

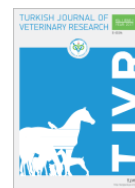


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Determination of *Malassezia* spp. infection and flea allergy incidences in pet dogs found in Kırıkkale and Ankara provinces

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ABSTRACT

Objective: The aim of this study is to determine the incidences of *Malassezia* infection and flea allergy in dogs with dermatitis complaints which were brought to veterinarians for examination in Ankara and Kırıkkale provinces.

Materials and Methods: The material for this study consists of 40 pet dogs of different breeds genders and ages found in Ankara and Kırıkkale provinces of Turkey. *Malassezia* examination was conducted by staining smear samples taken with the acetate band method with Modified Wright's Stain method. Flea existency examination was performed by using a flea comb.

Results: Out of the 40 dogs with dermatitis which were included in this study, 6 dogs (15%) were found to have *Malassezia* infection, 12 dogs (30%) were found to have flea infestation and 4 dogs (10%) were found to have together.

Conclusion: In light of the results that were found in this study, *Malassezia* and flea infestation hold an important place for dogs which were brought with complaints of dermatitis to veterinary clinics for examination in Ankara and Kırıkkale provinces. Clinical veterinarians must keep in mind that, there is high incidence rates of these 2 dermatitis causing agents and also remember to especially consider them in the list of differential diagnosis.

Keywords: Ankara, Kırıkkale, pet dog, dermatitis, *Malassezia*, flea

INTRODUCTION

Malassezia species are lipophilic yeasts which are found in the natural microbiota of the skin and mucous membranes of the animals and human beings (Silva et al., 1981). *Malassezia* yeasts are opportunistic pathogens. In the presence of predisposing factors, *malassezia* plays a role in creation of various diseases (Faergemann, 2002; Çomak and Ceylan, 2018).

Yeasts from the *Malassezia* genus have a grooved inner surface, a multi layered membrane and a thick (0.12 µm approx.) cell wall and the cell wall forms 26-37% of the total cell volume. The protoplasmic

membrane is tightly adherent to the cell wall (Kantarcioglu et al., 2005).

Malassezia dermatitis is a secondary problem that can develop after allergic diseases like flea allergy, atopic dermatitis and also after recurrent pyoderma, hyperadrenocorticism, hyperthyroidism, diabetes mellitus, cutaneous, internal neoplasia or other metabolic diseases (Bajwa, 2017).

The most common clinical symptom is average or extreme itchiness that may partially recover as a response to the corticosteroids and antibiotics. As a result of the itchiness the patient rubs their faces,

shake their heads, lick their feet or chew them (Aytuğ, 2012). In some dog's behavioral problems due to the intensity of the itching can be misinterpreted as a neurologic or a behavioral problem which results in an incorrect diagnosis (Patterson et al., 2002).

Lesions usually seen on the ventral parts of body such as neck, abdomen, inguinal and axillary area and in ears, lips, mouth and medials of the extremities are the other important clinical signs (Şentürk et al., 2001; Conkova et al., 2011). The skin and fur can become oily and alopecia may be observed as a result of this. In chronic cases, lichenification may be observed prominently. Hyperpigmentation can be variable among different breeds (Bond et al., 2020). In chronic cases secondary lesions are observed due to itching and licking (Kamaljyoti et al., 2017).

The most practical diagnosis method for *Malassezia* dermatitis is cytological examination. Samples collected through various methods such as swap smear, superficial skin scrapes, acetate band smear, impression smear are examined under the microscope (Bajwa, 2017).

Focal lesions can be treated with antifungal creams or ear drops. Substances containing chlorhexidine, climbazole, miconazole, boric acid and shampoos are recommended for topical treatment of diffuse infections. Oral antifungals are used when topical treatment cannot be used or provide effective treatment. Itraconazole, ketoconazole and terbinafine, flucanazole are the most commonly used drugs (Carlotti, 2005; Paterson, 2008).

