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STUDY OF CLINICAL AND INFECTION RATE OF *MALASSEZIA PACHYDERMATIS* IN PET DOGS WITH AND WITHOUT OTITIS EXTERNA IN DIYALA GOVERNORATE

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ABSTRACT

Malassezia pachydermatis is commensal yeast of the skin and external ear canal of dogs that may become an opportunistic pathogen under some conditions. This study was conducted from beginning of October 2015 to last of April 2016 in dogs of K9 police and housed dogs in different area of Diyala Governorate. Clinically, alopecia, crusts or scales, erythema, dermatitis, erosion, hyperkeratinization and lichenification of the ear epithelium, pruritus and drooling of ear was the most clinical signs of infection. The diagnosis was based on the presence of skin lesions compatible with these entities, in cooperation with the cytological examination. Significantly high infection rate recorded in diseased dogs 90.2% than healthy dogs 70.5%, also right and left ear 96.2 %, 88.9 % was the most anatomical site of lesion in diseased dogs than scalps 60.0% and trunk 71.4%. Epidemiological data showed that German shepherd dogs 97.5 % was the more breed susceptible to infection than crossbreeds 77.8%. The infection rate of higher than normal amounts did not correlate significantly at $P < 0.05$ with sex, and age of pets dogs. Whereas, relation of *M. pachydermatis* infection with months of year showed high infection in January 94.7% and February 94.4% than December 84.6%.

Keywords: Pets Dogs; *Malassezia pachydermatis*; Otitis externa

Introduction

Malassezia pachydermatis is yeast that is normally found in low numbers in the external ear canals, in perioral areas, in perianal regions, and in moist skin folds. Also, it is a non-lipophilic, non-mycelial, unipolar budding yeast characterized by a thick cell wall (Scott *et al.*, 2001; Batra *et al.*, 2005; Cabañes *et al.*, 2007; Mircean *et al.*, 2010). Otitis externa is inflammation of the skin and adnexal structures of the ear canal. This condition is one of the most common and frustrating problems encountered in small animal practice. *Malassezia pachydermatis* is considered to be a permanent agent of otitis externa which exacerbates the inflammatory process and can maintain the disease after eliminating the underlying factor. They can induce permanent pathologic changes to the ear canal and are the main reason for treatment failure in otitis externa (Noxon 2005; Sugita *et al.*, 2010; Morris, 2014). It is a commensal on animal skin, but may become pathogenic under the influence of predisposing factors (Cafarchia *et al.*, 2005 & 2012; Miceli *et al.*, 2011) leading to cases of otitis externa and different clinical forms of dermatitis in domestic animals (Batra *et al.*, 2005 Cafarchia *et al.*, 2005 and Bond *et al.*, 2010).

The clinical disease occurs when there is an excessive multiplication of *M. pachydermatis* due to alterations in the host defense mechanism. Itchiness is a major and consistent sign. (Ashbee 2007; Nardoni *et al.*, 2005). Face rubbing, head-shaking, foot-licking and chewing, and scooting may occur in dogs. The affected areas may be localized (ears,

around the anus, muzzle, around the eyes, feet) or generalized (Emily *et al.*, 2011). *Malassezia dermatitis* can occur in dogs of any sex, age and breed, with some breeds appearing to be predisposed (Scott *et al.*, 2001 Mircean *et al.*, 2010). *M. dermatitis* is one of most common skin diseases in dogs, with severe infections usually requiring prolonged treatment and/or high doses of antifungal agents (Bond *et al.*, 2010; Sugita *et al.*, 2010.) Including ketoconazole and itraconazole (Bond *et al.*, 2010). The aim of study was to investigation infection rate of *Malassezia pachydermatis* in dogs with and without of external otitis.

