Prevalence and infestation degree of yeast in canine otitis externa

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Abstract

Otitis externa is a persistent and recurrent problem, and the resistance of the involved pathogens to conventional drugs has made this a serious issue in veterinary medicine. This is an inflammation of the external ear canal in dogs that can be caused by bacteria or yeast. Often, these two causative agents are isolated together in combination. This study aimed to determine the prevalence of yeast and the degree of infestation of the isolated pathogens in canine otitis externa at veterinary clinics for small pets at the Department of Veterinary Medicine, University of Novi Sad, Serbia. Samples were taken from 60 dogs with clinical symptoms of otitis externa using a sterile swab and sent to laboratory for further mycological and bacteriological examination. In order to isolate the bacteria and yeast, conventional methods were used under aerobic conditions. Of the total samples, 53 (88.33%) were positive for at least one of the causative agents of otitis externa. Yeasts were isolated from 36 (52.94%) samples. The study showed that in comparing mixed and independent types of infection, in most cases 15/23 (65.22%) the degree of infestation with yeast was highest when the infection was mixed with bacteria. In relation to dog breeds, their ear forms and type of infection, more yeast samples (either as solo or mixed infection) were isolated from dogs with pendulous ears. It has been confirmed that *Malassezia pachydermatis* is the most common yeast pathogen of otitis externa in dogs, compared to other species of this genus, and it occurs in a higher degree of infestation in mixed infections than in solo infections. Dog breeds with pendulous ear form are more predisposed to yeast otitis externa than erect eared dog breeds.

Key words: *otitis externa; Malassezia spp.; dog breed*

Introduction

Otitis externa of dogs is an acute or chronic inflammation of the external ear canal including the ear pinna and is one of the most common reasons why owners take their dogs to the vet (Woodwars, 2022). Usually, bacteria and yeast are considered to be secondary causes of this disease (Lyskova et al., 2007). Moreover, small number of these potentially pathogenic microorganisms can be found normally in the external ear canal, but when an environmental or primary factors disturb the normal conditions in the ear, the

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potential for their virulent action is increased (De Martino et al., 2016).

Additionally, bacteria are frequently present in ear affected by otitis externa and the most commonly isolated species from dog ear canals are *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Escherichia coli* and other *Enterobacteriaceae* (Zamankhan Malayeri et al., 2010; Ebani et al., 2017; Bajwa, 2019). Furthermore, bacteria and yeast are often present together in combination (Little, 1996).

One of the most frequent agents in canine otitis externa is *Malassezia* yeast, with a prevalence as a sole causative agent between 8% and 26% (Rougier et al., 2005; King et al., 2018). Furthermore, the genus *Malassezia* includes seven species: *M. pachydermatis*, *M. globosa*, *M. furfur*, *M. slooffiae*, *M.sympodialis*, *M. obtusa* and *M. restricta*. All species except *M. pachydermatis* are lipid-dependent for growth (Gueho et al., 1996). Furthermore, *M. pachydermatis* is the most common isolated yeast from affected ears and in stained smears appears like bottle- or 'bowling pin'- shaped cells (Weidman, 1925) and is often attached to exfoliated epithelial cells (Kowalski, 1988).

In dogs, persistent or recurrent *Malassezia* dermatitis is usually associated with failure to identify and correct predisposing or perpetuating factors (Guillot and Bond, 2020). Although skin changes may be more prevalent in a certain area, larger parts of the body are usually affected, such as the limbs, underside, ears and face. In the case of otitis occurring at the same time with dermatitis, there are changes in the external ear canal that imply glandular hyperplasia and dilation, epithelial hyperplasia and hyperkeratosis which leads to lichenification, scaling and increasing yellow or brownish cerumen production (Bond et al., 2010; Bajwa, 2019).