Fleas are obligatory hematophagous ectoparasites of mammals. They are arthropods with small, flattened from the sides and wingless bodies. They have an important role as pathogen carriers in the world (Bitam et al., 2010). Fleas, which are important parasites in both veterinary and human medicine, act as vectors of allergy causing agents in both humans and animals such as *Bartonella*, *Yersinia* and *Rickettsia*. Furthermore, they cause damage to *Dipylidium caninum*, *Hymenolepis dimunata* ve *Hymenolepis nana* by acting as an intermediate host. Fleas, which are more concentrated in the mild temperate zone, multiply fast, parallel to the increasing temperature rates (Aciöz and Aydın, 2020).

Fleas, unlike lice, do not spend all their life's on the host. Egg, larva, pupa and adult stages are seen in fleas (Kandemir et al., 2019). Fleas that find a host such as a dog or a cat do not leave their host unless

they are exposed to insecticides. Fleas that live on cats have a minimum movement (Dryden, 2014).

Fleas, may cause skin diseases like dermatitis and cause anemia in the pets and their owners (Lam and Yu, 2009). Clinical symptoms related to flea allergy vary in accordance with the frequency of exposure to the fleas, secondary and simultaneous skin diseases, degree of hypersensitivity and effects of previous treatments. Itchiness related to the flea allergy dermatitis in dogs is intense and spreads throughout the body. Caudal and medial parts of the thigh, ventral abdomen, neck and ears develop a sensitivity in the affected dogs. Due to the itching and licking of these regions, color changes in the fur and fur loss is observed. The first lesion observed in flea allergy dermatitis is the papule formation at the flea bite spot. Alopecia may also be observed simultaneously with a red-brown crusted papule. Injuries which occur in the hair roots may secondarily lead to pyoderma. Pyotraumatic dermatitis may form as a result of intense itching by the affected dogs. Potential chronic diseases may present themselves with symptoms like diffuse alopecia, severe seborrhea, hyperkeratosis and hyperpigmentation. In cats however, the first lesion is a typical milier lesion which is seen on the face, the back and the neck (Dryden and Blakemore, 1989). Erythema, alopecia, excoriation, papules, crusts and itchiness is seen in dogs with flea allergy dermatitis while alopecia, milier dermatitis and itchiness is observed in cats with the same disease (Traversa, 2013).

Anamnesis, physical examination and clinical findings, flea or flea excreta, intradermal tests and elimination of other possible dermatologic diseases are the most important methods for the diagnosis of flea allergy dermatitis (Dryden, 2014). Diagnosis is usually done by detection of flea on a pet's body. Fleas and their excreta may be observed by using a flea comb. Flea excreta is reddish black. When excreta put on a moist towel forms a varying range of reddish brown colors when crushed. Flea excreta is generally cylindrical or comma shaped (Dryden, 2014).

In the first step of flea allergy dermatitis treatment, prevention of flea bites in the pets is important. Flea collars are commonly used for this purpose, although they are not effective enough as the only prevention method. Hence, orally and topically applicable products are preferred (Paterson, 2008; Bruner, 2011; Aytuğ, 2012).

The aim of this study is to determine the incidences of *Malassezia* infection and flea allergy in dogs with dermatitis complaints which were brought to veterinarians for examination in Ankara and Kirikkale provinces.

MATERIALS and METHODS

Animal material

The experiments were conducted according to ethical guidelines and under the supervision of Kirikkale University Local Ethics Committee of Animal Experiments.

The animal material for this study includes 40 dogs of different ages, breeds and genders that were brought to the Kirikkale University Veterinary Faculty Animal Hospital and Başkent Animal Hospital with a complaint of dermatological problems.

Clinical examination findings and sample collection

Upon general examination of dogs that were brought to the above mentioned places with complaints of skin rash, itching, alopecia, hyperkeratosis etc., no signs of a different disease were found. A systematic dermatological examination was done afterwards and the findings were noted down on a dermatological evaluation form.