Material and Methods

Dogs and sampling procedures

From beginning of October 2015 to last of May 2016, a total of 200 samples were collected from the ear canal and anatomical sites of 100 different breed dogs (80 samples from 40 German shepherd dogs and 120 samples from 60 different breed of housed dogs) were clinically examined for *Malassezia pachydermatis* and grouped as follows:

- Healthy dogs: 48 privately owned dogs were in good general health with no history of skin or ear diseases. Dogs in this group were aged between 4 month and 6 years: were females and males.
- Diseased dogs: 52 privately owned dogs with otitis externa (n = 28) or skin lesions (n = 24) localized on groin, back and chest anatomical site were included in this study. Dogs in this group were aged between 6

month and 4-5 years: were females and males All the dogs included in the study collected from outpatient admitted to the Faculty of Veterinary Medicine, Veterinary private clinics of different region of Diyala governorate. Samples were collected from 5 anatomical sites (scalp, back, trunk, groin and external ear canal) using sterile cotton swabs moistened with sterile saline solution (0.9% NaCl) for the external ear canal, and scraping with a scalpel for other body sites.

Cytological examination and mycological culture

Malassezia pachydermatis yeasts was evaluated by using sterile swabs, rubbing the anterior part of the ear canal and preparer smear then air-drying and fixed with methyl alcohol for 2-3 minute then staining by Giemsa stain for 30 minute later washing the smear under tape water and drying, which was analyzed under a microscope (1000X) in search for cells with morphology compatible to *M. pachydermatis*. Direct examination was done by counting the cells which were similar to the yeast in the microscopic field; six fields were observed.

The yeast cell/ field absence was considered negative (-), and the following scores were considered positive:

- a) (+) up to five cells/field
- b) (++) , six to ten cells/field

c) (+++) more than ten cells/microscopic field.

All samples (ear swabs and skin scrabing) were cultivated in Sabouraud dextrose agar with chloramphenicol (0.4 g/L) and cycloheximide (0.5 g/L) that kept at 37 °C for up to seven days; their macroscopic characteristics were observed daily.

Statistical Analyses: Chi-square test was performed using SPSS software (version 12.0) and differences were considered significant at $p < 0.05$.

Results

Clinical examination

The clinical examination of infected dogs show alopecia, crust or scaly, erythema, dermatitis, erosion, hyperkeratinization and lichenification of the ear epithelium (Fig. 1: A,B, C). Increase of cerumenous and waxy material in ear with scaly and drooling of ear in some cases (Fig. 2:A, B,C). On the other hand no lesion of skin scaly and other previously signs showed in healthy dogs (Fig. 2: D). The most common clinical signs of *M.pachydermatis* in diseased dogs included (28 dogs with Otitis externa and 24 dogs with Skin lesion(*Malassezia* dermatitis) were listed in (Fig. 3).



Fig. 1: A,B,C : A, B: A German shepherd dog with scales , crust, dermatitis, ulcer and loss of hair on different part of body (shoulder ,back, groin, leg). C: Otitis externa, scaly and alopecia of puppy infected by *Malassezia pachydermatis*.

