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Antimicrobial susceptibility of Staphylococcus spp. isolated from dogs with pyoderma

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Summary

The aim of this study was to evaluate antimicrobic resistance in canine staphylococci to the major classes of antimicrobials used in veterinary dermatology. The data was collected in 2002-2006 in the Dr L. Kriauceliunas small animal clinic of the Lithuanian Veterinary Academy. Clinical evaluation of 163 dogs was performed together with microscopical, mycological and bacteriological examinations. The clinical evaluation included the age of the dog, breed, sex, hair structure and season of the year. The authors found out that during the 5 year period males got the disease more often than females (52.98% versus 42.02%) and dogs at the ages of 1-5 years were the most common patients (43.9%). Bacterial skin diseases were more common in short-haired dogs (61.81%, $p < 0.05$). Long-haired dogs were more likely to be affected by bacterial skin diseases in the cold season ($p < 0.05$). Bacteriological examination revealed that the most common cause of bacterial skin infections was Staphylococcus intermedius (71.76%, $p < 0.05$) and Staphylococcus aureus (23.68%, $p < 0.05$). Mixed cultures (Staphylococcus spp., Streptococcus spp., Pseudomonas spp. and Escherichia coli) were found to be the cause of 4.56% ($p < 0.05$) of all bacterial skin diseases. S. intermedius and S. aureus strains exhibited the best sensitivity to ciprofloxacin, amikacin, methicillin (100%) and to cephadroxil (respectively 91.75% and 100%). S. intermedius strains were most resistant to sulphanilamides/trimethoprim (82.28%), S. aureus – to amoxicillin (86.0%).

Keywords: dogs, pyoderma, antimicrobic resistance

The incidence of pyoderma is one of the most common causes of canine skin diseases (3, 4, 14). For many years four coagulase-positive staphylococcus species have been recognized and documented as pathogens: *Staphylococcus aureus* (*S. aureus*), *Staphylococcus intermedius* (*S. intermedius*), *Staphylococcus hyicus* (*S. hyicus*) and *Staphylococcus schleiferi* (*S. schleiferi*) subspecies coagulans (23). The predominant pathogen of canine pyoderma is *S. intermedius* (up to 90%) (3, 4, 11, 14), a normal inhabitant of the skin and hair coat in dogs (14, 21). The virulence of *S. intermedius* is the sum of complex interactions between the host and pathogen (16). *S. aureus* is the predominant pathogen of humans, seldom found (6-15%) in dogs presented with bacterial skin diseases (3, 11, 14, 19). *S. schleiferi* subspecies is also known to be pathogenic in dogs and humans (11). Other bacterial pathogens, including Gram-negative organisms such as *Proteus* spp., *Pseudomonas* spp. and *Escherichia coli*, may be involved in deep pyodermas (14).

Systemic use of antibiotics is the cornerstone in the treatment of pyoderma. Currently, a clinically significant number of staphylococcus species that infect humans and domestic animals exhibit some degree of antimicrobic resistance and these can be extremely difficult to treat (2, 22, 23). Sensitivity profiles of staphylococcus strains have shown that multiresistance was more common in dogs with pyoderma (12, 13, 15, 17). Resistance of canine staphylococci to commonly used antimicrobic agents is widespread and comparable in many countries of Europe and USA (5, 9, 13, 15, 23). The relatively high resistance of canine staphylococci might be explained by an increased use of antimicrobic agents in dogs during the last decades (3, 13, 15, 21). There are special cases, such as chloramphenicol, which was hardly if ever used in the UK to treat canine pyoderma but is in use in Croatia with very low and high resistance rates, respectively (17). In order to detect early changes in bacterial susceptibilities before a high prevalence of resistance is selected or developed, regular monitoring of antimicrobial resistance both among pathogenic bacteria and normal flora of companion animals will be needed (4, 11, 15).

Literature data reveals different *Staphylococcus* spp. resistance against antimicrobic agents, depending upon

the country. In Lithuania it had never before been investigated which bacteria are the most common cause of bacterial skin infections, as well as their sensitivity to antimicrobic agents. The aim of the study was to establish which bacteria usually cause bacterial skin diseases in dogs, what is their sensitivity and resistance against various antimicrobic agents and to find out if there is a tendency for the development of increased resistance. Which dogs – short- or long-haired, males or females – are more prone to develop bacterial skin conditions was analyzed, as well as how these diseases are influenced by the season of the year.