The patients were combed with a fine toothed comb. The red-black colored flea excreta and adult fleas that were got collected on the comb were put aside for further tests. Variations in the reddish-brown colors that formed in the collected excreta when in contact with a moist towel were noted and evaluated.

Samples for dermatological tests were taken from the periorbital, perioral, external ear canal, interdigital and ventral abdominal regions of the dogs using acetate band smear and skin scraping methods. Dermatological samples that were taken using the acetate band smear were stained with Modified Wright's Stain. Samples which had 5 or more than 5 *Malassezia* agents were accepted as positive for *Malassezia* dermatitis.

Laboratory analysis

Samples taken using the acetate band smear method were stained with Modified Wright's Stain and put on the microscopic slide. The samples taken with the acetate band smear method were and examined with oil immersion under 100x magnification. From the samples included in the

analysis, the ones which had 5 or more than 5 *Malassezia* agents were accepted as positive for *Malassezia* dermatitis.

Statistical analysis

Chi-Square test was used for the comparison of proportional data and analysis results were interpreted in accordance with the Pearson Chi-Square or Fisher's Exact Test. 0.05 was taken into account as the significance level. SPSS (version 23) program was used for data analysis.

RESULTS

The data of dogs which submitted to the study were shown in Table 1.

Table 1. Distribution of 40 dogs included in the study by age, gender and breed.

Case Number	Age	Gender	Breed
1	3	Female	Pug
2	6	Female	Golden Retriever
3	1	Male	Husky
4	4	Female	German Shepherd
5	7	Male	Bull Terrier
6	2	Male	Labrador Retriever
7	4	Female	Pekingese
8	8	Male	Mixed
9	3	Male	Spitz
10	4	Female	Golden Retriever
11	6	Female	Mixed
12	1	Male	Pug
13	2	Female	Pug
14	9	Female	Golden Retriever
15	3	Female	Terrier
16	4	Male	Cavalier King Charles
17	1	Female	Mixed
18	4	Female	Maltese Terrier
19	6	Male	Pekingese
20	7	Male	Mixed
21	1	Male	Cocker Spaniel
22	2	Female	Golden Retriever
23	3	Male	Jack Russel
24	3	Female	Cavalier King Charles
25	5	Female	Pitbull
26	4	Male	Mixed
27	7	Female	Mixed
28	10	Male	Golden Retriever
29	3	Male	Kangal
30	2	Female	Chow Chow
31	6	Male	Maltese Terrier
32	4	Female	German Shepherd
33	7	Female	Labrador Retriever
34	3	Male	Cocker Spaniel
35	1	Female	Poodle
36	5	Female	Setter
37	4	Female	Mixed
38	2	Male	Bernese
39	3	Female	Mixed
40	6	Male	Golden Retriever

Table 2. Skin lesions of cases.

Skin lesions	Case Number																																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40						
Itching without lesion								+										+					+																							
Primary Skin Lesions																																														
Erythema		+		+						+	+			+			+	+			+		+			+	+	+	+					+												
Papul			+					+				+	+		+			+				+					+		+	+					+							+	+	+	+	
Pustula			+		+										+							+		+						+					+								+	+		
Secondary Skin Lesions																																														
Epidermal collarete					+									+							+							+														+	+			
Crusting				+	+		+	+						+	+							+							+	+		+			+	+				+	+		+	+	+	+
Staining with saliva		+								+												+																								
Alopecia		+			+		+		+	+		+	+	+	+		+		+			+		+			+		+					+					+				+	+		
Lichenification								+				+		+		+		+					+			+		+			+	+						+								
Hiperpigmentation					+		+		+			+	+	+		+		+								+	+						+							+	+					

No any other pathology was seen except dermatologic problems after the clinical examinations performed. The examination findings were recorded and classified for each patient after the dermatological examination. While in 3 of the patient no existence of dermatologic lesions determined, only itching was observed as a symptom and in the reminder of the 37 patient were seen different dermatological lesions. The determined dermatologic lesions were shown in Table 2.

