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An update on microbiological causes of canine otitis externa in Campania Region, Italy

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ABSTRACT

Objective: To update the recent knowledge of the microbiological causes of canine otitis externa in Campania Region (Italy) and the antibiotic susceptibility patterns of the isolated strains.

Methods: A total of 122 dogs were examined by otoscopy, and auricular swab samples were collected from both ears in 74 dogs presenting clinical bilateral otitis and from single ears in 48 dogs displaying clinical unilateral otitis. Cytological examination, bacteriological analysis and antimicrobial susceptibility tests were performed.

Results: Thirty-one out of 122 dogs were positive for yeast species (25.4%, 95% confidence interval (*CI*): 18.2%–34.2%) with a higher prevalence of *Malassezia pachydermatis* (21/31 isolates, 67.7%, *CI*: 48.5%–82.7%), and a total of 91 out of 122 dogs were positive for bacterial species (74.6%; *CI*: 65.8%–81.8%) with a higher prevalence of *Staphylococcus pseudintermedius* (45/143 isolates, 31.5%, *CI*: 24.1%–39.8%). These results are the first description of *Streptococcus agalactiae*-associated otitis. The yeasts isolated showed high levels of susceptibility to all antifungal agents tested; on the contrary all the isolated bacterial strains were highly resistant to at least four out of ten antimicrobial classes. Both Gram-positive and Gram-negative bacteria showed high resistance to amoxicillin/clavulanate and kanamycin hence they are not recommended as initial empirical therapy for the otitis treatment.

Conclusions: This update illustrates an increase in antibiotic resistances providing an insight into the current knowledge of the therapeutic procedures followed on canine otitis externa in Italy. It also emphasizes the importance of considering the results of the microbiological and sensitivity tests to decide on an appropriate antibiotic therapy.

1. Introduction

Otitis externa is the most common ear disease of dogs, being up to 20% of the dog population affected by this disease. It has a multifactorial etiology but it is predominantly a microbial infection. Clinical signs, such as exudates and frequently erythema, oedema, offensive odour and pruritus are seen. Microorganisms belonging to the normal microbial flora of the auricular area can become pathogenic when environmental and/or primary conditions determine faster expression of their virulence factors.

Malassezia genus are commonly isolated in auricular canal of dogs and cats, having otitis externa or in cases of dermatitis disorders. The yeasts of the *Malassezia* genus are

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All experimental procedures involving animals were conducted in accordance to guide for use and care of animals that visit our clinic and approved by The Local Ethical Committee of The Faculty of Veterinary Medicine, University of Naples "Federico II" (authorisation reference number Prot. 2011/0123330).

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opportunistic microorganisms and can cause both human [1,2] and animal infections [3]. Particularly *Malassezia pachydermatis* (*M. pachydermatis*) has been reported as the predominant causative agent of canine otitis externa [4].

Furthermore, among bacterial agents, it is known that *Staphylococcus*, *Pseudomonas*, *Escherichia*, and *Proteus* species are considered important pathogens causing otitis externa in dogs [5]. Among Gram-positive bacteria, *Staphylococcus pseudintermedius*, (*S. pseudintermedius*) a coagulase-positive staphylococcal species, is frequently associated with pyoderma, otitis externa, urinary tract infections, and opportunistically infected sites in dogs [6]. Among Gram-negative bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*) plays a major role in otitis externa also because of the increasing number of multiresistant strains [7].

Regular treatment at home with disinfecting ear washes should become part of the pet's grooming-routine with a correct antimicrobial therapy. It has been described a high sensitivity to beta-lactams and aminoglycoside-aminocyclitols for Gram-positive bacteria, while for Gram-negative bacteria has been suggested the use of aminoglycoside-aminocyclitols, polymyxin B and enrofloxacin [8].

In recent decades it was reported that the high frequency of multidrug-resistant of the isolated bacteria could become a high risk factor for owners and veterinary professionals. Therefore, a rational policy of antibiotic prescription in order to prevent the selection of resistant strains is needed.

The purpose of the present study is to evaluate the presence of microorganisms involved in otitis externa in dogs from Campania Region, in southern Italy, in order to update data and to investigate the presence of multi-resistant bacterial strains [9].