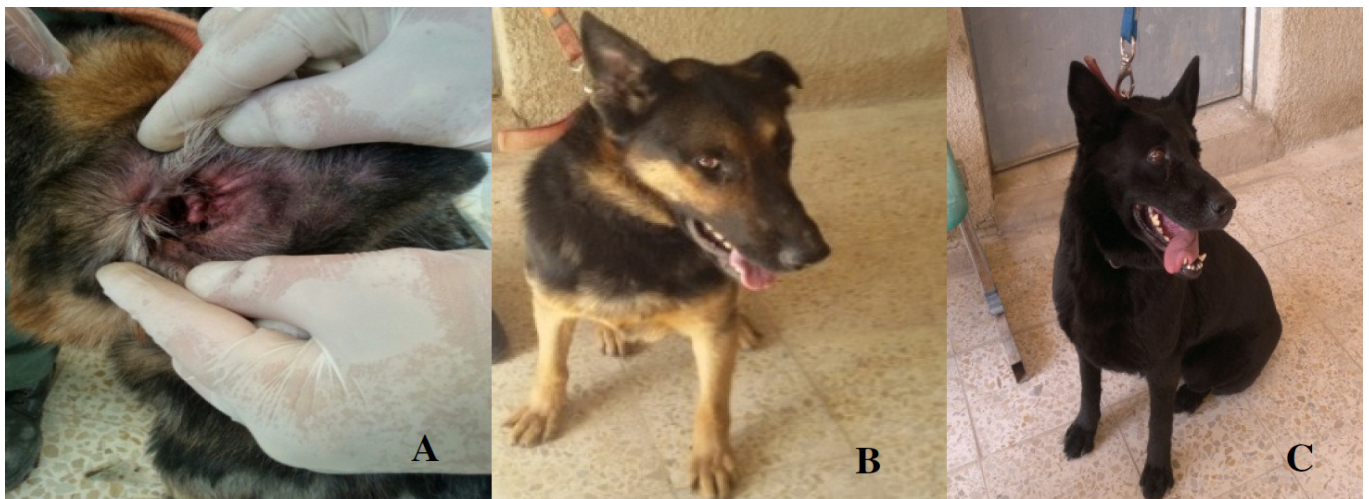


Fig. 2: A,B,C : A: German shepherd dog passing otitis externa ,excessive of cerumenous and black dried blood mixed with waxy material with erythema and crust infected by *Malassezia pachydermatis* B: German shepherd dog with otitis externa and drooling of left ear. C : K.9 Police healthy dog without otitis and skin lesion.

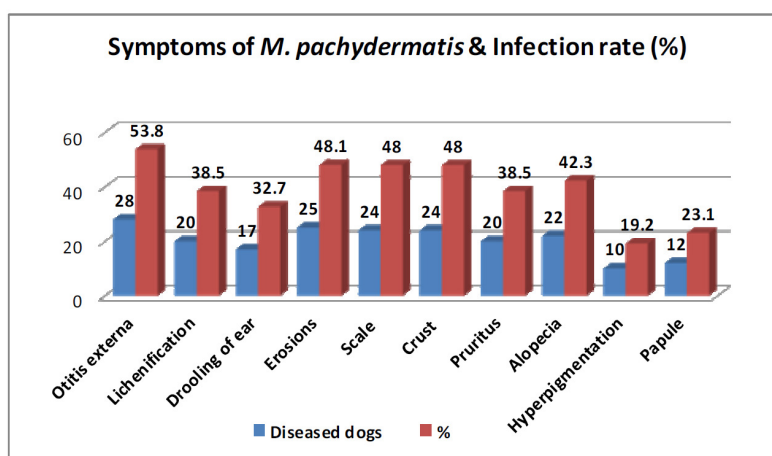


Fig. 3: The most common clinical signs of *M. pachydermatis* in diseased dogs.

Epidemiological data:

1. Infection rate *Malassezia pachydermatis*

The infection rate of *Malassezia pachydermatis* was high in 90.2% diseased dogs than 70.5% healthy dogs significantly at $p < 0.05$ (Table, 1).

Table 1 : Infection rate of *Malassezia pachydermatis* in healthy and diseased dogs.

Animals	N.D.E.	N.S.	N.P.	Infection rate (%)	P. value	X ² value
Diseased dogs	52	112	101	90.2%	0.0004	12.72 S.D P<0.05
Healthy dogs	48	88	62	70.5%		
Total	100	200	163	83.3%		

N.E.D= Number of dogs examined; N.S = Number of samples; N.P= Number of positive samples; P.V= Probability value, X² value= Chi-square, SD= Significant difference at P<0.05.

As for the location of the lesion the study show high infection rate in right ear than left ear in diseased dogs than healthy dogs. Moreover, the anatomical site of lesion represented by back and groin was recorded high infection rate than scalp of diseased dogs as in (Table, 2).

