

Original Article

Therapeutic trials to evaluate the efficacy of topical Clotrimazole and Nystatin on clinical cases of otitis externa in dogs caused by *Malassezia pachydermatis* in district Lahore and its suburbs in Pakistan

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Malassezia pachydermatis (*M. pachydermatis*) otitis is an important infectious disease of dogs throughout the world. In the present study, the presence, diagnosis, clinical signs and chemotherapy of *M. pachydermatis* were studied in clinical otitic dogs of Lahore and its suburbs. During two-year study period, a total of 200 ear cerumen samples from otitic dogs were examined microscopically. Of these, 46 (23%) were found positive for *M. pachydermatis*. The difference in the prevalence of infection between the pendulous ear and erected ear dogs as well as sex predilection was found non-significant ($P>0.05$). However, a significantly ($P<0.05$) higher prevalence (86.90%) was recorded in dogs of more than one years of age group. The animals determined positive for the *M. pachydermatis* were divided into two groups (A and B) and treated with Clotrimazole and Nystatin, respectively. Efficacy of both the antifungals was evaluated on the basis of reversal of clinical signs scoring and cytological examinations at 7, 14, 21-day post treatment. The overall efficacy of Clotrimazole and Nystatin was 73% and 68%, respectively. Clotrimazole showed better results as compared to Nystatin in accomplishing cure rate from mycological infection. It was concluded that *M. pachydermatis* is a significant cause of otitis in dogs wherein Clotrimazole proves to be a more effective drug in eliminating the infection in the affected Dogs.

Key words: otitis, malassezia pachydermatis, clotrimazole, nystatin, dogs.

Introduction

The inflammation of external ear canal (otitis externa) of dogs is frequently seen in veterinary practice and

thought to affect 5 to 20% of the total dog population, and the condition is difficult to cure [1]. There are mainly three types of causes of otitis in dogs. One of them is the predisposing factor which includes ear anatomy, systemic disease and inappropriate treatment. The second cause is the primary factor that includes ectoparasites, foreign bodies, abnormal growth like tumor, allergies, autoimmune disease, and third cause is the secondary factor which plays its role in provoking the inflammation of ear along with other factors such as microbes like bacteria, fungus, yeast and histopathological changes [2]. Yeasts are considered as one of the main causes of ear canal infection wherein mainly four genera of Yeast are associated with otitis in dogs i.e., *Malassezia*, *Candida*, *Saccharomyces* and *Rhodotorula*. *Malassezia* species are the part of normal biota of human and animal skin and mucous membranes [3, 4]. Almost 50% of healthy dogs are carriers of *Malassezia* which can be found in the external ear canal [5]. However, it may become an opportunistic pathogen due to alterations of the ear canal microclimate [6]. Presently, 13 species of genus *Malassezia* have been discovered, but *M. pachydermatis* has been believed to be one of the several etiologies in developing otitis externa in dogs [7] and responsible for 30~80% cases of otitis externa in dogs [8]. Mostly, the chronic *M. pachydermatis* otitis in dogs is accompanied by redness, thickening of the medial surface of the ear due to excessive itching and rubbing as well as abundant foul smelling brownish discharge of thicker cerumen from the ear canal [9]. It is difficult to diagnose and treat otitis externa caused by *M. pachydermatis* based on clinical signs alone because they are quite variable and easily confused with other causes of otitis externa as predispos-

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ing and primary factors. Treatment of ear canal infection in canines is usually topical rather than systemic therapy [1]. Many topical antifungal drugs like ketoconazole, miconazole, itraconazole, nystatin, terbinafine, clotrimazole, and pimaricin etc. are commonly used worldwide [10]. But still it is challenging to choose most efficacious drugs among these. Keeping in view the importance of otitis externa due to *M. pachydermatis* in dogs and degree of damage it causes, the present study was designed to find out the presence of *M. pachydermatis* in dogs, its clinical symptoms and comparative efficacy of two most commonly available medicines. This study could help to inform field veterinary practitioners regarding the selection of most appropriate treatment of otitis externa.

