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Investigation and Antimicrobial Susceptibility of Microbial Agents of External Otitis in Dogs

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Abstract: The purpose of this research was to investigate the presence of secondary bacterial or yeast contamination of otitis externa in dogs and to analyze the antimicrobial susceptibility of those microorganisms. Clinical manifestations of external otitis were evaluated. Samples from the ear canal of 42 dogs with otitis externa were submitted to direct microscopic examination and cultured in blood agar and Sabouraud dextrose agar with chloramphenicol and cycloheximide. Otitis externa was more frequent in dogs between 2 and 5 year old 19 cases (55.9%) followed by up to 2 years 9 cases (26.5%). The most common dog breeds affected from otitis externa were crossbred (23.8%), German shepherd (16.6%) followed by cocker spaniel (14.3%) and Fox terrier (11.9%). Among 42 swab ear samples from dogs with otitis externa, microorganisms were isolated in 50% of cases as mono-infections (21 cases), involving mainly *Staphylococcus pseudintermedius* (10 cases), followed by *Malassezia pachydermatis* (8 cases), *Streptococcus* spp. (2 cases) and *Staphylococcus aureus* (one case) and other cases (13) as poly-infections (31%). The other microbial agents *Proteus mirabilis* in 4 cases, *Pseudomonas aeruginosa* in 5 cases and *Staphylococcus aureus* in 2 case were found also in poly-infection. All of the bacterial isolates tested were highly susceptible to amoxicillin-clavulanic acid, gentamycin, chloramphenicol and resistant to ampicillin, lincomycin and streptomycin.

Key words: Antimicrobial susceptibility, microbial agents, otitis externa, dogs, Europe

INTRODUCTION

Otitis externa is the inflammation of the external ear canal. The canine auditory canal is an environment vulnerable to any change, anatomic and physiologic alternations can set off the development of a microclimate which favors microorganism proliferation (August, 1988; Gotthelf, 2000). The disease is more frequent in dogs than in cats and can be caused by many different factors. Some of these factors (such as parasites, Foreign bodies and allergies) appear to directly cause the inflammation while others (such as certain bacteria and yeasts) perpetuate the condition. Dogs with long pendulous ears are most commonly affected (August, 1988). Microorganisms are seen as perpetuating factors as they are responsible for the aggravation of the process. Bacteria and yeasts are opportunists and not primary pathogens causing solely otitis externa but they found a favorable medium for growth provided that another cause is present (Rosser, 2004). Common organisms isolated from dogs with otitis externa include *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Streptococcus* spp., *Escherichia coli*, *Klebsiella* spp. and *Pasteurella* spp. (Petersen *et al*., 2002). *Malessezia pachydermatis*is an important pathogen in veterinary medicine. This yeast is also one of the most frequent microorganisms associated to external otitis in dogs (Crespo *et al*., 2002). In most of the previous studies, the antibiotic susceptibility of the microorganisms isolated from dogs with otitis externa is variable (Martin *et al*., 2000; Petersen *et al*., 2002; Harihan *et al*., 2006). The objectives of this research were to investigate the microbial agents that complicate otitis externa in dogs, assess the frequency of these microorganisms in relation to age and breed and to determine their susceptibility to the antibiotics.

MATERIALS AND METHODS

The study was performed in 42 dogs with clinical signs of external otitis, classified according to age and breed. We paid attention to patient with unilateral or bilateral ear dropp, head shaking, pruritus, erythema, pain when palpated, thickening of the ear mucosa and increased amount of cerumen. All infected dogs had not

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received any topical and/or systemic antibacterial or antifungal medication according to their recorded medical history. Samples were collected from different private veterinary clinics in Kosovo. All dogs were examined before sampling through general clinical and otoscopic examination. The samples were taken by sterile cotton swabs and were immediately transferred to the laboratory for culture. Samples from the swabs were cultured onto modified Dixon medium and Sabouraud dextrose agar containing chloramphenicol (0.5%) and cycloheximide (0.5%). All the plates were incubated at 37°C for about 7 days. For bacterial isolation the samples were cultivated on blood agar containing 5% sheep blood, MacConkey agar (Difco) and Mannitol salt agar. Cultures were incubated aerobically for 24-48 h at 37°C. After incubation, isolated microorganisms were identified according to conventional microbiological methods and according to biochemical test. This study was conducted between April 2014 and March 2015. Isolates were tested for antimicrobial susceptibility on Mueller Hinton Agar (HIMEDIA) by disk diffusion method. The following antibiotic disks were tested: penicillin P (10 IU), chloramphenicol (30 μg), ampicillin A (10 μg), amoxycillin-clavulanic acid AMC (30 μg), oxytetracycline T (30 μg), lincomycin (15 μg), streptomycin S (10 μg) and gentamycin CN (10 μg).