On 6 of the patients, 5 or more *Malassezia* spp were detected and accepted as *Malassezia* positive. On the 10 of patient adult fleas were determined either on inspectional examination and on testing with flea comb manipulation. On 2 of the patients no flea feces was determined by the flea comb grooming. On 4 of the patients (case no 1-7-11-12) both *Malassezia* spp and flea existence were determined together (Table 3).

Table 3. *Malassezia* spp. and flea detected case numbers and percentages.

	<i>Malassezia</i> spp.	Flea	<i>Malassezia</i> spp. and Flea
Case Numbers	1-7-9-11-12-25	1-7-11-12-14-20-27-29-31-34-37-39	1-7-11-12
Percentages	15%	30%	10%

DISCUSSION

Malassezia species are lipophilic yeast which exists in the natural microbiota of the skin and mucous membranes of the animals and human beings (Silva *et al.*, 1981). *Malassezia* yeasts are opportunist pathogens. They play a role in various diseases via various predisposing factors (Faergemann, 2002). These predisposing factors are excess production of and the decreasing quality of sebum, destruction of epidermis, increasing of humidity, persistence of skin folds, changing of the cutaneous pH levels, application of antibiotic treatments and long term corticosteroid medications (Bajwa, 2017). *Malassezia pachydermatidis* could be found in ear channel, anal glands, lips, chin, vagina, rectum and even skin and causes alopecia, both localized or generalized erythema, papulas and maculas, and crustaceous and squamous appearance in face, body perianal and interdigital areas and also in skin folds (Patteron *et al.*, 2002).

There is no age and gender predisposition on *Malassezia* dermatitis. The predisposing breeds are; Westhighland White Terrier, Basset Hound, English

Setter, Poodle, American Cocker Spaniel, Jack Russel Terrier, Shih Tzu, Silky Terrier, Boxer, German Shepherd, Maltese, Australian Terrier, Chihuahua, Shar Pei, Shetland Sheepdog, Lhaso Apso, Springer Spaniel and Daschund (Çamak and İçen, 2010). In our study, 6 dogs suffered from dermatitis which got diagnosed *Malassezia* spp., were not belong to any of predisposed breeds. The mentioned 6 dog's breeds were as Pug, Pekingese, Spitz, Pitbull and mix. The average ages of 4 female and 2 male dogs who got *Malassezia* diagnosed were 3.66 years.

Because of the itching, patient rubs his face to certain surfaces, shakes his head, licks or bites his legs (Aytuğ, 2012). Lesions usually seen on the ventral parts of the body such as neck, abdomen, inguinal and axillary area and in ears, lips, mouth and medials of the extremities. Usually there is an unpleasant and soury smell exists (Jasmin, 2011). The other important findings are erythema, plaque, papule and nodule formation, hyperpigmentation, seborrhoea, otitis externa which are relevant on the areas rich from the sebum glands (Şentürk *et al.*, 2001; Conkova *et al.*, 2011). The red-brown discharge is very specific in cases of *Malassezia* related otitis externa (Şentürk *et al.*, 2001). Alopeci were seen in all 6 dogs included to the study and got diagnosed *Malassezia*. The other skin lesions determined frequently were as erythema (5 into 6), hyperpigmentation, liquenification and papule formation (3 into 6), getting stain with saliva (2 into 6) and pustula formation (1 into 6).

Demir and Sancak (2013), diagnosed *Malassezia* dermatitis on the 16 dogs of 566 dermatologic patient (2.82%). Canpolat *et al.* (2018), diagnosed *Malassezia* dermatitis in only two of the 653 dermatologic patients (0.3%). In the presented study in 6 of the 40 dermatology patients *Malassezia* was diagnosed and the incidence of *Malassezia* was found as 15%. The results obtained from this study about *Malassezia* coincidence were determined severely high from the other studies.