Materials and Methods

The dogs selected for the study were handled according to animal welfare regulations of Pet center, University of Veterinary and Animal Sciences, Lahore, Pakistan. The present study was conducted during October 2012 to June 2014 at the University of Veterinary and Animal sciences, Lahore, Pakistan. A total of 200 dogs suffering from otitis externa, of different age, sex and breeds were randomly selected from different private and government pet clinics. Temperature and humidity during this study period were 28°C to 40°C and 60%, respectively, in the region under study. The cerumen samples were taken from the dogs showing the following peculiar signs of otitis externa, dark pungent discharge from the ear canal, swollen and painful ear canal mucosa, intense pruritus, scratching and rubbing of the ears. After the clinical examination, a sterile cotton-tipped applicator was employed to acquire specimens of auricular exudates from the external ear canal. The applicator was placed in the plastic storage tube. The samples were labeled for identification and transferred immediately to Medicine Laboratory, UVAS, Lahore for cytological examination and yeast culture and isolation. The applicator was rolled on a clean glass slide to make the direct smear and then stained with Gram staining for microscopic examination. The morphological identification criteria used to identify *Malassezia* spp. were the cell shape, size, and the budding pattern [11, 12]. The population of yeast for the severity of disease was calculated from consecutive microscopic fields at 40× magnification by using a semi-quantitative scale [1].

To isolate the yeast, samples were inoculated onto the plates of Mycobiotic agar containing Sabouraud dextrose agar without lipid enrichment, chloramphenicol (0.05 g/L) and cycloheximide (0.5 g/L) and incubated at 32°C for 7 days [13]. Identification of *Malassezia* yeast was done at the species level by catalase test and tryptophan assimilation test [14].

The animals positive for the *M. pachydermatis* were divided into two groups; group A and B and consequently treated with Clotrimazole and Nystatin, respectively. In group A, Clotrimazole (Dermosporin drops 1%, Nabiqasim Industries Pvt. Ltd Pakistan) was applied topically for 3 weeks, and group B was treated with Nystatin (Monilstat drops 100,000 I.U/ml, Ellahi Pharma, Pvt. Ltd. Pakistan) topically 4 drops < 15 kg and 8 drops ≥ 15 kg body weight, thrice a day for 3 weeks. Samples of both groups were collected at 0, 7, 14 and 21 days [15]. Efficacy of both antifungals was determined on the basis of reversal of clinical signs such as scoring and cytological examinations at days 7, 14 and 21 post-treatment.

Data on gender, age, breed and therapeutic trials were analyzed by completely randomized design through analysis of variance technique. The means were compared for significance according to the least significant differences and therapeutic trials data were also analyzed by one-way ANOVA using Statistical Package for Social Sciences (SPSS); $P < 0.05$ was considered significant.

Results

Out of the total 200 animals, 23% (46) were found positive for *M. pachydermatis* infection, whereas 154 dogs were negative. The highest prevalence (86.95%) of *M. pachydermatis* was observed in more than one year of age, and 13.04% was observed in less than one year of age. The prevalence of *M. pachydermatis* was higher in male than female dogs but statistically, the association between the two sexes was non-significantly different ($P > 0.05$). The morphological identification criteria for the *Malassezia* spp. were the cell shape, size, and the budding pattern at 40× magnification by using a semi-quantitative scale (Fig. 1). The prevalence of *Malassezia*

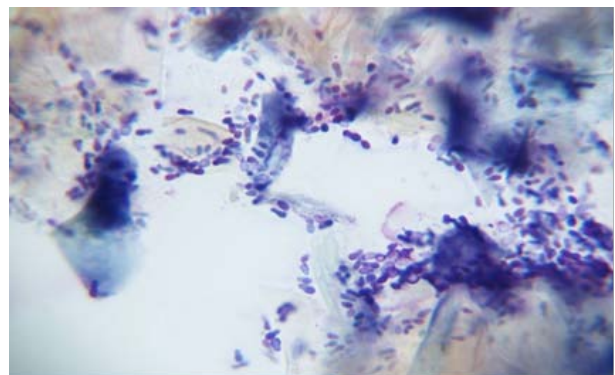


Fig. 1. Pictorial presentation of *Malassezia* yeast during the cytological examination on a clean glass slide (direct smear) from an otitic swab with Gram staining. The morphological identification criteria for the *Malassezia* yeast were the cell shape, size, and the budding pattern at 40× magnification by using a semi-quantitative scale.

otitis was significantly higher ($P<0.05$) in dogs possessing pendulous ears such as St. Bernard and Cocker spaniel than the ones having erected ears like German shepherd and Russian breeds groups (Table 1). The dogs of group A were treated with Clotrimazole, and group B were treated with Nystatin. Efficacy of drugs was measured on the basis of reversal of clinical signs and cytology examination. The efficacy of Clotrimazole and Nystatin was recorded as 73% and 68%, respectively at the completion of therapeutic trial. There was no significance difference ($P>0.05$)

