Otitis externa in dogs: microbiology and antimicrobial susceptibility

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Summary

The aims of the study were to determine the prevalence of bacterial/yeast otitis externa in dogs and to analyse antimicrobial susceptibility. Among 257 swab ear samples from Bulgarian dogs with otitis externa, bacteria and yeast species were isolated in 93.77% of cases as mono-infections (109 cases) involving mainly coagulase-positive staphylococci (60 strains), Malassezia pachidermatis (97 strains) and Pseudomonas aeruginosa (42 strains) and more often as poly-infections (132 cases). The other agents scarcely identified were β-haemolytic Streptococcus spp., Proteus mirabilis and Escherichia coli, the 2 last species being almost exclusively found in poly-infections. The sensitivity of isolated bacteria to antimicrobial drugs, most commonly used treatment of otitis externa has been tested. The high sensitivity to beta-lactams and aminoglycoside-aminocyclitols was established in Gram positive bacteria, while Gram negative bacteria were sensitive to aminoglycoside-aminocyclitols, polymyxin B and enrofloxacin. These results highlight the relative high frequency of yeasts and the necessity of coupling antimicrobial susceptibility tests to bacterial isolation.

Keywords: Otitis externa, dogs, bacterial isolation, yeast, antimicrobial susceptibility.

Introduction

Otitis externa is an acute or chronic inflammation of the external ear. The disease is more frequent in dogs than in cats. Dogs with long pendulous ears are most commonly affected [2]. Ear infections are among the ten most frequent reasons for dogs to be presented to veterinarians and may affect up to 20% of dogs [1, 6]. A number of predisposing factors are identified such as anatomical ear canal stenosis, hair in the ear canal, pendulous ears, hair concave side of ears, increased humidity (hinting dogs), moisture retention, washing, injury during manipulations, foreign bodies, prolonged antibiotic treatment, obstructive diseases (neoplasms) and systemic conditions (immunosuppression) [17]. Other causes include permanent factors such as bacteria (coagulase positive Staphylococcus spp., β-haemolytic Streptococcus spp., Pr. mirabilis, Pseudomonas aeruginosa, Escherichia coli), yeasts (Malassezia pachidermatis, Candida albicans), as well as progressing pathological alterations (hyperplasia, oedema, fibrosis [17]. The normal ear canal microflora consists of coagulase-positive, coagulase-negative Staphylococcus spp., β-haemolytic Streptococcus spp., Pr. mirabilis, E. coli, Ps. aeruginosa etc. The last agent is isolated in only 1% of healthy dogs and in up to 20% of dogs with chronic otitis externa [7, 18]. In healthy feline ears, Pasteurella multocida could be also isolated. Apart these bacterial agents, M. pachidermatis, Microsporum canis and Otodectes cynotis could be also present without clinical signs of inflammation [5]. It should be noticed that bacteria and yeasts are opportunists and not primary pathogens causing solely otitis externa, but they found a favourable medium for growth provided that another cause is present [17, 18]. The successful treatment of the disease requires appropriate medications including anti-inflammatory drugs, antifungal agents and chemotherapy with relation to the sensitivity of the aetiological agents [7, 9].
The purpose of the present study was to determine the microbial aetiological agents of canine otitis externa, their prevalence and behaviour to antibacterial drugs that are most commonly used for treatment of this disease.

Material and methods

ANIMALS AND SAMPLES

The study included 193 dogs from both genders, from different breeds and from 8 months to 14 years old, all with clinical signs specific for otitis externa. Patients were presented with unilateral or bilateral dropping of ears, head shaking, pruritus, pain when palpated, erythema and swelling of ear skin or of ear canal with increased amount of cerumen. A total of 257 samples of ear secretion were collected using sterile cotton swabs. The study was performed between January 2007 and December 2011.

MICROBIOLOGICAL TESTS

Samples were cultivated on blood agar containing 5% sheep blood (Bul-bio Base, National Institute of Parasitic and Infectious Diseases, Sofia) and on McConkey agar (Difco). Cultures were incubated aerobically for 24-48 hours at 37°C. Mycological tests included aerobic cultivation of the samples on Sabouraud dextrose agar (supplemented with 0.4 g/L chloramphenicol and 0.5 g/L cyclohexamide) for 2-7 days at 37°C. After incubation, isolates were identified according to conventional microbiological methods [16].

The behaviour of isolates to antimicrobial drugs was tested by the disk diffusion method and the results were interpreted by the three-score system of Bauer et al. [3], as per requirements of NCCLS [14]. Disks loaded with antimicrobial drugs: amoxicillin/clavulanic acid (20/10 µg), ampicillin/cloxacillin (6.69/18.31 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), enrofloxacin (5 µg), chloramphenicol (30 µg), doxycycline (30 µg), lincomycin/spectinomycin (9 / 100 µg), and polymyxin B (10 µg) were used.