RESULTS

A total of 42 cases of otitis externa in dogs were included in study. The age of the affected dogs ranged from 2 years to >8 years. As shown in Table 1, the frequency of otitis externa was found more in dogs between 2 and 5 years old 19 cases (55.9%) followed by up to 2 years 9 cases (26.5%). In the present investigation, the most common dog breeds affected from otitis externa were crossbred (23.8%), German shepherd (16.6%) followed by cocker spaniel (14.3%) and Fox terrier (11.9%). Out of the 42 ear swab samples that were taken from dogs with otitis externa, 8 of the samples did not contain pathogenic microorganisms while 34 (80.9%) were processed and different microorganisms were isolated. The distribution of microorganisms by species are reported in Table 2. Among the 34 positive samples for microbiological tests, mono-infection were observed only in 21 samples (50%). *Staphylococcus pseudintermedius* was isolated as a single microbial species in 10 (23.8%) cases of dogs of the otitis externa, followed by *M. pachydermatis* in 8 cases (19%), *Streptococcus* spp. in 2 case (4.8%) and *Staphylococcus aureus* in one case (2.4%). Two and three microbial species were involved and isolated from 18 samples: 16 samples with 2 microbial specie and 2 with three pathogens. The most frequent microbial agents involved in poly infections (with 2 and three microbial species) were also *M. pachydermatis* in 9 (21.4%) cases of animals of the otitis externa and *Staphylococcus pseudintermedius* in 8 cases (19.1%). Other bacteria such as *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were involved and isolated only in 4, 2 and 5 cases of dogs of the otitis externa, respectively, in association to *Staphylococcus pseudintermedius* or *M. pachydermatis* in poly-infections. The results of the antimicrobial susceptibility testing of the 5 common bacterial isolates in this study are reported in Table 3. In present study, *Staphylococcus pseudintermedius* isolates tested (18) from dogs with otitis externa were highly susceptible to amoxicillin-clavulanic acid (89%), gentamycin (61%), chloramphenicol (75%) and the most resistant to ampicillin (61%), lincomycin (67%) and streptomycin (66%).

Table 1: Isolation of bacteria/yeast from dogs with otitis externa based on breed and age

Breeds	No. of samples examined	Positive sample isolate	Age of dogs			
			Up to 2 year	2-5 year	5-8 year	>8 year
German shepherd	8	7 (16.6%)	2	4	1	0
Rottweiler	4	4 (9.5%)	1	3	0	0
Pekingese	2	1 (2.4%)	0	1	0	0
Crossbred	12	10 (23.8%)	2	5	2	1
Siberian husky	2	1 (2.4%)	0	1	0	0
Cocker spaniel	8	6 (14.3%)	2	4	0	0
Fox terrier	6	5 (11.9%)	2	1	2	0
Total	42	34 (80.9%)	9 (26.5%)	19 (55.9%)	5 (14.7%)	1 (2.9%)

Table 2: Microorganisms isolated from the otitis externa of 42 infected dogs

Microbial scpecies	Infected dogs no. of isolates	Percentage
Single specie: *Staphylococcus pseudintermedius*	10/42	23.8
M. pachydermatis	8/42	19.0
Streptococcus spp.	2/42	4.8
Staphylococcus aureus	1/42	2.4
2 species: *Staphylococcus pseudintermedius+M. pachydermatis+Proteus mirabilis*	4/42, 2/42	9.5/4.5
M. pachydermatis+Pseudomonas aeruginosa+Staphylococcus aureus	3/42, 2/42	7.1/4.8
3 species: *Staphylococcus pseudointermedius+Pseudomonas aeruginosa+Proteus mirabilis*	2/42	4.8
No growth	8/42	19.0
Total	34/42	100