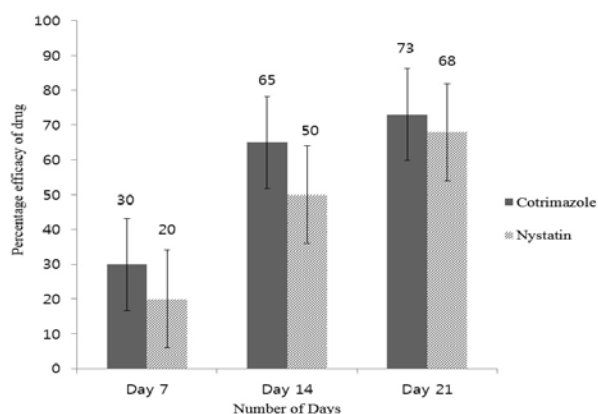


Fig. 2. Percentage efficacy of Clotrimazole and Nystatin against *M. Pachyderma* in otitic dogs at different days. The dogs of group A were treated with Clotrimazole, and group B were treated with Nystatin. Efficacy of drugs was measured on the basis of reversal of clinical signs and cytology. There was no significance difference ($P<0.05$) between the efficacy of the two drugs on the completion of the trial.

between two treatment groups on the completion of the trial (Fig. 2).

Discussion

In the present study, 46 (23%) cases of otitis externa were found positive for *M. pachydermatis* infection. Similar findings were reported by the previous studies [16-18] showing 24.6% prevalence in dogs. Another study described comparable prevalence of *M. pachydermatis* in cats i.e. 23.1% in Spain. This resembling level of infection in dogs and cats might be due to the matching climatic conditions in the areas of study.

In the present study, a non-significant difference ($P>0.05$) was observed between the pendulous ear and erected ear dogs. The highest percentage of *Malassezia* otitis externa was recorded in drooping ear dogs (56.52%) than in dogs with erected ears and medium hair on the ears (43.47%). Our study was strengthened by the results of [19] which also pointed out identical findings in pendulous ear (55.2%) and in erect ear breed (53.6%) groups. They also concluded that pendulous ears entrap more humidity within the ear canal along with ceruminous discharges which become infected secondarily and predispose the ears to *Malassezia* organisms' replication. Likewise, Conkova and coworkers [20] found higher prevalence of *M. pachydermatis* in samples from long-haired (51.5%) and short-haired (45.9%) dogs compared to smooth haired (21.4%) dogs. The higher prevalence i.e. 86.9% in the current study was observed in age

Table 1. Breeds, ages and sex of the clinically otitic dogs sampled in the study

	Breeds			Age				Gender				
	Breeds Names	Number of examined animal	Number of positive animal	%	< 1year	%	> 1year	%	Male	%	Female	%
Pendulous Ear Breeds	Cocker spaniel	22	08	36.3	01	4.5	07	31.8	03	13.6	05	22.7
	Gaddi	6	02	33.3	-	0.0	02	33.3	02	33.3	-	00.0
	Labrador	48	10	20.8	02	4.1	08	16.6	06	12.5	04	8.3
	Rottweir	16	03	18.7	01	6.2	02	12.5	02	12.5	01	6.2
	St. Bernard	02	01	50	-	0.0	01	50	-	0.0	01	50
	Poodle	08	02	25	-	0.0	02	25	02	25	-	00.0
Erected Ear Breeds	German shepherd	68	13	19.1	01	1.4	12	17.6	07	10.2	06	8.8
	Non-script	18	04	22.2	01	5.5	03	5.55	03	16.6	01	5.5
	Russian	12	03	25	-	0.0	03	25	01	8.33	02	16.6
Total		200	46		06		40		26		20	

group of more than one years, which is very similar to the results of [11-13] who observed the peak incidence of otitis externa in dogs of the same age group, while a lower incidence was seen in animals less than one year old (13.04%).

In the present investigation, higher efficacy (73%) was recorded for Clotrimazole which showed a significant ($P < 0.05$) reduction in yeast population and reversal of clinical signs of otitis, whereas Nystatin showed an efficacy of 68% as compared to Clotrimazole. This is in agreement with the study [2] which observed the efficacy of Clotrimazole to be 58.3% on day 14. In terms of availability, both drugs are readily available in the market with a number of products by different companies at different economical costs. Clotrimazole is a costly product as compared to Nystatin, but it showed better curative performance than Nystatin from mycological infection in Dogs. Moreover, it is concluded that *Malassezia* otitis is predominantly present in young age and pendulous ear dog breeds against which Clotrimazole is the most effective drug of the two.