Table 2 : Anatomical site of samples collection and infection rate of *Malassezia pachydermatis* in healthy and diseased dogs.

Anatomical site of sample	N.S.E.		N.P.		(%)		P. value	X ² value
	D.D	H.D.	D.D	H.D	D.D	H.D		
	52	48	32	28	88.9 %	70.0 %		
Left ear	36	40	32	28	88.9 %	70.0 %	0.0007	38.85 S.D P<0.05
Right ear	52	48	50	34	96.2 %	70.8%		
Scalp	5	0	3	0	60.0%	0		
Back	4	0	4	0	100%	0		
Trunk	7	0	5	0	71.4%	0		
Groin	8	0	7	0	87.5%	0		
Total	112	88	101	62	90.2%	70.5%		
	200		163		81.5%			

D.D: Diseased dogs; H.D: Healthy dogs; N.E.D= Number of dogs examined; N.S = Number of samples; N.P= Number of positive samples ; P.V= Probability value , X² value= Chi-square ,SD= Significant difference at P<0.05.

2. Relation of *M. pachydermatis* infection with breed of dogs:

High infection rate of *M. pachydermatis* recorded in in D.D. GSD 97.5 % and H.D 83.3 % than D.D. C.B.D 77.8% and H.D CBD with no significant difference at P<0.05 (Table,3).

Table 3: Relation of *M. pachydermatis* infection with breed of dogs.

Breed of Dogs	Diseased dogs D.D		Healthy dogs H.D		(%)		P. value	X ² value
	N.S.E	N.P	N.S.E	N.P	D.D	H.D		
	40	39	24	20	97.5	83.3		
German shepherd dogs (G.S.D)	40	39	24	20	97.5	83.3	0.679	9.273 N.S.D P<0.05
Greyhound (Hunting dogs)	14	12	10	6	87.5	60.0		
Belgian Shepherd	20	18	18	14	90.0	77.8		
Siberian Husky	20	18	16	12	90.0	75.0		
Crossbreed dogs (CBD)	18	14	20	10	77.8	50.0		
Total	112	101	88	62	90.2%	70.5%		

N.E= Number of sample examined ; N.P= Number of positive samples ; P.V= Probability value , X² value= Chi-square ,NSD= No Significant difference at P<0.05.

3. Relation of *M. pachydermatis* infection with age of dogs

The differences in frequency of the *M. pachydermatis* infection in relation to age and sex of the dogs were not statistically significant. Positive results for *Malassezia pachydermatis* were more prevalent in adult dogs (1 to 3 years old and 3to 8 years and lower in 3 to 12 month old) as in table (5).

Table 4: Relation of *M. pachydermatis* infection with ages of dogs.

Age of dogs	D.D		H.D		(%)		P. value	X ² value
	N.S.E.	N.P	N.S.E	N.P	D.D	H.D		
3-12 months	48	45	34	28	93.8	83.3	0.6629	4.102 N.S.D P<0.05
1-3 years	30	26	24	14	86.7	60.0		
3-8 years	34	30	30	20	88.2	77.8		
Total	112	101	88	62	90.2%	70.5%		

N.E= Number of sample examined; N.P= Number of positive samples; P.V= Probability value, X² value= Chi-square, NSD= No Significant difference at P<0.05.

4. Relation of *M. pachydermatis* infection with sex of dogs

In addition to what was mentioned, the relationship of sex with the infection rate showed a higher infection in diseased and healthy female dogs than male dogs (Table, 5).

Table 5: Relation of *M. pachydermatis* infection with sex of dogs.

Sex of dogs	D.D		H.D		(%)		P. value	X ² value
	N.S.E.	N.P	N.S.E	N.P	D.D	H.D		
Male	64	57	50	32	89.1	64.0	0.469	2.532 N.S.D P<0.05
Female	48	44	38	30	91.7	78.9		
Total	112	101	88	62	90.2%	70.5%		

N.E= Number of sample examined; N.P= Number of positive samples; P.V= Probability value, X² value= Chi-square, NSD= No Significant difference at P<0.05.

