MICROBIAL FLORA OF THE DOG EYES

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Summary. The purpose of this investigation was to isolate microbial flora of the normal dog eyes and eyes with clinical signs of external ocular disease.

Samples were collected from 46 dog eyes (92 samples). Pathogens were isolated from 58 eye samples of dogs without clinical signs and 34 – from dogs with clinical signs of one or both eyes. Among the isolated pathogens from eyes of dogs without clinical signs were *Staphylococcus* spp. (55%), *Pseudomonas* spp. (11.4%) and *Corynebacterium* spp. (6.8%). Among the isolated pathogens from eyes of dogs with clinical signs of external ocular diseases commonly isolated microorganisms were *Staphylococcus* spp. – 58%. *Staphylococcus aureus* was the most frequent microorganism isolated (20.5%) of total isolates. We detected certain pathogens (*Staphylococcus* spp.) which are commonly related to dog eyes microbial flora.

The antibiotic susceptibility tests were carried out according to the Kirby–Bauer method.

Our data showed that *Staphylococcus aureus* were the most susceptible to Methicillin, Oxacillin and Amoxicillin with clavulanic acid.

According to the results of dispersive analysis (ANOVA) we determined that *Pseudomonas aeruginosa* isolation frequency depends on a dog hair lenght and breed ($p<0.05$).

Keywords: *Staphylococcus* spp., microorganisms, dog, eye.

ŠUNŲ AKIŲ MIKROFLORA

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Santrauka. Tyrimo tikslas – išskirti mikroorganizmus, esančius sveikų šunų akyse, ir akyse su klinikiniais simptomais.

Akių mikroflora ištirta 46 šunų akyse, t.y. 92 akių konjunktyvos mėginiuose. Penkiasdešimt aštuoni mėginiai tyrimui paimti iš sveikų šunų akių, 34 mėginiai – iš šunų akių su išoriniais klinikiniais požymiais. Atlikę mikrobiologinį tyrimą nustatėme, kad iš sveikų šunų akių daugiausia išskirta *Staphylococcus* spp. (55%), *Pseudomonas* spp. (11,4%) ir *Corynebacterium* spp. (6,8%) genčių mikroorganizmų padermių. *Staphylococcus* spp. (58%) mikroorganizmų padermės (iš jų *Staphylococcus aureus* – 20,5%) taip pat vyrauja sergančių išorinėmis akių ligomis šunų akyse. Atlikę išskirtų mikroorganizmų padermių jautrumo antimikrobinėms medžiagoms tyrimą pagal Kirby – Bauer metodiką, nustatėme, kad išskirtos *Staphylococcus aureus* padermės jautriausios meticilinui, oksacilinui ir amoksicilinui su klavulanine rūgštimi.

Pagal vienfaktorinę dispersinę analizę (ANOVA) nustatėme, kad *Pseudomonas aeruginosa* išskyrimui įtakos turėjo šuns plaukų ilgis ir veislė ($p<0,05$).

Raktažodžiai: *Staphylococcus* spp., mikroorganizmai, šuo, akys.

Introduction. Bacterial and fungal flora of the normal conjunctival have been reported in cows (Samuelson et al., 1984), pigs (Davidson et al., 1994), birds (Miller et al., 1995), rabbits (Cooper et al., 2001), horses (Moore et al., 1988), bison (Davidson et al., 1999). Most reports of normal ocular flora in dogs show a predominance of nonpathogenic, mainly gram-positive organisms (Haghkhan, et al., 2005). However, gram-negative and fungal species are also found as part of the normal ocular flora of the dog, and are thought to be transitory and related to fungal organisms present in the surrounding environment. The microbiota of ocular surface depend on the age of the dog, geographical location and the climate (Grahn, 2004). Many causes of external bacterial ocular diseases result in increased numbers of normal flora, or in growth of common pathogens (Whitley, 2000). During assesment of a patient with these ocular diseases it is important to provide historical information, physical examination and bacterial examination (Slatter, 2001). As reported by Prado (2005), in Brasil the most commonly isolated microorganisms from healthy dog eyes were *Staphylococcus intermedius*. Among the isolated pathogens were *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Lactobacillus* spp., *Neisseria* spp., *Alcaligenes faecalis*, *Pasteurella canis*, *Klebsiella* spp., *Francisella* spp. (Haghkhah et al., 2005; Massa et al., 1999; Miller and Murphy, 1995; Salisbury et al., 1995). In accordance with studies the most commonly isolated microorganisms from eyes with clinical signs were *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Escherichia coli*, *Corynebacterium* spp. and *Bacillus cereus.*