Results

The microbiological tests conducted on 257 samples showed that only 16 did not contain pathogenic microorganisms. From the other 241 samples, 388 microbial agents were isolated. Their distribution by species is shown on figure 1. Coagulase-positive staphylococci were the prominent species, representing 43.6% of the microbial agents identified and were found in more than 70% (169/241) of dogs with bacterial otitis externa. *Malassezia pachidermatis* represented 25% of the identified germs and was isolated in 40% of patients whereas *Pseudomonas aeruginosa* (17%), *Proteus mirabilis*, *Escherichia coli* and β-haemolytic streptococci were more rarely observed and were isolated in 17%, 11% and 10% of dogs with bacterial otitis externa respectively. In addition, *Pseudomonas aeruginosa* and *Proteus mirabilis* were isolated only from animals with chronic otitis or from dogs that failed to respond to previous antibiotic treatment. As already mentioned, the other bacterial species (β-haemolytic streptococci and *E. coli*) rarely caused otitis externa independently, but were rather seen in association with staphylococci or *Pseudomonas aeruginosa*.

Among the 241 positive samples for microbiological tests, mono-infections were observed only in 109 samples (45.2%). Coagulase-positive staphylococci were most commonly identified when a single organism was isolated from otitis patients corresponding to 60 cases (25% of all diseased dogs) followed by *M. pachidermatis* in 31 cases (13%). The other bacterial species (β-haemolytic streptococci, *E. coli* and *Pseudomonas aeruginosa*) rarely caused mono-infections, only in 11, 2 and 5 cases, respectively. By contrast, *Proteus mirabilis* was not detected as a sole agent of canine otitis.

Two and multiple microbial species were isolated from 132 samples: 119 samples with 2 microbial species, 11 with 3 germs and 2 with 4 pathogens (Table I). The most frequent microbial species involved in poly-infections were again Coagulase positive Staphylococcus spp (109/132, i.e. 82.58%), *Malassezia pachidermatis* (50.00%) and *Pseudomonas aeruginosa* (28.03%). However, among the species scarcely isolated in bacterial otitis externa, *Proteus mirabilis* and *E. coli* were often associated with other germs in 100% and 92.86% of positive samples whereas the proportions of mono-infections with the β-haemolytic *Streptococcus* spp. and of poly-infections involving them remained roughly similar (44% and 56%, respectively). Animals with 3 or 4 microbial agents simultaneously isolated exhibited chronic otitis.

The behaviour of bacterial isolates to tested antibacterial drugs is summarized in Table II. The analysis of strains behaviour towards antibiotics showed preserved high sensitivity to β-lactam combinations amoxicillin/clavulanic acid and ampicillin/cloxacillin in staphylococci and streptococci. Similar behaviour was exhibited by Gram + bacteria towards aminoglycoside-aminocyclitols gentamicin, amikacin and tobramycin, with percentage of sensitive strains between 74% and 91%. With regard
to enrofloxacin, chloramphenicol and polymyxin B, the sensitivity of staphylococci and streptococci was different. All streptococcal isolates were resistant to enrofloxacin while 68% of staphylococci were sensitive. The opposite was true for chloramphenicol: all streptococci were sensitive whereas 40% of staphylococci were resistant to this antibiotic. Some staphylococci (34%) were sensitive to polymyxin B, but all streptococci were resistant. The sensitivity to tetracyclines as well as to the combination lincomycin/spectinomycin was higher for streptococci than for staphylococcal isolates.

Gram - bacteria showed a very different behaviour to beta-lactam combinations. All Ps. aeruginosa, Pr. mirabilis and E. coli strains were resistant to ampicillin/clavulanic acid. At the same time, more than 2/3 of Proteus and E. coli isolates were sensitive to amoxicillin/clavulanic acid, whereas all Pseudomonas isolates were resistant to this combination. The resistance pattern to aminoglycosides was similar to that of Gram + bacteria although the percentage of sensitive strains was usually higher (ranging from 72% to 100%). The majority of Gram - bacteria was highly sensitive to enrofloxacin (from 62% to 86%). All Pseudomonas and the majority of Pr. mirabilis strains were resistant to chloramphenicol (74%) and tetracyclines (81%), while E. coli strains were highly sensitive. Ps. aeruginosa and Pr. mirabilis isolates were often resistant to lincomycin/spectinomycin (40% and 55% respectively).
BACTERIAL OTITIS EXTERNA IN DOGS

whereas all E. coli strains were sensitive. All Pseudomonas and E. coli isolates were sensitive to the polypeptide antibiotic polymyxin B while the sensitivity of Proteus spp. strains was however low (< 20%).