The fleas which are the obligatory hematophagous ectoparasites of mammals and birds. They are small insects that flattened from lateral sides and wingless. They have big importance because of they are pathogenic carriers all over the world (Bitam *et al.*, 2010). As an important parasite for both human and veterinary medicine, they cause allergic reactions and serve as vectors for *Bartonella*, *Yersinia* and *Rickettsia* kind of agents. Besides they cause harm as they are intermediate hosts for *Dphylidium caninum*,

Hymenolepis diminuta, and *Hymenolepis nana* (Acıöz and Aydın, 2020).

Fleas can cause allergic dermatitis kind of dermatologic diseases and anemia both on the animals and their owners (Lam and Yu, 2009). The flea bite dermatitis caused allergic itching could be intense and generalized all over the body in the dogs. The sensitivity develops on the caudal and medial femoral areas, ventral abdomen, neck and ears in the effected dogs. The color changes and hairloss can be seen on these areas due to licking and itching. The first lesion seen in allergic flea bite dermatitis is the hyperemic areas occurred on the bite site. These lesions can become as a papula formation. Besides the red brown crustous covered papulas and alopecia can be seen. The damage occurred on the hair follicules can cause pyoderma secondarily. Also due to the self-itching trauma, wet dermatitis can develop. As the disease getting chronical, generalized alopecia, severe seborrhoea, hyperkeratosis and hyperpigmentation become evident. In cats, the primary lesion is the typical miliary dermatitis where seen on the face, back and neck (Dryden et al., 1989; Traversa, 2013). The lesions of the dogs which flea agent were frequently seen determined as papula (9 into 12), erythrema and incrustation (8 of 12), hyperpigmentation (6 of 12), alopecia (5 of 12), liquenification (4 of 12), pustula and staining with saliva (1 of 12). The average age of dogs was found as 4.66 year overall 12 dogs (7 female and 5 male) which no existence of predispositon neither gender nor breed.

It has been stated that, the 10-20% of the allergic dog disease are flea bite allergies (Bourdeau et al., 2004). Acıöz and Aydın (2020), performed a flea scanning on the 142 dogs at the localization Datça, Muğla, and submitted the flea infestation on 27 dogs (19%). Canpolat et al. (2018), informed that, on the study conducted with 1000 dogs which suffered from dermatological conditions, the rate of the “flea hypersensitivity” is 38% among the ichthy dermatoses and 10% of overall dermatologic diseases. Demir and Sancak (2013), reported 29 allergic flea bite dermatitis (5.12%) between on total of 566 dogs with dermatologic problems. In Yılmaz et al’s study (2002), including 7831 dogs which registered for the distribution of several diseases, the incidence of flea allergies was reported as 5%. In our study 40 dogs with dermatologic problems were subjected, and on 12 of these dogs (30%) even flea or feces of flea were determined. Based on the evaluation of the results obtained from our study, it can be state that the incidence of flea existency on

the ichthy dermatoses is higher than the results of Bourdeau (2004), which is 10-20%, and is lower than the results of Canpolat et al. (2008), which is 38%. An average rates reached according to these two studies. Despite the other research results given, were about to survey of the flea infestation on overall population, the results obtained from our study were evaluated between only the dogs with dermatologic problems and that why a severely high incidence was determined.

CONCLUSION

In conclusion, the incidence of *Malassezia* infection and flea infestation in dogs brought to clinics in the Ankara and Kırıkkale provinces with a dermatitis complaint was found to be 15% and 30% respectively. The results obtained have shown that the incidences of these two agents are very high and that they need to be placed in the list of differential diagnosis. Especially the 30% occurrence rate of flea infestation in pet dogs, found in this study, raises a question about whether ectoparasite treatments are done on an adequate level or not.

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