The ideal basis for selection of an ocular antibiotics, is

the identification of the responsible organisms and its antibiotic sensitivity. Often, however, obtaining this information cannot be justified either because of expense or because treatment must be instituted before the results are available. Therefore, knowledge of the most likely organisms, their sensitivity, effective antibiotics is necessary (Gerding et al., 1993).

An impact of various factors on frequency of specific microbial flora isolation was not much evaluated. As reported by Whitley (2000), in mixed breed dog eyes there were more microorganisms than in Poodles eyes. In the eyes of the dog kept in the yard there were more microorganisms then in the eyes of dogs kept in the room. In the eyes of dogs over 2 years of age there were more microorganisms than in the eyes of dogs under 2 years (Whitley, 2000).

We did not find the data of dog conjunctival flora and details of various microbes species in the local literature. The purpose of this investigation was to isolate microbial flora of the normal dog eyes and eyes with clinical signs of external ocular disease.

Materials and methods. The study was carried out in 2004–2005. The samples of 46 dogs were examined (92 samples of the eyes). Fifty eight eye samples were taken from clinically healthy dogs, 34 – from dogs with clinical signs of one or both eyes. Fifty samples were taken from long-haired dogs, 42 – from short-haired dogs; 48 – from female, 44 – from male; 38 – from mixed breed dogs, 54 from purebred dogs; 26 – from dogs kept in the yard, 66 – from dogs kept in the room; 48 – from up to 2 years of age dogs, 44 – from over 2 years of age dogs. Forthy four eye samples were examined in autumn, 48 – in spring.

The samples for microbiological examination were taken with sterile cotton swab from normal eye and from eye with clinical signs of disease. The swabs were passed back and forth on the surface of the lower palpebral conjunctiva or directly on the corneal ulcer of each eye. Swabs were transferred to the microbiological laboratory (LVA, Department of Infectious diseases). Samples of eyes were inoculated in Meat Peptone Broth (MPB) and parallel were inoculated on solid media: blood agar (BA) and Mac – Conkey agar (Oxoid, England). After inoculation Petri plates were incubated at 37°C temperature, for 24–48 hours. The evaluation of microorganisms colonies was done. The smears from microorganisms cultures were stained by Gram („Diagnostica Merck“, German) and microscopy for determination of microorganisms morfology was performed. *Staphylococcus* spp. were identified by Coagulase 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). For enterobacteria identification up to species, selective medium Manitoli agar, Hectoen enteric agar, Brilliant Green, XLD (Oxoid, England), Oxidase test Bactident® Oxidase (Merck, German) and biochemical test – BD BBL™ Enterotube™ II (Becton. Dickinson, GmbH. Diagnostic systems, Germany), Hygicult E/β/ - Gur (Orion diagnostica, Finland) were used.

Antimicrobial susceptibility was obtained according to the technique reported by Kirby – Bauer (1966). 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 unit of McFarland with Mini Shaker MS 1 („Crystal Spec“, USA). Bacteria suspension of 0.25 ml was inoculated on plate with Mueller Hinton II Agar (Oxoid, England) and the discs of antibiotics: Penicillin-G 10 U, Ampicillin 10 μg, Oxacillin 1 μg, Methicillin 5 μg, Amoxicillin 30 μg , Amoxicillin with clavulanic acid 30 μg, Amikacin 30 μg, Gentamicin 10 μg, Cephalothin 30 μg, Cephalexin 30 μg, Tetracycline 30 μg (Oxoid, England), Oxytetracycline 30 μg (Liofilehem, Italy), Erythromycin 15 μg, Chloramphenicol 30 μg, Furazolidon 50 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 interpretating results shown in tables of firms.