In case of otitis externa treatment caused by yeast, despite antifungal therapy, many cases do not respond and remain unresolved for long periods, until they are resolved spontaneously or became a more serious problem (Bernardo et al., 1998). However, the evidence that reduced susceptibility of *M. pachydermatis* to commonly used antifungal drugs may develop under both field and laboratory conditions highlights the need for surveillance and vigilance for the emergence of clinically-relevant resistance (Guillot and Bond, 2020).

Although *M. pachydermatis* is mainly associated with animals, transmission and infections to newborn babies and immunocompromised people is known (Chang et al., 1988; Mani and Maguire, 2009). Interestingly, it could represent a One Health issue since the transference from animals to human is determined.

Hence, the aim of the present study was to determine the prevalence of the yeast in canine otitis externa at veterinary clinics for small pets at the Department of Veterinary Medicine, University of Novi Sad in Serbia, and the degree of infestation of the isolated pathogens.

Materials and methods

Sample collection

The research was conducted at the Veterinary Clinic of the Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Serbia over a two-year period (October 2020 to October 2022). In total, 60 samples from the external ear canal from dogs with clinical symptoms of otitis externa were examined mycologically and bacteriologically. Dogs were of different breeds, age and gender. Furthermore, clinical symptoms of otitis externa were determined in at least one

ear, established by the anamnesis from the owner and by physical and otoscopic examination of the dogs. Samples were collected with sterile cotton-tipped swabs, transferred to a tube with transport media (EUROTUBO®) and maintained at 4°C until transport the same day to the Vetlab veterinary laboratory in Belgrade for further testing.

Isolation and identification of pathogens

To isolate bacteria, conventional microbiological methods were used under aerobic conditions (Columbia agar, Biomerieux, France), commercial biochemical tests were used for identification, and antibiotic susceptibility testing was performed by disc diffusion method according to European Society of Clinical Microbiology and Infectious Diseases (EUCAST) guidelines.

To isolate yeast, the conventional method was used under aerobic conditions (SDA agar, Promedia, Serbia). Confirmation of the identification and concentration of *Malassezia pachydermatis* in otitis externa is additionally performed with microscopy. The concentration, precisely the degree of infestation is estimated based on the average number of yeast cells per epithelial cell on the cytological preparation (Figure 1).

Results and discussion

Bacteriological and mycological examination (Figure 2) found that of the 60 samples, 53 (88.33%) were positive for at least one of the infectious secondary causative agents of otitis externa. In the remaining 7 samples (11.67%), neither bacteria nor yeast were found. Furthermore, 23 (38.33%) samples were with a mixed infection, bacteria and yeast, 19 (31.67%) samples were positive for G+ bacteria, while the remaining samples 4 (6.67%) were mixed with G- bacteria. These results are similar to those obtained by Aalbæk et al. (2010), where 23 of 55 samples were mixed cultures. Also, Saridomichelakis et al. (2007) reported that at least one of these organisms was present in 93 of 100 cases, whereas the coexistence of two or more was observed in 33 of 100 dogs. Contrary to our findings, other research results have shown that mixed infections occurred in more than half of otitis externa affected dogs, where the combination of *Staphylococcus intermedius* and *Malassezia pachydermatis* was most common (Oliveira et al. 2008; Petrov et al., 2013). Moreover, Kiss et al. (1996) stated that this phenomenon might be related to the capability of these two microorganisms to utilise each other's metabolic products, i.e., the

