Factors Associated with the Presence of Malassezia spp. in Dogs with Atopic Dermatitis in Quito, Ecuador

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ABSTRACT

Yeasts of the genus *Malassezia* are commonly isolated from different areas of the body in dogs diagnosed with atopic dermatitis. The proliferation of these yeasts may trigger clinically relevant hypersensitivity responses in patients with CAD. There are also reports that there is some association with certain body areas and predisposing factors such as gender, race, or age, among others. The objective is evaluate the factors associated with the presence of *Malassezia* spp. yeasts in dogs with atopic dermatitis in Quito, Ecuador. A total of 800 patients who attended a dermatological clinic at Veterinary Care Centers in Quito were evaluated, among whom 10% (80 dogs) were diagnosed with CAD. Epidemiological data were collected and cytological samples comprising ear culture swabs, imprints, or Scotch tape samples from different areas of the body, were taken. Each sample was stained and analyzed under the microscope.

The 86.2% of dogs with CAD presented *Malassezia* spp. Dogs aged less than two years (32/80) had an association with the presence of *Malassezia* (Chi-square = 35.83, P = 0.0001), as did dogs from Quito (52/80), (Chi-square = 41.02, P = 0.0001). Both ears, right (32/80) and left (35/80), showed association with a severe or stern load of *Malassezia* spp. Age and residence in the Quito area are factors associated with the presence of CAD. Ears are areas that are severely affected by *Malassezia* spp.

INTRODUCTION

Canine atopic dermatitis (CAD) is a pruritic inflammatory skin disease with a genetic predisposition. It is a multifactorial pathology involving IgE mediated allergic reactions to various environmental allergens; alterations of skin barrier func-

Table I. *Factors Associated with the Prevalence of Malassezia in dogs*

Evaluated factor	Positive (%) n= 69	Negative (%) n= 11	Chi-Square	*P*-value
Sex				
Male	30 (37.5)	4 (5)		
Female	39 (48.7)	7 (8.8)	0.19	0.65
Age				
<2 years	32 (40)	0 (0)		
2.5-5 years	27 (33.75)	0 (0)	35.83	0.0001
>6 years	10 (12.50)	11 (13.75)		
Ears				
Erect	22 (27.50)	3 (3.75)		
Pendulous	47 (58.75)	8 (10)	0.09	0.75
Hair				
Short	27 (33.75)	5 (6.25)		
Long	42 (52.50)	6 (7.50)	0.15	0.69
Reproductive status				
Castrated	28 (35)	6 (7.5)		
Not castrated	41 (51.3)	5 (6.2)	0.75	0.38
City				
Concolo	1 (1.25)	0 (0)		
Cumbaya	10 (12.5)	3 (3.75)		
El Tingo	0 (0)	1 (1.25)		
Quito	52 (65)*	1 (1.25)	41.02	0.0001
San Golqui	1 (1.25)	5 (6.25)		
San Rafael	1 (1.25)	1 (1.25)		
Tumbaes	4 (5)	0 (0)		

**P= 0.05*

tion; microbial colonization; and abnormal cutaneous immunity.[1] The diagnosis of CAD is based on clinical signs, skin scrapings, and cytology. Allergy testing can be performed to identify the responsible allergens in order to perform specific allergen immunotherapy.[2] Different prevalences of CAD have been reported, ranging from 3.3% to 27%.[3]

Malassezia spp. comprises a group of commensal yeasts that usually colonize the superficial layers of the epidermis, *Malassezia* pachydermatis (also known as *Malassezia* canis, Pityrosporum pachydermatis or Pityrosporum canis) and is commonly found in skin, auditory passages, mucosal surfaces (oral, anal), anal sacs, and vagina of healthy animals, and can be isolated from the skin of puppies aged 3 days.[3] These yeasts can proliferate and trigger immediate, clinically relevant hypersensitivity responses.[4] This hypersensitivity to *Malassezia* spp. is recognized as a triggering factor for canine atopic dermatitis in some dogs, and hypersensitivity can be determined using the intradermal test (IDT).[5] Allergenic reactivity is higher in atopic patients with cytological evidence of *Malassezia* spp.[6] The reason for the high *Malassezia* spp. sensitivity in patients with CAD compared with healthy individuals is still unclear.[7] Therefore, the aim of the present investigation was to evaluate factors associated with the presence of *Malassezia* spp. in dogs with atopic dermatitis in Quito, Ecuador.

