk B., J. Leibner, W. Barańska-Rybak, A. Samet, R. Nowicki and J. Kur. 2007. ADSRRS-fingerprinting and PCR MP techniques for studies of intraspecies genetic relatedness in *Staphylococcus aureus*. *J. Microbiol. Meth*. 71: 114–122.

Król J. 1998. Characterization of staphylococci isolated from healthy and infected dogs (in Polish). Zeszyty Naukowe Akademii Rolniczej, Wrocław, Weterynaria LVIII, 344: 17–47.

Mahoudeau I., X. Delabranche, G. Prevost, H. Monteil and Y. Piemont. 1997**.** Frequency of isolation of *Staphylococcus intermedius* from humans. *J. Clin. Microbiol.* 35: 2153–2154.

Małachowa N., A. Sabat, M. Gniadkowski, J. Krzysztoń-Russjan, J. Empel, J. Międzobrodzki, K. Kosowska-Shick, P.C. Appelbaum and W. Hryniewicz. 2005. Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence

typing for clonal characterization of *Staphylococcus aureus* isolates. *J. Clin. Microbiol.* 43: 3095–3100.

Międzobrodzki J., N. Małachowa, T. Markiewski, A. Białecka and A. Kasprowicz. 2008**.** Differentiation of *Staphylococcus aureus* isolates based on phenotypical characters (in Polish). *Postępy Hig. Med. Dośw.* 62: 322–327.

Nagase N., A. Sasaki, K. Yamashita, A. Shimizu, Y. Wakita, S. Kitai and J. Kawano. 2002. Isolation and species distribution of staphylococci from animal and human skin. *J. Vet. Med. Sci.* 64: 245–250.

Ruscher C., A. Lubke-Becker, C.G. Wleklinski, A. Soba, L.H. Wieler and B. Walther. 2009. Prevalence of methicillin-resistant *Staphylococcus pseudintermedius* isolated from clinical samples of companion animals and equidaes. *Vet. Microbiol.* 136: 197–201.

Sasaki T., K. Kikuchi, Y. Tanaka, N. Takahashi, S. Kamata and K. Hiramatsu. 2007a. Reclassification of phenotypically identified *Staphylococcus intermedius* strains. *J. Clin. Microbiol.* 45: 2770–2778.

Sasaki T., K. Kikuchi, Y. Tanaka, N. Takahashi, S. Kamata and K. Hiramatsu. 2007b. Methicillin-resistant *Staphylococcus pseudintermedius* in a veterinary teaching hospital. *J. Clin. Microbiol.* 45: 1118–1125.

Shopsin B., B. Mathema, J. Martinez, E. Ha, M.L. Campo, A. Fierman, K. Krasinski, J. Kornblum, P. Acabes, M. Waddington, M. Riehman and B.N. Kreiswirth. 2000. Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the community. *J. Infect. Dis.* 182: 359–362.

Simoons-Smit A.M., P.H.M. Savelkoul, J. Stoof, T.M. Starink and C.M.J. Vandenbroucke-Grauls. 2000. Transmission of *Staphylococcus aureus* between humans and domestic animals in a houshold. *Eur. J. Clin. Microbiol. Infect. Dis.* 19: 150–152.

Stepanovic S., V. Dimitrijevic, D. Vukovic, I. Dakic, B. Savic and M. Svabic-Vlahovic. 2001. *Staphylococcus sciuri* as a part of skin, nasal and oral flora in healthy dogs. *Vet. Microbiol.* 82: 177–185.

Szewczyk E.M. 2005. Bacteriological diagnostics (in Polish). Wydawnictwo Naukowe PWN, Warszawa, pp 20–23.

Talan D.A., D. Staatz, A. Staatz and G..D. Overturf. 1989. Frequency of *Staphylococcus intermedius* as human nasopharyngeal flora. *J. Clin. Microbiol.* 27: 2393–2399.

Van Hoovels L., A. Vankeerberghen, A. Boel, K. Van Vaerenbergh and H. De Beenhouwer. 2006. First case of *Staphylococcus pseudintermedius* infection in a human. *J. Clin. Microbiol.* 44: 4609–4612.

Weese J.S., R. Poma, F. James, G. Buenviaje, R. Foster and D. Slavic. 2009. *Staphylococcus pseudintermedius* necrotizing fascilitis in a dog. *Can. Vet. J.* 50: 655–656.

Wieliczko A., J. Król, T. Piasecki, M. Mazurkiewicz and Z. Staroniewicz. 2002. Occurence and characteristics of staphylococci isolated from poultry (in Polish). *Med. Weter.* 58: 348–352.

Władyka B., M. Bišta, A.J. Sabat, E. Bonar, S. Grzeszczuk, W. Hryniewicz and A. Dubin. 2008. A novel member of the thermolysin family, cloning and biochemical characterization of metalloprotease from *Staphylococcus pseudintermedius*. *Acta Biochim. Pol.* 55: 525–536.