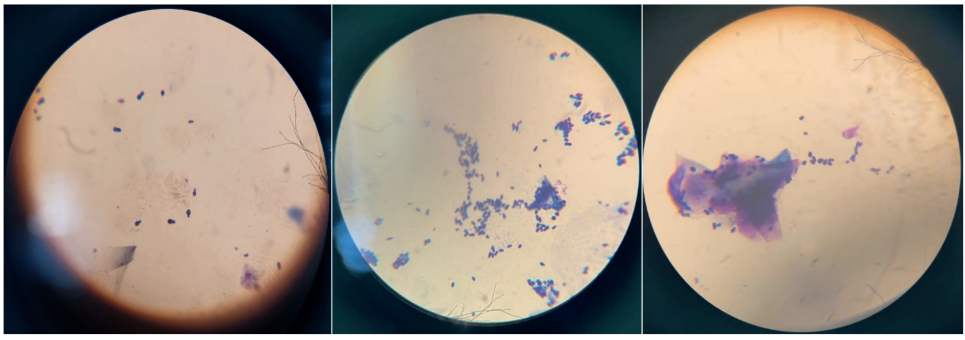

Figure 1. Microscopic morphology of *Malassezia pachydermatis*

proteolytic products of *Malassezia* or the nicotinic acid produced by staphylococci. The results presented here show that samples where only bacteria were isolated were present in 17 (28.33%) cases, of which 12 (20%) were with G+ and 5 (7.35%) with G- bacteria.

Perry et al. (2017) established that yeasts were associated with the majority of infections (82.0%), either as sole or mixed infections. In the present study, yeasts were isolated from 36 (52.94%) samples, though only solely yeast infection in 13 (19.12%) cases. Among the yeast pathogens, the most common was *Malassezia* yeast species, as the only type isolated. In a Hungarian study conducted on 210 isolates, it is determined that the most common isolate was the yeast *M. pachydermatis*, which was isolated in pure culture in 43.30% of cases and in association with different bacteria in 33.00% of cases (Kiss et al., 1997).

The type of infection and degree of infestation of *Malassezia pachydermatis* in dogs with diagnosed otitis externa are shown in Table 1. In cases where the yeast appeared independently, a first-degree infection was present in most cases 8/13 (61.54%), second degree in 3/13 (23.08%), while third-degree infection cases occurred the least with 2 of 13 cases (15.38%). These results show that the most dominant in independent otitis externa infection was a first-degree infestation. Regarding the comparison between mixed and independent types of infection, in most cases 15/23 (65.22%) the degree of infestation with yeast was highest when the infection was mixed with bacteria. The reason might be that mixed infection could provoke otitis externa infection to be more serious with more complicated clinical symptoms. Although most owners and veterinarians recognize ear infections, the ongoing

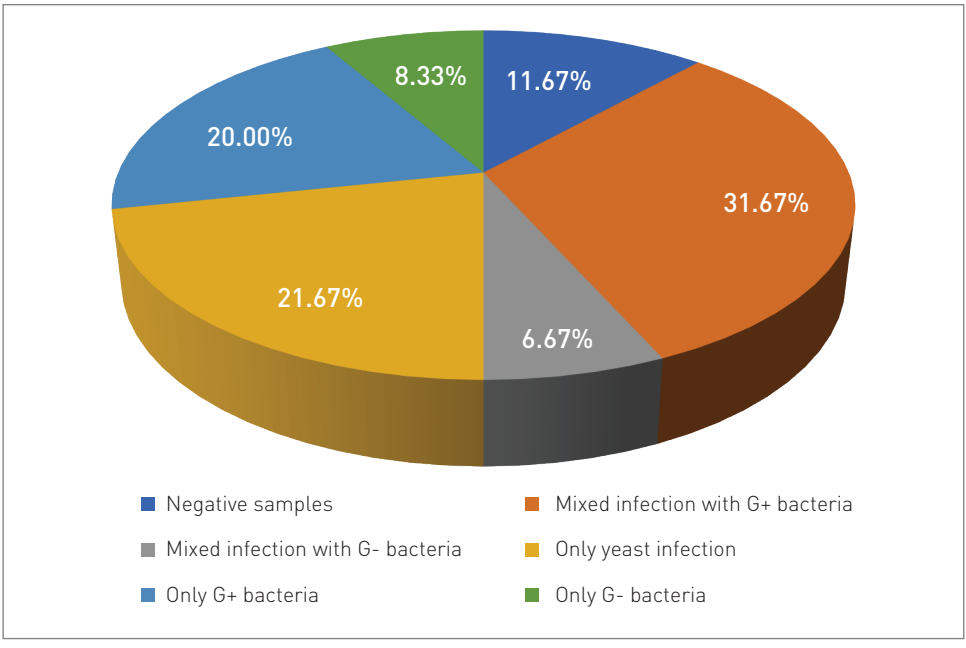

Figure 2. Prevalence of otitis externa causing pathogens in the collected samples