MATERIALS AND METHODS

Location of Research

The present investigation was carried out in 16 veterinary centers in the Metropolitan

Table II. *Body Area Associated with the Presence of Malassezia in dogs*

Body area	Positive (%) n= 69	Negative (%) n= 11	Chi-square	*P*-value
Lips				
Absent	36 (45)	3 (3.75)		
Mild	12 (16.25)	5 (6.25)		
Moderate	13 (16.25)	2 (2.50)	4.20	0.24
Severe	7 (8.75)	1 (1.25)		
Perineum				
Absent	29 (36.25)*	3 (3.75)		
Mild	17 (21.25)	7 (8.75)	7.69	0.05
Moderate	15 (18.75)	1 (1.25)		
Severe	8 (10)	0 (0)		
Axil				
Absent	41 (51.25)	4 (5)		
Mild	8 (10)	4 (5)	4.83	0.18
Moderate	12 (15)	2 (2.50)		
Severe	8 (10)	1 (1.25)		
Right Ear				
Absent	10 (12.50)	2 (2.50)		
Mild	19 (23.75)	3 (3.75)	9.97	0.01
Moderate	8 (10)	5 (6.25)		
Severe	32 (40)*	1 (1.25)		
Left Ear				
Absent	9 (11.25)	0 (0)		
Mild	19 (23.75)	4 (5)	13.18	0.004
Moderate	6 (7.50)	5 (6.25)		
Severe	35 (43.75)*	2 (2.50)		

**P= 0.05*

District of Quito, Ecuador, from June 2015 to February 2016.

Animals

From a sample of 800 patients with dermatological problems diagnosed based on Carlotti theory (2005), 10% of the sample, treated in 16 veterinary attention centers in Quito DM city and its valleys, were diagnosed with CAD. Epidemiological data such as age, race, weight, gender, and place of origin were recorded.

Identification of Patients with CAD

CAD diagnosis was made through the patient's anamnesis and clinical history, which suggested recurrent and chronic skin processes, dermatological examinations, diagnostic tests, as well as the use of the criteria of Favrot et al[8] and Hensel et al.[9]

Cytological Sampling

Cytological samples were taken from the 80 patients comprising left and right ear swabs[10] and imprints or samples obtained with Scotch tape at the labial commissure, perineum, interdigits of the right and left pelvic limbs, right and left thoracic limbs, and armpits.[11] Each sample was stained with Diff-Quick and analyzed under a microscope with a 100x objective, in the LABVET laboratory of the Teaching Hospital of the University of San Francisco in Quito, to evaluate the presence of *Malassezia* spp. The samples were classified by the number of yeasts per optical field into four categories — absent, mild, moderate, and severe.

Data Analysis

According to the distribution of the data,

Table III. *Interdigital area by member associated with the presence of Malassezia in dogs*

Member	Positive (%) n= 69	Negative (%) n= 11	Chi-Square	*P*-value
LFM				
Absent	29 (36.25)	4 (5)		
Mild	13 (16.25)	4 (5)	2.30	0.51
Moderate	23 (28.75)	2 (2.50)		
Severe	4 (5)			
RFM				
Absent	33 (41.25)	5 (6.25)		
Mild	11 (13.75)	2 (2.50)	0.18	0.98
Moderate	15 (18.75)	2 (2.50)		
Severe	10 (12.50)	2 (2.50)		
LBM				
Absent	29 (36.25)	8 (10)		
Mild	14 (17.50)	1 (1.25)	6.30	0.09
Moderate	13 (16.25)	2 (2.50)		
Severe	13 (16.25)	0 (0)		
RBM				
Absent	28 (35)*	6 (7.50)		
Mild	13 (16.25)	0 (0)	9.32	0.02
Moderate	16 (20)	5 (6.25)		
Severe	12 (15)	0 (0)		

*LFM (Left front member), RFM (Right front member), LBM (Left back member), RBM (Right back member). *P= 0.05*

the Chi-square test was performed to find associations between the prevalence of *Malassezia* spp. and the factors considered in the study. For data analysis, the statistical software JMP 8.0 was used.

RESULTS

Of the 800 dogs evaluated, 10% were positive for CAD. Of these 80 dogs, *Malassezia* spp. were present in 86.2%. When analyzing the association of factors considered in this study with the presence of *Malassezia* spp., it was found that the genus, ear type, hair type, and reproductive status showed no association with the presence of *Malassezia* spp. However, an age of less than 2 years (32/80) was associated with the presence of *Malassezia* (Chi-square = 35.83, P = 0.0001), and originating from Quito (52/80) also showed association (Chi-square = 41.02, P = 0.0001) as shown in Table I.

Table II shows the areas of the body where samples were taken to isolate *Malassezia* spp. Each body site and individual were classified in the categories absent, mild, moderate, and severe depending on the level of *Malassezia* spp. present. The lips and armpits showed no association with the presence of *Malassezia* spp., while the perineum area was associated with the absence of *Malassezia* spp. (29/80; Chi-square = 7.69, P = 0.05). This was interpreted as *Malassezia* spp. not being found in the perineum. In contrast, both ears (right, 32/80; and left, 35/80) showed association with a severe or moderate load of *Malassezia* spp. The interdigital area was analyzed separately for each member (Table III). The anterior and posterior left limbs showed no association with *Malassezia* spp., while the right hind limb was associated with the absence of *Malassezia* spp.