Material and methods

The data was collected in 2002-2006 in the Dr. L. Kriauceliunas small animal clinic of the Lithuanian Veterinary Academy. 1249 of them were diagnosed with skin diseases (163 of these being of bacterial origin – 86 males, 77 females, 99 short-haired and 64 long-haired dogs). Clinical evaluation was performed together with microscopical, mycological and bacteriological examination that were performed in the department of infectious diseases of the LVA. The clinical evaluation included the age of the dog, breed, sex, hair structure and season of the year.

The samples for microbiological examination were taken with a sterile cotton swab. Samples were inoculated in Meat Peptone Broth (MPB) and in a parallel manner were inoculated on solid media: blood agar (BA) and MacConkey agar (Oxoid, England). After inoculation Petri plates were incubated at 37°C temperature, for 24-48 hours. The evaluation of colonies of microorganisms was conducted. The smears from microorganism cultures were stained by Gram (Diagnostica Merck, German) and microscopy for determination of microorganism morphology was performed and *Staphylococcus* spp. were identified by caogulase activity (Liofilchem, Italy), Staphytest Plus (Oxoid, England). *Staphylococcus aureus* strains producing beta lactamase were determined by Beta Lactamase Test (Liofilchem, Italy). *Streptococcus* spp. were identified by Streptococcal Grouping Test (Oxoid, England). Hygicult E/β Gur (Orion diagnostica, Finland) was used for enterobacteria identification. For *Pseudomonas* spp. identification up to *Pseudomonas aeruginosa*, selective medium Pseudomonas agar P, Pseudomonas agar F and Drigalsky lactose (Oxoid, England) agars were used.

Antimicrobial susceptibility was obtained according to the technique reported by Kirby-Bauer. Cultures of microorganisms were re-inoculated to MPB and incubated at 37°C temperature, for 24-48 hours. The density of microorganisms was evaluated by a unit of McFarland with Mini Shaker MS 1 (Crystal Spec, USA). Bacteria suspension of 0.25 ml were inoculated on plates with Mueller Hinton II Agar (Oxoid, England) and discs of antibiotics: amoxicillin 30 μg, amoxicillin with clavulanic acid 30 μg, methicillin 5 μg, gentamicin 10 μg, amikacin 30 μg, lincomycin 2 μg, clindamycin 2 μg, erythromycin 15 μg, cephalexin 30 μg, cephalothin 30 μg, cefadroxil 30 μg, oxytetracycline 30 μg (Liofilehem, Italy), chloramphenicol 30 μg, enroxil 5 μg, ciprofloxacin 5 μg and sulfonamides 300 μg (Oxoid, England), were placed, when the unit of McFarland was 0.5. Inoculated plates were incubated at 35-37°C temperature, for 18-24 hours. Resistance to antibiotics was evaluated calculating inhibition zones and interpreting the results shown in the table (13).

The data were analyzed statistically. The arithmetic average values ($\bar{x}$), standard deviation (SD), and coefficient of variation (CV) were calculated for all data. Dispersive analysis (ANOVA) was used to assess the influence of separate factors upon the bacterial skin diseases morbidity. The results are considered to be reliable when $p < 0.001$, $p < 0.01$ and $p < 0.05$.

Results and discussion

In 2002-2006 bacterial skin diseases in dogs among other skin disorders amounted to: in 2002 – 11.8% ($p < 0.05$), in 2003 – 11.9%, ($p < 0.05$) in 2004 – 8.4% ($p < 0.05$), in 2005 – 15.9% ($p < 0.05$) and in 2006 – 16.3% ($p < 0.05$).

The incidence of pyoderma is one of the most common causes of canine skin disease worldwide when compared with other species of domestic animals (4, 11, 19). Canine *stratum corneum* is thinner and more compact and the intercellular spaces are permeated with a lesser amount of protective emulsion than in other species. In addition, unlike other species, canine hair follicle infundibula are not filled with protective emulsion and thus are relatively unprotected (10).