Table 1. Type of infection and degree of infestation of *Malassezia pachydermatis* in dogs with diagnosed otitis externa

Dog breed	Mixed infection with bacteria	Type of the yeast	Degree of infestation
PUG	YES	*M. pachydermatis*	+++
LR	YES	*M. pachydermatis*	+
PUG	YES	*M. pachydermatis*	+++
GSD	YES	*M. pachydermatis*	+++
SHIH	NO	*M. pachydermatis*	+
POM	NO	*M. pachydermatis*	+
MLT	YES	*M. pachydermatis*	+
MIXED	YES	*M. pachydermatis*	+++
AK	NO	*M. pachydermatis*	+
POODL	NO	*M. pachydermatis*	+
MIXED	YES	*M. pachydermatis*	+++
MLT	YES	*M. pachydermatis*	+++
PUG	YES	*M. pachydermatis*	+++
BT	YES	*M. pachydermatis*	+++
MIXED	NO	*M. pachydermatis*	+
DH	YES	*M. pachydermatis*	+
DOGO	YES	*M. pachydermatis*	+
SBT	YES	*M. pachydermatis*	+++
BF	YES	*M. pachydermatis*	+
LAGR	YES	*M. pachydermatis*	+++
APBT	YES	*M. pachydermatis*	++
MIXED	NO	*M. pachydermatis*	++
BGL	NO	*M. pachydermatis*	+
DH	NO	*M. pachydermatis*	++
PULI	YES	*M. pachydermatis*	+++
HWV	NO	*M. pachydermatis*	++
MLT	YES	*M. pachydermatis*	+
WT	YES	*M. pachydermatis*	+++
HWV	NO	*M. pachydermatis*	+
BOL	NO	*M. pachydermatis*	+++
WHWT	YES	*M. pachydermatis*	+++
MIXED	NO	*M. pachydermatis*	+++
MLT	NO	*M. pachydermatis*	+++
GR	YES	*M. pachydermatis*	+++
GSD	YES	*M. pachydermatis*	++
APBT	YES	*M. pachydermatis*	+++

*PUG-Pug, LR-Labrador Retriever, GSD-German Shepherd Dog, SHIH-Shih Tzu, POM-Pomeranian, MLT-Maltese, MIXED-Mixed Breed, AK-Akita, POODL-Poodle (Toy & Miniature), BT-Boston Terrier, DH-Dachshund (Miniature, Standard), DOGO-Dogo Argentino, SBT-Staffordshire Bull Terrier, BF-Bichon Frise, LAGR-Lagotto Romagnolo, APBT-American Pit Bull Terrier, BGL-Beagle, PULI-Puli, HWV-Hungarian Wire-Haired Vizsla, WT-Welsh Terrier, BOL-Bolognese, WHWT-West Highland White Terrier, GR-Golden Retriever

Table 2. Type of infection in dog breeds in relation to ear form

Dog breed	Type of the ear	Total number of samples	Mixed infection with bacteria	Only yeast infection
MLT	P	4	3	1
MIXED	P	4	2	2
PUG	P	3	3	-
GSD	E	2	2	-
DH	P	2	1	1
APBT	E	2	2	-
HWV	P	2	-	2
LR	P	1	1	-
GR	P	1	1	-
POM	E	1	-	1
MIXED	E	1	-	1
POODL	P	1	-	1
SHIH	P	1	-	1
AK	E	1	-	1
BT	E	1	1	-
DOGO	P	1	1	-
SBT	E	1	1	-
BF	P	1	1	-
LAGR	P	1	1	-
BGL	P	1	-	1
PULI	P	1	1	-
WT	E	1	1	-
BOL	P	1	-	1
WHWT	E	1	1	-