Table 3: Antimicrobial susceptibility rate of bacteria isolated from dogs with otitis externa

	Tested antimicrobial drug													
	Amoxicillin/ clavulanic acid		Ampicillin		Gentamycin		Chloramphenicol		Penicillin G		Lincomycin		Streptomycin	
Bacterial isolates	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
S. pseudintermedius n = 18	89	11	39	61	61	39	75	45	56	44	33	67	44	66
S. aureus n = 3	100	0	33	67	67	33	67	33	33	67	33	67	33	67
Steptococcus spp. n = 2	100	0	50	50	100	0	100	0	50	50	50	50	0	100
Proteus mirabilis n = 4	75	25	25	75	75	25	75	25	100	0	0	100	50	50
Ps. æruginosa n = 3	100	0	0	100	67	33	67	33	100	0	33	67	33	67

Streptococcus spp., isolates were the most susceptible to amoxicillin-clavulanic acid, gentamicin and chloramphenicol (100%) but resistant to ampicillin, penicillin G, lincomycin (50%) and streptomycin (100%). All isolates of *Pseudomonas aeruginosa* were sensitive to amoxicillin-clavulanic acid, penicillin G but, resistant to ampicillin.

DISCUSSION

Otitis externa infection in dogs has become a problem among dogs in our country in Kosovo. The results of this study showed that this infection occurred more in dogs between 2 and 5 year old in 19 cases (55.9%). These findings were almost in accordance with results of other studies (Lilenbaum *et al*., 2000; Choi *et al*., 2001). However, other researchers report a higher incidence in the 5-8 year old group of dogs with otitis externa (August, 1988). The most common dog breeds affected from otitis externa were crossbred (23.8%), German shepherd (16.6%) followed by cocker spaniel (14.3%) and Fox terrier (11.9%). These breeds are predisposed to recurrent otitis (Muse, 2000) and the findings in this study are almost similar with report of other researchers (Fraser *et al*., 1969; Sharma and Rhoades, 1975; Choi *et al*., 2001). Various microorganisms may be involved in the otitis externa infection in dogs. In our study, *Staphylococcus pseudintermedius* was the most commonly isolated microorganism involved in mono and poly-infection of otitis externa (16 cases or 48.1%), followed by *M. pachydermatis* (15 cases or 35.6%). Our data about the most frequent isolates in otitis externa of dogs are similar to those previously reported by other researchers (Petersen *et al*., 2002; Hariharan *et al*., 2006). *Malassezia pachydermatis* is considered as a secondary cause of dog otitis externa (Scott *et al*., 2001; Crespo *et al*., 2002). *Staphylococcus pseudintermedius* was the sole isolate in 10 (23.8%) cases of dogs with otitis externa, while in 4 (9.5%) cases were found to coexist with *M. pachydermatis* and in 4 (9.5%) cases with other microorganisms. *Malassezia pachydermatis* was the sole isolate in 8 (19%) cases of dogs with otitis externa while with other microorganisms was found to coexist in 9 (21.4%) cases of dogs with otitis externa. The findings in the present study were different to those reported by Bourtzi-Hatzopoulou *et al*. (2003) and Petrov *et al*. (2013). Other bacteria such as *Proteus mirabilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were involved and isolated with other microorganisms only in 4, 2 and 5 cases of dogs of the otitis externa, respectively. A similar species of bacterial infection in dogs with otitis externa were found by Sapierzynski (2009).

Otitis externa infection in dogs and its treatment is an important part of veterinary practice. Antibiotic sensitivity testing in veterinary practice is also an essential tool for treatment. All of the bacterial isolates tested (34) were highly susceptible to amoxicillin-clavulanic acid, gentamycin, chloramphenicol and resistant to ampicillin, lincomycin and streptomycin. All streptococcal isolates were sensitive to amoxicillin-clavulanic acid, gentamycin and chloramphenicol (100%) but resistant to streptomycin (100%). Resistance was most frequently detected to ampicillin, lincomycin (*S. pseudintermedius*, *S. aureus*, *Proteus mirabilis* and *Pseudomonas aeruginosa*), streptomycin (*S. pseudintermedius*, *S. aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*) and Penicillin G (*S. aureus*). All *Proteus mirabilis* isolates were sensitive to penicillin G (100%) but resistant to licomycin (100%). The majority of *Staphylococcus aureus* strains were resistant to streptomycin, lincomycin, penicillin G and ampicillin (67%). The findings of our study indicate that the antimicrobial sensitivies of the bacteria isolated from dogs with otitis externa are variable.