The authors found out that the sex of the animals had almost no influence on the morbidity. During the 5 year period males got the disease more often than females (52.98% versus 42.02%), but the data was not statistically reliable. According to Holm et al. (6) pyoderma is more common in males, although most scientists (1, 18, 20) found no statistically reliable difference between males and females.

Concerning the influence of age on the morbidity it was found out that dogs at the age of 1-5 years were the most common patients (43.90%, $p > 0.05$). In 2002 dogs 1-5-years-of-age made up 41% of all patients with bacterial skin diseases, in 2003 – 25%, in 2004 – 60%, in 2005 – 52% and in 2006 – 42%. These conditions were slightly less frequent at the age of 5-10 years (29.54%, $p < 0.05$) and up to 1 year (22.70%, $p < 0.05$). In 2002 dogs 5-10-years-of-age comprised 26% of all patients with bacterial skin diseases, in 2003 – 61%, in 2004 – 20%, in 2005 – 22% and in 2006 – 20%. Dogs up to 1-year-old in 2002 comprised 22% of such patients, in 2003 – 11%, in 2004 – 20%, in 2005 – 24% and in 2006 – 36%. Bacterial skin diseases were found most rarely in dogs over 10-years-of-age (3.84%, $p < 0.05$). Many authors (1, 3, 6, 18, 19) indicate that bacterial skin diseases are more common in dogs younger than 5-years-old (50% and more), dogs over this age and up to 1-year-old are less likely to get this condition.

Bacterial skin diseases were more common in short-haired dogs (61.81%, $p < 0.05$) than in long-haired dogs (38.19%, $p < 0.05$). In 2002 short-haired dogs

comprised 63% of all bacterial skin diseases patients, in 2003 – 61%, in 2004 – 70%, in 2005 – 54% and in 2006 – 62%. Literature reveals controversial data about breed predisposition to these diseases. Some authors (6) state that these diseases are more common in long-haired breeds; others (8, 20) say that short-haired breeds are more often affected. Scott et al. (20), Sentürk et al. (21) express the opinion that hair length doesn't influence the occurrence of skin diseases.

The season of the year was divided into warm (May--September) and cold (October-April). It seems that the influence of the season was insignificant, and the data obtained was not statistically reliable. Long-haired dogs were more likely to be affected by bacterial skin diseases in the cold season ($p < 0.05$). Literature presents limited data about how the manifestation of bacterial skin diseases is influenced by the season. Breathnach et al. (2), Holm et al. (6) state that seasons do not have any significant influence.

Bacteriological examination performed in 2002--2006 revealed that the most common cause of bacterial skin infections was *S. intermedius* (71.76%, $p < 0.05$): in 2002 *S. intermedius* was found in 77.8% cases, in 2003 – 71.4%, in 2004 – 65.0%, in 2005 – 65.8% and in 2006 – 78.8% of all cases. In 2002-2006 23.68% ($p < 0.05$) bacterial skin infections in dogs were caused by *S. aureus*. In 2002 *S. aureus* was found in 18.5% of all cases, in 2003 – 28.6%, in 2004 – 25.0%, in 2005 – 29.3% and in 2006 – 17.0%. Mixed cultures (*Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp. and *Escherichia coli*) in 2002-2006 were found to be the cause of 4.56% ($p < 0.05$) of all bacterial skin diseases. Many scientists indicate that *S. intermedius*, a Gram-positive, coagulase-positive bacterium, is the most common (79% and more) infectious agent cultured in canine pyoderma (3, 14, 18, 21). Occasionally, *S. aureus* is the causative agent, but it is cultured less frequently (6-15%) (3, 14, 19). Gram--negative microorganisms, such as *Proteus* spp., *Pseudomonas* spp. and *E. coli*, are usually secondary invaders in dogs with pyoderma (14).