Variety of the microorganisms species subject to breed, hair, gender and age of dogs, season and keeping conditions were defined by dispersive analysis (ANOVA). For this aim statistical models were created. An impact of hair, gender, breed and age of dog, keeping conditions and seasonal effects were calculated on the microbial rate, and statistical reliability was evaluated. The results are considered to be reliable when $p< 0.05$, $p<0.01$ and $p<0.001$. The data of investigations were evaluated statistically by the statistic model „R 2.20.“ (http://www.r-project.org) and *WinExcel* program.

Results of the investigations. Microorganisms were identified from 40 (69.0%) eye samples of healthy dogs. After investigation mixed microflora was determined. The bacteria isolated from dog eyes were mainly gram-positive (66%) and 34% gram-negative. *Staphylococcus* spp. were isolated from 55% of the growns. The isolates were identified as *Staphylococcus aureus* in 20.5% of cases.

The microbial flora from dog eye samples with clinical signs of disease was analyzed. Pathogens were isolated from 94.1% of the samples. Among the isolates pathogens, 71% were gram-positive and 29% were gram-negative. *Staphylococcus* spp. were isolated from 58% of the cases. *Staphylococcus aureus* was detected in 24% of the isolated strains. Among the gram-negative pathogens were *Pseudomonas aeruginosa* (7.9%), *Escherichia coli* (5.3%), *Enterobacter* spp. (5.3%), *Proteus mirabilis* (5.3%).

Results of susceptibility testing of the most commonly isolated microbes indicated that for *Staphylococcus* spp. the most efficacious antimicrobials were Methicillin, Oxacillin (100%); for β lactamase negative *Staphylococcus aureus* strains the most efficacious antimicrobials were Methicillin, Oxacillin, Amoxicillin with clavulanic acid, Cephalexin and Tetracycline (100%), for β lactamase positive *Staphylococcus aureus* strains – Methicillin, Amoxicillin with clavulanic acid and

Oxacillin (100%). All examined *Pseudomonas aeruginosa* strains were susceptible to Gentamicin and Cephalexin (100%). *Streptococcus* spp. were susceptible to Penicillin (100%).

After evaluation of impact of various factors on frequency of specific microbial isolation we determined that *Pseudomonas aeruginosa* isolation was subject to hosts hair – 26.80% ($p<0.01$) and breed – 27.50% ($p<0.05$). *Pseudomonas aeruginosa* microorganisms were isolated only from eye samples of long-haired dogs – 19.50% and from eye samples of thoroughbred dogs – 15.10%. We determined that *Corynebacterium* spp. isolation was subject to keeping conditions – 19.40% ($p<0.001$). *Corynebacterium* spp. microorganisms were isolated only from eye samples of dogs kept in the yard – 20.0%. *Proteus mirabilis* isolation was subject to hosts age and season. *Proteus mirabilis* microorganisms were isolated only from eye samples of the dogs up to 2 years of age – 9.10% and from eye samples of dogs evaluated in autumn – 8.0%. The season has influence on frequency of *Staphylococcus* spp. – 43.60% ($p<0.05$) and on total number of microorganisms isolation – 76.10% ($p<0.01$). *Staphylococcus* spp. microorganisms were isolated more frequently in autumn – 34.0% than in spring – 25.0%. The number of isolated microorganisms was more in autumn – 61.0%, than in spring – 39.0%.