CONCLUSION

The results of this study indicated that the most commonly isolated microbial agents from canine otitis externa were *Staphylococcus pseudintermedius* and *Malassezia pachydermatis*. The present study indicates that amoxicillin-clavulanic acid, gentamicin, chloramphenicol and penicillin G could be the most effective antibiotics for the treatment of otitis externa caused by these bacterial agents.

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REFERENCES

August, J.R., 1988. Otitis externa: A disease of multifactorial etiology. Vet. Clin. North. Am. Small. Anim. Pract., 18: 731-742.

Bourtzi-Hatzopoulou, E., E. Petridou and V. Psyhoyos, 2003. Association of *Malassezia pachydermatis* with the otitic and the normal ear canal of the dogs. J. Hellenic Vet. Med. Soc., 54: 34-40.

Choi, W., S. Lee and K.W. Lee, 2001. Aetiology and epidemiological features of canine dermatitis. Korean. J. Vet. Res., 40: 94-100.

Crespo, M.J., L. Abarca and F.J. Cabanes, 2002. Occurrence of *Malassezia spp.* in the external ear canals of dogs and cats with and without otitis externa. Med. Mycol., 40: 115-121.

Fraser, G., W.W. Gregor, C.P. Mackenzie, J.S.A. Spreull and A.R. Withers, 1969. Canine ear disease. J. Small Animal Practice, 10: 725-754.

Gotthelf, L.N., 2000. Small Animal Ears Diseases. 1st Edn., W.B. Saunders Company, Philadelphia, Pennsylvania, Pages: 270.

Hariharan, H., M. Coles, D. Poole, L. Lund and R. Page, 2006. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. Can. Vet. J., 47: 253-255.

Lilenbaum, W., M. Veras, E. Blum and G.N. Souza, 2000. Antimicrobial susceptibility of staphylococci isolated from otitis externa in dogs. Lett. Applied Microbiol., 31: 42-45.

Martin, B.J.L., G.P. Lupiola, L.Z. Gonzales and J.M. Tejedor, 2000. Antimicrobial susceptibility patterns of *Pseudomonas* strains isolated from chronic canine otitis externa. J. Vet. Med. Infect. Vet. Public Health, 47: 191-196.

Muse, R., 2000. *Malassezia pachydermatis*. In: Kirk's Current Veterinary Therapy: Small. Bonagura, J.D. (Ed.). W.B. Saunders, Philadelphia, Pennsylvania, pp: 574-577.

Petersen, A.D., R.D. Walker, M.M. Bowman, H.C. Schot and E.J. Rosser, 2002. Frequency of isolation and antimicrobial susceptibility patterns of *Staphylococcus intermedius* and *Pseudomonas aeruginosa* isolates from canine skin and ear samples over a 6-year period (1992-1997). J. Am. Anim. Hosp. Assoc., 38: 407-413.

Petrov, V., G. Mihaylov, I. Tsachev, G. Zhelev and P. Marutsov *et al.*, 2013. Otitis externa in dogs: Microbiology and antimicrobial susceptibility. Revue. Med. Vet., 164: 18-22.

Rosser, E.J., 2004. Causes of otitis externa. Vet. Clin. Small Anim., 34: 459-468.

Sapierzynski, R., 2009. Otitis externa in dogs. Med. Weterynaryjna, 65: 552-556.

Scott, D.W., W.H. Miller and C.E. Griffin, 2001. In Small Animal Dermatology. 6th Edn., Philadelphia, Pennsylvania, Philadelphia, Pennsylvania, Pages: 1204.

Sharma, V.D. and H.E. Rhoades, 1975. The occurrence and microbiology of otitis externa in the dog. J. Small. Animal Practice, 16: 241-247.