5. Relation of *M. pachydermatis* infection with Months of year of dogs

Correlation of months of year with Malasseziasis showed higher percentage of infection January and February (94.7 & 94.4 %) in diseased dogs and healthy dogs (80.0 % & 76.9%) Whereas, lower infection in November (84.6%) in D.D and (58.3%) in H.D. as in (Figure, 4).

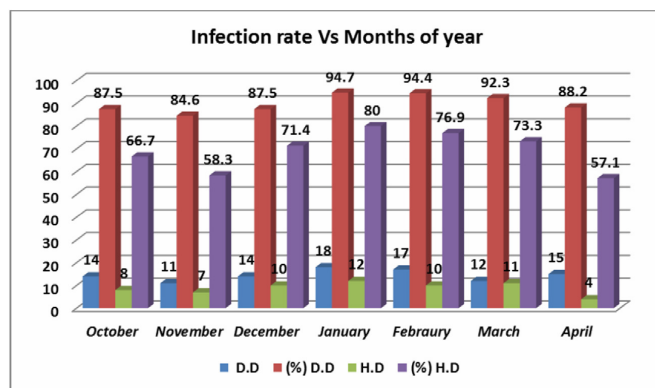


Fig. 4: Relation of *M. pachydermatis* infection with Months of year of dogs.

Laboratory examination:

Direct microscopic examination and Macro- Micro morphological cultural

Direct microscopic examination of ear swabs and scrabing staining by Geimsa stain show positive result to *Malassezia pachydermatis* when examined under the microscope at 100x reveal large number organisms (in purple color look yeast resembling a footprint or a peanut) as in (Fig.3).

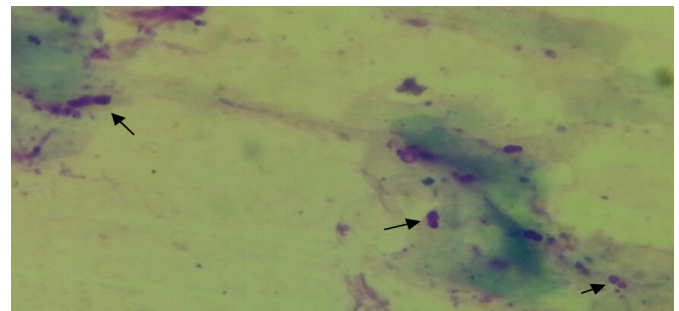


Fig. 3 : *Malassezia pachydermatis* staining by Geimsa stain (purple color, yeast resembling a footprint or a peanut) (+++) more than ten cells/microscopic field at (1000X).

On the other hand, the characteristic features of *Malassezia pachydermatis* colony was round, convex and creamy to yellow color develop on Sabouraud's dextrose agar as in figure (4:A,B).

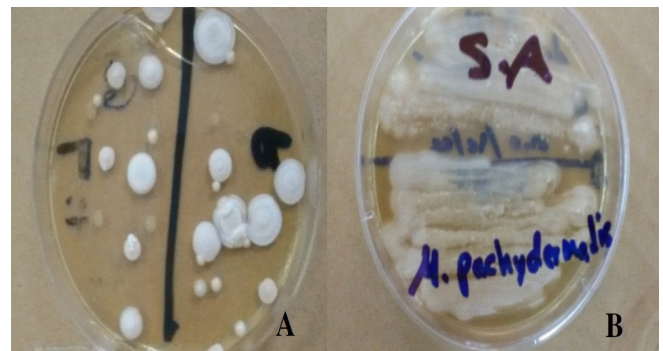


Fig.(4: A,B) A: Show the characteristic features of *Malassezia pachydermatis* colony rounded shape after cultivated for 7 days at 37 C⁰ .B Subculture and streaking spread of *M. pachydermatis* on Sabouraud's dextrose agar at 37 C⁰.