DISCUSSION

In the present study, *Malassezia* spp. showed a prevalence of 86.25% (69/80) in dogs that were positive for CAD, lower than the prevalence reported by Morris and DeBoer,[12] who reported a 100% prevalence of M. pachydermatis. Nardoni et al[10] also reported 100% prevalence in a study where they quantified and determined the distribution of *Malassezia* in 41 dogs with CAD, and took samples from 16 anatomical sites. Although they obtained 100% prevalence of M. pachydermatis, although at least 10 species were found alongside *Malassezia* pachydermatis .[13]

In the results obtained in the present study, there was no association between prevalence and gender as previously reported by Machado et al[14] and later by Sihelská et al.[15] Both groups assessed the presence of *Malassezia* in dogs and found no association with the gender variable, although it is worth mentioning that the samples were taken from healthy dogs. Data analysis by Machado et al[14] showed an association between prevalence and age <2 years (32/80), although Sihelská et al[15] found no relationship between prevalence and age in a study to identify *Malassezia* yeasts. They found 84 positive dogs, and of these, 76 with *Malassezia* pachydermatis.

On the other hand, Nardoni et al16isolated *Malassezia* most frequently from dogs aged 1 to 5 years, with a presence in 85 of 120 dogs (70.8%), therefore, determining association with age <5 years. In another study by Machado et al[14] the authors reported that healthy dogs aged between 6 and 7 years were more frequently positive for *Malassezia*, while Girao et al[17] report that the majority of the dogs showing a presence of *Malassezia* pachydermatis were aged between 1 and 3 years, in agreement with our results. In this work, we considered the variable ear type (pendulous or erect), which showed no association with the presence of *Malassezia* spp. although, Sihelská et al[15] previously reported a higher frequency in dogs with pendulous ears (32.8%) compared with dogs with erect ears (14.2%).

The type of hair (long or short) was not associated with the presence of *Malassezia* in this study, although Layne and DeBoer18 mention that retriever (long hair breed) dogs with CAD are frequently affected by *Malassezia*. Girao et al[17] report that there is a relationship between the poodle breed and the presence of *Malassezia* pachydermatis, although other studies do not agree with this assertion, e.g., Machado et al.,[14] who, as in the present study, did not find any association. The place of origin of the patient (city) was associated with *Malassezia* prevalence, specifically dogs from the city of Quito were more likely to be affected.

We also looked for association with the prevalence of *Malassezia* in different areas of the body. The study by Sihelská et al[15] reported a higher prevalence of *Malassezia* in dogs with otitis externa (36.0%), followed by dermatitis (24.5%), interdigital dermatitis (16.4%), and infected anal sacs (14.3%), with *Malassezia* counts being higher in the ears, followed by the anus, interdigital areas, and perianal glands. These results confirmed the extensive colonization by this species of yeast in different body sites of atopic dogs, a result similar to that obtained in the present study, where both ears (right, 32/80, and left, 35/80) showed association with a severe or moderate load of *Malassezia* spp., as well as in the study by Campbell et al[19] where the fungal otic microbiota in healthy, atopic and otitis externa dogs was characterized.

The authors mention that there were no differences between the study groups. However, it was more likely that *Malassezia* was isolated from atopic dogs or those with otitis presenting dermatological signs of inflammatory skin disease and/or pyoderma. The prevalence of *Malassezia* species in healthy dogs is generally lower than in dogs with lesions, as mentioned by Nardoni et al,[16] who studied the presence of *Malassezia* spp. in different areas of the body of dogs with and without dermatological problems. They found *Malassezia* yeasts in 63.4% of 244 dog samples examined, although the counts

of colonies obtained from dermatologically diseased dogs showed more intense fungal development compared with the level of fungal growth observed in healthy dogs.

In the present study, the perineum area was associated with the absence of *Malassezia* spp. (29/80). However, other authors have studied the pathogenicity of yeasts recovered from seven anatomical sites of dogs with healthy and diseased skin in which they isolated *Malassezia* species from at least one site in 45 dogs (51.7%), with a higher prevalence (61.1%) in dogs with lesions than in healthy dogs (36.4%). The perianal region was the most frequently colonized area (60.6%) and the inguinal region (3.0%) the least.[20]

The presence of *Malassezia* spp. is a clinical problem that is commonly associated with patients with CAD. Knowledge about the factors predisposing or associated with the presence of *Malassezia* spp. can be a valuable tool for diagnosis in dogs with CAD and improve their treatment. In this study, the presence of *Malassezia* spp. in dogs with CAD was found to be associated with animals aged less than 2 years and in those originating from the Quito area. The body areas most associated with severe infection by *Malassezia* spp. were the right and left ears.

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