*PUG-Pug, LR-Labrador Retriever, GSD-German Shepherd Dog, SHIH-Shih Tzu, POM-Pomeranian, MLT-Maltese, MIXED-Mixed Breed, AK-Akita, POODL-Poodle (Toy & Miniature), BT-Boston Terrier, DH-Dachshund (Miniature, Standard), DOGO-Dogo Argentino, SBT-Staffordshire Bull Terrier, BF-Bichon Frise, LAGR-Lagotto Romagnolo, APBT-American Pit Bull Terrier, BGL-Beagle, PULI-Puli, HWV-Hungarian Wire-Haired Vizsla, WT-Welsh Terrier, BOL-Bolognese, WHWT-West Highland White Terrier, GR-Golden Retriever

*P-Pendulous ears, E-Erect ears

inflammation is often missed. This can lead to chronic infection or otitis media that is more difficult to treat and takes longer to resolve (Nuttall, 2016). Moreover, first-degree yeast infections were present in 6/23 (26.09%) cases and only 2/23 (8.70%) were present with second-degree yeast infections.

The relation between dog breeds, their ear forms and type of infection are shown in Table 2. Isolates from dogs with pendulous ears were present in 25 of 36 samples (69.44%), while 11/36 (30.56%) were isolated from erect eared dogs. In the pendulous group, yeast otitis externa occurred most often in Maltese 4/36 (11.11%), Mixed breed 4/36 (11.11%) and Pug 3/36 (8.33%), while in the erect ear group German Shepherd Dog and American Pit Bull Terrier, both

with 2 of 36 (5.55%) cases, had a larger number of otitis cases than other dog breeds. Moreover, this study also shows that the proportion of mixed and yeast infections was not significantly different among dog breeds in relation to ear form, and mixed infection were dominant in both groups. In pendulous ear breeds, mixed infection was present in more samples 15/25 (60%) than only yeast infection 10/25 (40%). The same is the case with erect ear breeds where mixed infection was present in almost all samples 8/11(72.73%) while the remaining 3/11 (27.27%) samples was with only yeast infection.

Yeast otitis externa has been recognized as a very common cause of dermatitis in dogs (Larsson et al., 1988; Bond et al., 1995), though this is not equally prevalent in different dog breeds (Perry et al., 2017). Factors that increase the risk of this disease include form of the ear canal and pinna, hair growth, stenotic ears, increased cerumen production, ear trauma and increase in humidity (Paterson, 2016; Bajwa, 2019). Some dogs may develop an allergic reaction to *Malassezia* spp., which can consequently lead to discomfort and pruritus (Bajwa, 2019) and, when it not correctly treated, the condition can turn into otitis media (Boone et al., 2021). Furthermore, *Malassezia* yeast and bacteria such as *Staphylococcus* and *Pseudomonas* are common perpetuating factors that do not initiate inflammation but lead to exacerbation of the inflammatory process and maintain ear disease even if the primary factor has been identified and corrected. Moreover, if infection travels to the tympanic bulla, the presence of this infection in the middle ear can also act as a perpetuating factor, leading to recurrent external ear infections. Perpetuating factors are often the main reason for treatment failure in dogs affected by recurrent otitis externa (Bajwa, 2019).

Keeping in mind the routine sensitivity and in terms of absence of standard methods suitable for the *Malassezia* genus, diagnostic testing in veterinary practice should preferably be based on cytological than on culture methods (Guillot and Bond, 2020). In addition, topical antimicrobial therapy is preferred to systemic treatment in the case of otitis externa, but frequently it is not successful because of the resistance of bacteria and yeast agents to conventional drugs (Ebani et al., 2023). Also, such an application is often not dosed correctly (Bernardo et al., 1998). Moreover, effective therapy, together with the correction of predisposing factors and concurrent diseases, is mandatory to prevent relapses (Guillot and Bond, 2020). That is the reason why the agreed reference methods to assess antifungal susceptibility of *M. pachydermatis* are required to assist veterinary practitioner in the management of chronic cases (Guillot and Bond, 2020).