Whereas, microscopic morphology of colony after culture show odidi of *M. pachydermatis* as in (Fig. 5).

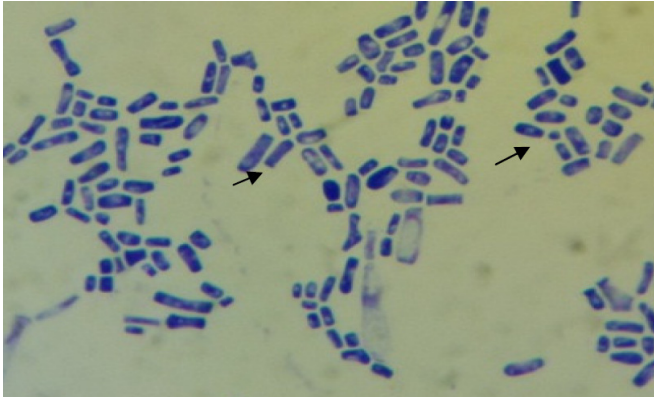


Fig. 5 : Microscopic morphology of odidi of *M. pachydermatis* staining with lactophenol cotton blue stain at (1000X).

Discussion

Yeast infection in dogs, especially *Malassezia pachydermatis*, is a natural resident of the skin and is an opportunistic pathogen (Bond, 2010; Hedayati *et al.*, 2010).

Carriage of *M. pachydermatis* has been extensively studied in dogs because of its importance as an obvious dermatologist in these animals. Clinically diseased dogs with *Malassezia* dermatitis were represented by alopecia, scaling and crusting pruritis and hyperpigmentation agreed with (Scott *et al.*, 2001; Mircean *et al.*, 2010; Minnat *et al.*, 2015). While dogs with otitis externa represented by lichenification and drooling of ear agree with (Minnat *et al.*, 2015).

According to (Table, 2) the present research show there was a significant difference between the positive culture results and the various anatomical sites of lesions in diseased and healthy dogs. This result in part agrees with (Gupta *et al.*, 2001 & 2004) those suggested that different *Malassezia* species tend to be found on different anatomical sites in both normal and diseased skin.

The epidemiological interpretation of our results has shown that high *M. pachydermatis* percentage (90.2%) in canine external otitis cases was similar to those previously found by (Nascente *et al.*, 2004). Although most breeds known to be susceptible to this infection Mircean *et al.* (2010), we recovered that the German shepherd and Belgian dogs were most susceptible than crossbreed dogs to infection.

In addition, our data showed the density of *Malassezia pachydermatis* cells on the lesions of diseased dogs in the age group of 3-12 months is significantly higher in comparison with age groups 3-8 years

Sex predisposed factor has not been reported significantly in our study and by other authors (Scott *et al.*, 2001; Mircean *et al.*, 2010). Correlation of months of year with *Malassezia* showed higher percentage of infection in diseased dogs and healthy dogs in January and February and lower infection in November can be explained by alteration in infection rates is due to low temperatures and high humidity in Jan. and Feb. this agreed with (Bond, 2010; Mircean *et al.*, 2010).

On the other hand, the direct microscopical and cultures examination presenting a large number of colonies were accounted in this study. Therefore, the diagnosis was based

on the presence of skin lesions compatible with these entities, in cooperation with the cytological examination these finding agree with (Gupta *et al.*, 2004; Bond, 2010; Mircean *et al.*, 2010; Minnat *et al.*, 2015).

Conclusions

The present work confirms the presence of *M. pachydermatis* as the most prevalent species in the skin and external ear canal of healthy and diseased dogs. Therefore, we think that *Malassezia pachydermatis* might be playing a causative role in the etiology of canine otitis externa.

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