Discussion. In the majority of reports detailing conjuctival microflora, gram-positive species are predominant. Ninety two samples of dog eyes were cultured in this study. The most general bacteria were gram-positive, including *Staphylococcus* spp., *Bacillus* spp., *Corynebacterium* spp., *Streptococcus* spp. These bacteria have been commonly isolated form the conjunctiva healthy dogs and dogs with clincal sign. The most general isolated bacteria are considered non-pathogenic, however, they can become pathogenic as a result of underlying ocular pathology. We detected certain pathogens, which are commonly related to eyes infection (*Staphylococcus* spp., *Staphylococcus aureus*). This observation reinforces the need for more care with spreading of contagious microorganisms. We evaluated the microbial flora of healthy dog eyes and eyes with clinical sign. We found that the bacteria isolated from eyes without clinical sign were mainly gram-positive (66%). As observed by Prado (2005), from eye samples without clinical sign, gram-positive microorganisms have been isolated more frequently – 86.5%, than gram-negative microorganisms – 13.5%. *Staphylococcus* spp. (55%) were the most frequently isolated microorganisms, this is consistent with previous surveys (Haghkhah et al., 2005; Whitley, 2000). *Bacillus* spp. (26.98%), *Streptococcus* spp. (38.4%), *Pseudomonas aeruginosa* (11.4%) and *Corynebacterium* spp. (6.8%) were the next most frequently isolated microorganisms (Haghkhah et al., 2005). As reported by Whitley (2000), *Pseudomonas aeruginosa* and *Corynebacterium* spp. microorganisms were not isolated. Our data showed that 2.3% of *Streptococcus* spp. were isolated from dog eyes. We found that the microorganisms isolated from dog eyes with clinical signs in comparison with other studies are slightly different. There are many reports stating that *Staphylococcus* spp. and *Streptococcus* spp. are predominant isolated microorganisms. As observed by Gerding (1988), *Staphylococcus* spp. – 39.3% and *Streptococcus* spp. – 25.2% were the most common isolates. In this study, *Staphylococcus* spp. were isolated from 58% of samples, *Streptococcus* spp. were detected rarely – in 5.3% of the cases. Our data showed that *Staphylococcus aureus* is one of the most common islolate from dog eyes with clinical signs (24%) and healthy eyes (20.5%). *Pseudomonas aeruginosa* was isolated from 7.9% of investigated samples. The results (9.4%) are comparable with earlier observations (Gerding et al., 1990). We found that there is considerable population of microorganisms which spread by direct or indirect contact.

The ideal basis for selection of an ocular antibiotic is an identification of the responsible organism and its antibiotic sensitivity. The evaluation of microorganisms susceptibility to antibiotics revealed that *Staphylococcus* spp. were the most susceptible to Methicillin and Oxacillin. As reported by Haghkhah (2005), for *Staphylococcus* spp. the most effective antibiotics were Tylosin, Chloramphenicol, Oxytetracycline and Gentamicin. As observed by Slatter (2001), for *Staphylococcus aureus* the most effective antibiotics were Gentamicin, Oxacillin, Methicillin, Cephalosporines and Fluorochinolones. In this study isolated *Staphylococcus aureus* strains were susceptible to these antibiotics too; for isolated *Pseudomonas aeruginosa* strains the most effective antibiotics were Amikacin, Gentamicin and Cephalexin. As observed by Gerding, for *Pseudomonas aeruginosa* the most effective antibiotics were Tobramycin, Polymyxin B, Amikacin and Gentamicin (Gerding et al., 1988).

After evaluation of impact of various factors on frequency of specific microflora isolation, we determined that *Pseudomonas aeruginosa* was subject to hosts breed. We determined that *Corynebacterium* spp. isolation was subject to keeping conditions of dog. *Proteus mirabilis* isolation was subject to hosts age. As reported by Whitley (2000), keeping conditions, breed and age had influence on total number of isolated microorganisms. In mixed breed dog eyes there were more (83.0%) microorganisms than in Poodle eyes (40.0%). In the eyes of the dogs kept in the yard there were more (93.3%) microorganisms than in the eyes of dogs kept in the room (69.0%). In the eyes of dogs over 2 years of age there were more (100%) microorganisms than in eyes of dogs under 2 years of age (Whitley, 2000). The season has influence on frequency of *Proteus mirabilis* and *Staphylococcus* spp. isolation. According to quotable sources breed and season have not influence on frequency of microbes isolation (Andrew et al., 2003; Venables and Smith, 2005).

Conclusions:

1. In this study, microflobial flora were cultured from healthy and with clinical signs dog eyes. *Staphylococcus* spp. were the most frequently isolated microorganisms from dogs with healthy eyes (55%) and dog eyes with external ocular diseases (58%).

2. The most efficacious antimicrobial agents for *Staphylococcus* spp. were Methicillin, Oxacillin, for *Staphylococcus aureus* – Amoxicillin with clavulanic acid and Oxacillin.

3. The variety of *Pseudomonas aeruginosa* was subject to breed, hair and age of dog, season and keeping conditions ($p < 0.05$).

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