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References

- Rigaut D, Sanquer A, Maynard L, Reme CA. Efficacy of a topical ear formulation with a pump delivery system for the treatment of infectious otitis externa in dogs: A randomized controlled trial. *Int J Appl Res Vet Med* 2009;9:15–28.
- Rougier S, Borell D, Pheulpin S, Woehrl F, Boisrame B. A comparative study of two antimicrobial/anti-inflammatory formulations in the treatment of canine otitis externa. *Vet Dermatol* 2005;16:299–307.
- Ashbee R, Evans GV. Immunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev* 2002;15:21–57.
- Crespo MJ, Abarca ML, Cabanes FJ. Isolation of *Malassezia furfur* from a cat. *J Clin Microbiol* 1999;37:1573–1574.
- Guillot J, Bond R. *Malassezia pachydermatis*: a review. *Med Mycol* 1999; 37 :295–306
- Scott DW, Miller WH, Griffin CE. Muller and Kirk's small animal dermatology. Fungal skin diseases. 6th ed. Philadelphia, PA: W.B. Saunders; 2001. P.336-422.(Book chapter Fomat)
- Eidi S, Khosravi AR, Jamshidi S. A comparison of different kinds of *Malassezia* species in healthy dogs and dogs with otitis externa and skin lesions. *Turk J Vet Anim Sci* 2011;35:345–350.
- Nowakiewicz A, Ziolkowska G. Comparative analysis of protein profiles of *Malassezia pachydermatis* strains isolated from healthy dogs and dogs with otitis externa. *Bull Vet Inst Pulawy* 2013;57:41–46.
- Bernardo FM, Martins HM, Martins ML. A survey of mycotic otitis externa of dogs in Lisbon. *Rev Iberoam Micol* 1998;15:163–165.
- Uchida Y, Mizutani M, Kubo T, Nakade T, Otomo K. Otitis externa induced with *Malassezia pachydermatis* in dogs and the efficacy of pimaricin. *J Vet Med Sci* 1992;54:611–614.
- Cafarchia C, Gallo S, Romito D, Capelli G, Chermette R, Guillot J, Otranto D. Frequency, body distribution, and population size of *Malassezia* species in healthy dogs and in dogs with localized cutaneous lesions. *J Vet Diagn Invest* 2005;17:316–322.
- Cafarchia C, Gallo S, Capelli G, Otranto D. Occurrence and population size of *Malassezia* spp. in the external ear canal of dogs and cats both healthy and with otitis. *Mycopathologia* 2005;160:143–149.
- Cafarchia C, Figueredo LA, Iatta R, Montagna MT, Otranto D. In vitro antifungal susceptibility of *Malassezia pachydermatis* from dogs with and without skin lesions. *Vet Microbiol* 2012;155:395–398.
- Prado MR, Brito EHS, Girao MD, Monteiro AJ, Sidirim JJC, Rocha MFG. Higher incidence of *Malassezia pachydermatis* in the eyes of dogs with corneal ulcer than in healthy dogs. *Vet Microbiol* 2004;100:115–120.
- Cole LK, Kwochka KW, Kowalski JJ, Hillier A, Hoshaw-Woodard SL. Evaluation of an ear cleanser for the treatment of infectious otitis externa in dogs. *Vet Ther* 2003;4:12–23.
- Crespo MJ, Abarca ML, Cabanes FJ. Otitis externa associated with *Malassezia sympodialis* in two cats. *J Clin Microbiol* 2000;38:1263–1266.
- Crespo MJ, Abarca ML, Cabanes FJ. Atypical lipid-dependent *Malassezia* species isolated from dogs with otitis externa. *J Clin Microbiol* 2000;38:2383–2385.
- Crespo MJ, Abarca ML, Cabanes FJ. Occurrence of *Malassezia* spp. in the external ear canals of dogs and cats with and without otitis externa. *Med Mycol* 2002;40:115–121.
- Masuda A, Sukegawa T, Mizumoto N, Tani H, Miyamoto T, Sasai K, Baba E. Study of lipid in the ear canal in canine otitis externa with *Malassezia pachydermatis*. *J Vet Med Sci* 2000;62:1177–1182.
- Conkova E, Sesztakova E, Palenik L, Smrco P, Bilek J. Prevalence of *Malassezia pachydermatis* in dogs with suspected *Malassezia* dermatitis or otitis in Slovakia. *Acta Vet Brno* 2011;80:249–254.