Research conducted in 2002-2006 showed different sensitivity of the bacteria to antimicrobic agents. During this period it was found that the bacteria showed the least sensitivity against amoxicillin (86.0% of *S. aureus* and 54.9% of *S. intermedius* were resistant). Harvey and Hunter (5) indicate the increase of β-lactamase producing staphylococcus, which explains why labile penicillins are insufficient for treating bacterial skin diseases. It has been noted (9, 17) that the resistance of staphylococcus against penicillins reaches 80%. The bacteria show the least resistance against cephadroxil (0% resistant *S. aureus* and 8.75% *S. intermedius*). Literature data indicates that pathogenic staphylococci appear to be slow in acquiring resistance to cephalosporins (21). Retrospective surveys of antimicrobic sensitivity of staphylococci isolates from dogs *in vitro* have been published and they indicate a very low level of resistance against these drugs (14, 17, 21). Low resistance was also found against other first generation cephalosporins: cephalotin (6.7% *S. aureus* and 23.9% *S. intermedius*), cephalexin (9.52% *S. aureus* and 22.66% *S. intermedius*).

24.46% of *S. auerus* and 14.76% of *S. intermedius* strains exhibited resistance against amoxicillin-clavulanic acid compounds. Many investigators also note the effectiveness of these compounds for skin diseases treatment (4, 11, 17).

Macrolides and lincosamides are used in veterinary dermatology but their main disadvantage is rapid resistance development (13). Cross-resistance between macrolides and lincosamides, and among the molecules of the same class, is common (13, 17). Some authors found a significant increase in resistance rates after six years to erythromycin (increase 7-21.5%) and lincomycin (increase 22.5-29%), whereas others did not observe any difference after 11-12 years (13). Research results showed that staphylococci are rather resistant to antibiotics such as: gentamycin (51.66% of resistant *S. aureus* and 32.29% of *S. intermedius* strains), erythromycin (52.44% of *S. aureus* and 49.72% of *S. intermedius* strains), clindamycin (50% of *S. aureus* and 68.22% of *S. intermedius* strains) and linkomycin (33.34% of *S. aureus* and 52.38% of *S. intermedius* strains).

Resistance to fluoroquinolones are reportedly rare (11, 21), but their frequent usage may lead to increased resistance in some cases of up to 17% (7, 18). The results of this investigation are similar – the authors found that 9.78% of *S. aureus* and 14.92% of *S. intermedius* strains are resistant to enrofloxacin.

It was found that 74.30% of *S. aureus* and 55.92% of *S. intermedius* strains were resistant to oxytetracycline, 47.78% of *S. aureus* and 37.48% of *S. intermedius* strains resistant to chloramphenicol and 33.34% of *S. aureus* and 82.28% of *S. intermedius* strains were resistant to sulphanilamides/trimethoprim. Other authors also produce similar data. The incidence of resistance of staphylococci to trimethoprim/sulpha varies from 10 to 56.9% (9, 11, 15), to tetracycline up to 40-90% (9, 15, 17) and to chloramphenicol up to 18% (9).

In 2002-2006 not a single bacteria strain resistant to ciprofloxacin, amikacin and methicillin was found. According to other investigators staphylococci are always sensitive to methicillin (4, 11) and the more recently developed fluoroquinolones (18, 21).

It has been noticed that refusing to administer antibiotics empirically and using them only based on the culture sensitivity test results has lately resulted in decreased resistance to oxytetracycline, erythromycin, clindamycin, sulphanilamides/trimethoprim, lincomycin. But *S. aureus* resistance to amoxicillin-clavulanic acid compounds and *S. intermedius* resistance to enrofloxacin and cephadroxil increased. The probable cause of this increase is that these substances have lately often

been used without the culture sensitivity test that is necessary to be able to choose the proper antimicrobic agent. Excessive use of animicrobic agents results in the development of resistant strains.

Conclusions

1. 1-5-year-old dogs are the ones most frequently affected by bacterial skin diseases; the sex of the dogs had no influence on the of bacterial skin diseases morbidity.

2. Bacterial skin diseases are more common in short-haired dogs than long-haired ones; long-haired dogs are more often affected by these diseases in the cold season.

3. The most frequent causes of bacterial skin diseases in dogs are *Staphylococcus intermedius* and *Staphylococcus aureus*.

4. *Staphylococcus intermedius* and *Staphylococcus aureus* strains exhibit the best sensitivity to ciprofloxacin, amikacin, methicillin and cephadroxil; *Staphylococcus intermedius* strains are most resistant to sulphanilamides/trimethoprim, *Staphylococcus aureus* – to amoxicillin.

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