In the prevention of otitis externa and its complications that may lead to chronic otitis, hearing loss, otitis media, and end-stage ear disease, there are few effective preventive measures. Otic examination of all patients presented for a physical examination can assist in early detection of mild and early cases of otitis. Moreover, when dogs are presented with early ear disease, thorough client education and detailed diagnostic workup, including frequent follow-up examinations, can help prevent development of complications (Bajwa, 2019).

Conclusion

In conclusion, this study aimed to determine the prevalence and degree of infestation of yeast in otitis externa in dogs.

The results showed that the only yeast species isolated was *Malassezia pachydermatis*. Isolation of higher levels of *Malassezia pachydermatis* appears to play a significant role in canine otitis externa, both in independent and mixed otitis externa infections. As bacteria become more and more resistant to antibiotics, which is currently a global problem, fungal infections should not be disregard and therefore further research is needed for *in vitro* testing of antifungal sensitivity of the isolated pathogens. The application of molecular typing methods will allow for recognition of more cases in the future in light of the One Health concept, since awareness of the potential for the transfer of *Malassezia* spp. to human patients is increased.

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Prevalencija i stupanj infestacije gljivica kod upale vanjskog ušnog kanala u pasa

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Kao perzistentan i rekurentan problem, zbog prisustva rezistencije patogenih uzročnika na konvencionalne lijekove upala vanjskog ušnog kanala predstavlja ozbiljan problem u veterinarskoj medicini. Upala vanjskog ušnog kanala u pasa može biti prouzročena bakterijama i gljivicama. Ova se dva uzročnika često mogu izolirati zajedno, u kombinaciji. Predmet je ovog istraživanja bio utvrđivanje prevalencije gljivica i stupnja infestacije izoliranih patogena kod upale vanjskog ušnog kanala u pasa u Veterinarskoj klinici za male životinje Departmana za veterinarsku medicinu u Novom Sadu, Srbija. Uzorci su uzeti od 60 pasa s kliničkim simptomima upale vanjskog ušnog kanala pomoću sterilnog brisa, a poslani su u Laboratorij na daljnje mikološko i bakteriološko ispitivanje. Da bi se izolirale bakterije i gljivice, uporabljene su konvencionalne metode u aerobnim uvjetima. Od ukupnog broja uzoraka, 53 (88,33 %) su bila pozitivna na prisustvo najmanje jednog od uzročnika upale vanjskog ušnog kanala. Gljivice su izolirane iz ukupno 36 (52,94 %) uzoraka. Istraživanje je pokazalo da je u slučaju usporedbe između miješanih i samostalnih tipova infekcije u najvećem broju slučajeva 15/23 (65,22 %) stupanj infestacije gljivicama bio najviši kada je infekcija bila pomiješana s bakterijama. U odnosu na pasmine pasa, njihove tipove ušiju i vrstu infekcije, utvrđeno je da je više uzoraka s gljivicama bilo kao samostalna ili miješana infekcija izolirano u pasa s visećim ušima. Potvrđeno je da je *Malassezia pachydermatis* najčešći gljivični patogen upale vanjskog ušnog kanala pasa, u usporedbi s drugim vrstama ovoga roda. Kod mješovite infekcije javlja se u većem stupnju infestacije nego kod samostalne infekcije. Pasmine pasa s visećim ušima su sklonije nastanku upale vanjskog ušnog kanala prouzročene gljivicama nego pasmine pasa s uspravnim ušima.

Ključne riječi: *upala vanjskog ušnog kanala*, *Malassezia* spp., *pasmine pasa*