2. Materials and methods

2.1. Samples

One hundred and twenty-two dogs with clinical signs of otitis externa, such as local pain, pruritus, erythema, ear discharge and desquamation of the epithelium, in at least one ear, were selected to collect auricular swabs, during a period of two years, and a total of 196 culture swabs were obtained. Precisely, samples were collected from both ears in 74 dogs with clinical bilateral otitis and unilaterally from 48 dogs with clinical unilateral otitis. Sterile cotton-tipped applicator was used to collect samples of ear exudates by inserting swabs into ear canal, rotating once through 360° and then rolling it out, and immediately transferred to Amies transport medium (Oxoid Ltd, UK) and maintained at 4 °C (not longer than 24 h) until processing. The samples were presented for screening at the Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production, University of Naples Federico II (Italy). The canine population studied aged from 2 months to 16 years and included 75 males and 47 females.

Dogs were excluded from the trial if they had previously received (i) topical treatment (within 10 days), (ii) systemic treatment with an antibiotic, antifungal or nonsteroidal anti-inflammatory (within 10 days), (iii) a steroidal anti-inflammatory treatment (within 14 days), or (iv) a long acting steroidal anti-inflammatory drug systemically (within 60 days). Informed consent was obtained from the owners of all dogs prior to their participation in the study. All experimental procedures were approved by The Local Ethical Committee of The Faculty of Veterinary Medicine, University of Naples Federico II (authorisation reference number Prot. 2011/0123330).

2.2. Cytological analysis

For cytological analysis cotton-tipped swabs of ear canal exudates were streaked onto glass slides, which were then heat fixed and stained with a modified Wright's stain (Diff Quik, Dade Behring, Deerfield, IL). All stained slides were examined under a Nikon Eclipse E600 Microscopy (Nikon Instruments Inc., Melville, NY) at 40× magnification. At least 10 fields were examined, and a number of yeast cells per field (> 5 yeast/field) represented excessive colonization by the organism and were considered positive (infection). A number of bacteria 25 per microscopy field (40×) were considered positive (infection) as previously described by Ginel *et al.* [10].

2.3. Microbiological analysis

Collected samples were plated on blood agar base supplemented with 5% sheep blood, selective medium used for the isolation of Gram-positive microorganisms, on mannitol-salt agar, selective medium to identify staphylococci, and on MacConkey agar, selective and differential medium to grow Gram-negative bacteria, which were incubated aerobically at 37 °C for 24–48 h. Sabouraud's dextrose agar with chloramphenicol was used to grow fungal flora and was kept at 30 °C for 7 days, then stained with Gram stain and examined microscopically. The plates were all microbiological media from Oxoid Ltd, UK. In the case of yeasts of the genus *Candida*, we performed subculture on Oxoid chromogenic candida agar media to differentiate species of *Candida*. Further confirmation of genus and species of yeasts were obtained by biochemical identification using Remel RapID™ yeast plus identification panel after pure culture growth on Emmons agar (Oxoid).

Bacteria were identified by macroscopic observation of the colonies, Gram staining, standard laboratory methodologies (catalase, staphylocoagulase tube test, aesculin), and miniaturized biochemical tests API system (bioMérieux SA, Marcy L'Etoile, France). The species identification by miniaturized biochemical tests was accepted when probability was > 88%. β-haemolytic cocci strains were identified as group B [*Streptococcus agalactiae* (*S. agalactiae*)] or group C *Streptococcus dysgalactiae* (*S. dysgalactiae*), using a streptococcus grouping kit (Streptex, Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The presence of the *mecA* gene was detected by growth on oxacillin-containing media (2 mg/L), agar diffusion with oxacillin disks (5 μg) and positive latex agglutination test (PBP2′ Test. Oxoid Ltd, UK).

2.4. Antimicrobial susceptibility testing

The antimicrobial susceptibility patterns of isolated bacterial strains were determined by disk diffusion test using Mueller–Hinton agar (Oxoid Ltd, UK). The inhibitory zone diameters obtained around the antibiotic disks were measured after incubation for 24 h at 37 °C and evaluated according to the Clinical and Laboratory Standards Institute [11]. An oxacillin (methicillin) susceptibility test of all isolates was performed by oxacillin disk diffusion and if assayed, the minimal inhibitory concentration was determined by standard methods.

Ten drug classes were included in this study, using commercial disks containing the drugs (Oxoid Ltd, UK). Penicillins were represented by benzylpenicillin (penicillin G) (10 IU),