Discussion

In this study, 43.56% of all microbial isolates belonged to the group of coagulase-positive staphylococci, among which Staphylococcus intermedius (recently called Staphylococcus pseudointermedius) and Staphylococcus aureus are already known as canine pathogens [7]. A survey of microbial agents of canine otitis externa in Bulgaria was performed in the period 1993-1995 by BORISSOV et al. [4] that demonstrated the presence of coagulase-positive staphylococci in 54% of all dogs with otitis. In this investigation, coagulase-positive staphylococci were present both in mono- or co-infections in 70% of dogs with otitis externa. This prevalence is higher than that reported by BORISSOV et al. [4] and similar to that found by HARIHARAN et al. [8], HARVEY et al. [9], LILENBAUM et al. [11], LYSKOVA et al. [12], and OLIVERA et al. [15]. ZAMANKHAN et al. [21] observed that 73.8% of isolates in their study belonged to Staphylococcus spp. vs. only 43.56% in ours. The authors have investigated the bacterial agents of otitis in a warmer climate (Iran) and this is a probably cause for the higher prevalence observed. Our data however are considerably different from those of BORNAND [5] who isolated S. intermedius in only 23% of canine otitis cases.

The incidence of Gram - bacteria in the present study was not very different from data reported in Bulgaria [4]: Ps. aeruginosa isolates were 9% of all strains vs. almost 11% in this study. The study of BORISSOV et al. [4] did not present data about the involvement of yeasts, but our data corresponded to those of BORNAND [5] in a similar setting in Switzerland, where M. pachidermatis was isolated from 56% of dogs with otitis (40% in our study). With regard to this species, the present results were closer to those obtained by LYSKOVA et al. [12] in the Czech Republic. Prevalences of Ps. aeruginosa, Pr. mirabilis and E. coli observed in the present study are similar to those reported by KROGH et al. [10], LYSKOVA et al. [12] and OLIVERA et al. [15]. The last research group report also data about the incidence of co-infections and microbial species involved. Their results are similar to ours in that mixed infections occurred in more than half of diseased dogs. The combination Staphylococcus intermedius + Malassezia pachidermatis was the most commonly seen.

The sensitivity of staphylococci isolated from dogs with otitis externa in the present study coincide with data of LILENBAUM et al. [11] with respect to ampicillin and gentamicin, except that the proportions of resistant strains to tetracyclines observed here was elevated (almost 50% vs. 7% in their study). The results in the present study on staphylococci resistance to amoxicillin/clavulanic acid, gentamicin, amikacin and enrofloxacin are also highly comparable to those of VANNI et al. [19] who has conducted a very detailed investigation on the resistance of Staphylococcus intermedius and Staphylococcus schleiferi in Italy.

The general opinion that Ps. aeruginosa is the most resistant microbial agent involved in the aetiology of canine otitis externa was also confirmed in the present study. Similar data are published by HARIHARAN et al. [8], demonstrating 38% resistance to enrofloxacin, 99% to chloramphenicol, 98% to doxycycline but also a rather higher resistance to aminoglycoside-aminocyclitols gentamicin (15%) and amikacin (11%), contrary to present results (2% and 0%). Nevertheless, ZAMANKHAN et al. [21] have obtained 100% resistance of Ps. aeruginosa strains to amoxicillin/clavulanic acid and lincomycin/spectinomycin combinations and 10% to gentamicin and 0% to amikacin, which are closer to our observations. They have also observed that 20% of isolates were sensitive to oxytetracycline and 30% to chloramphenicol whereas all isolates in the present study were resistance to these drugs. Others [13, 20] reported a higher resistance to aminoglycosides and fluoroquinolones, consequently to the frequent use of medications containing gentamicin and enrofloxacin and recommended to replace them with other drugs from the same class as tobramycin and marbofloxacin.

With regard to Pr. mirabilis and E. coli isolates, the current results were highly similar to those of HARIHARAN et al. [8] and ZAMANKHAN et al. [21].

As a conclusion, it could be assumed that the commonest bacterial agents isolated from canine otitis externa were coagulase-positive staphylococci. It should be however remembered that yeasts are also frequently involved in the aetiology of ear canal inflammations and that in many instances, clinical cases were caused by co-infections. Microbiological tests are always recommended for isolation and identification of microbial agents and for determination of their sensitivity to chemotherapeutics used for treatment of otitis externa in dogs.

References


Revue Méd. Vét., 2013, 164, 1, 18-22


