DETECTION OF *Malassezia*-LIKE YEASTS ON THE SKIN OF APPARENTLY HEALTHY DOGS USING CYTOLOGY

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ABSTRACT

The frequency, body distribution and population size of *Malassezia*-like yeasts were determined from 30 dogs with apparently healthy skin. Cutaneous swab samples obtained from nine sites of the body were associated with the breed, length of hair, sex and age of the animals. Of the thirty dogs, eleven (36.67%) were considered positive for *Malassezia*-like infections. No differences were noted in the frequency of infection between breeds, length of hair, sex and age (P>0.05). Of the fourteen positive cases based on body distribution, 5 (35.71%) and 4 (28.57%) were obtained from the right and left ears, respectively, 3 (21.43%) from the facial area, 1 (7.14%) from the medial surface of the arm and 1 (7.14%) from the medial surface of the thigh. Population size of *Malassezia*-like yeast cells ranged from 0 to 273 with no differences (P>0.05) among breeds, sex and age of dogs. The highest population counts were noted in the right ear followed by the left ear, then face, medial surface of the arm and thigh.

Keywords: cytology, dog, *Malassezia sp*., malasseziasis, yeast

INTRODUCTION

Malassezia sp. is a natural yeast inhabitant or commensal of the skin in dogs. It is lipophilic in nature and is considered an opportunistic pathogen, proliferating when the animal is immunocompromised or when the skin of the animal is challenged by allergic reactions and by other pathogenic organisms. Among the ten species under this genus, *Malassezia pachydermatis* is regarded as an opportunistic pathogen on the skin surface and in the ear canals of dogs. It is non-lipid-dependent, non-mycelial, thick-walled and with a distinct “shoe print”, “bottle-like” or “peanut-shaped” appearance upon microscopic examination (Cafarchia *et al*., 2005; Carter *et al.*, 1995; Scott *et al.*, 1995; Biberstein, 1999b; Carlotti, 2005; Ihrke, 2008). A pH range of 4.0-8.0 is most favorable for their growth; this makes the canine skin, which has a pH range of 5.5 to 7.5, conducive as a habitat (Scott *et al.*, 1995; Matousek *et al.*, 2003). The diagnostic methods by which *Malassezia spp*.

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can be detected and identified are through cytology and histopathology (Cafarchia *et al.*, 2005; Daigle, 2007). Histopathology through skin biopsy is not commonly used because of its lower sensitivity than cytology (Carlotti, 2006) and further confirmation may be done through isolation and culture (Carlotti, 2006; Ihrke, 2008).

Studies have been conducted regarding malasseziasis in the United States of America (Kennis *et al.,* 1996), Italy (Nardoni *et al.,* 2004; Cafarchia *et al.,* 2005) and Brazil (Girão *et al.,* 2006). In 2010, Macandili conducted a preliminary study at the University of the Philippines Veterinary Teaching Hospital (UP-VTH) in Diliman, Quezon City to establish the frequency, body distribution and population size of *Malassezia*-like infection in 30 dogs with cutaneous lesions. However, a similar study concerning dogs with apparently healthy skin has not yet been done. In this study, the frequency, body distribution and population size of *Malassezia*-like yeasts in dogs with apparently healthy skin was conducted using cytology and the findings were associated relative to the breed, length of hair, sex and age of the dogs. Hopefully, the results may serve as baseline data in determining whether the number of *Malassezia sp.* yeast cells found in a given sample is within the normal counts as found by previous researches or an overgrowth, which could be a prelude to possible skin infection.

MATERIALS AND METHODS

Collection and preparation of samples

Thirty dogs with apparently healthy skin brought to the University of the Philippines Veterinary Teaching Hospital (UP-VTH) in Diliman, Quezon City for vaccination and/or deworming were used in the study. Among these were 21 purebreds and 9 mixed breeds, 17 males and 13 females, with ages ranging from 2 months to 14 years.

Sterile double-tipped cotton swabs moistened with sterile normal saline solution were used to obtain samples from an area of approximately 0.5 square inch of skin of each dog at the following sites: 1) the face (specifically the dorsal plane of the planum nasale); 2) ventral neck; 3) concave lateral surface of the left ear; 4) concave lateral surface of the right ear; 5) medial surface of the arm; 6) abdomen (specifically the umbilical area); 7) medial surface of the thigh; 8) interdigital space of one limb; and 9) perianal area. A total of 270 samples were collected from 30 dogs. Clean glass slides were labeled and marked with masking tape to border an area of 0.5 square inch, such that two samples may be accommodated per slide (Figure 1). The swabs were rolled onto the slide on the same side as these markings. The slides were then stained with modified Wright's stain (HemaQuik®), air-dried, and viewed at 1000x magnification under oil immersion objective (Figure 2).

The sites from which samples were obtained were derived from those in the studies of Cafarchia *et al*. (2005), Nardoni *et al*. (2004) and Macandili (2010), and the number of microscopic fields to be viewed was based on the study conducted by Macandili (2010).

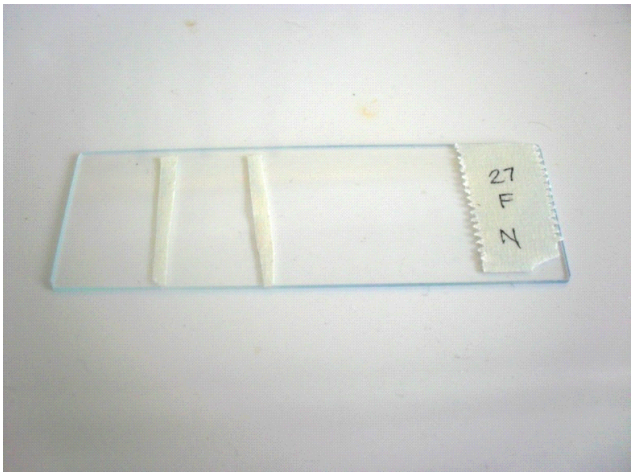

Figure 1. Unstained sample. Two strips of tape (arrows) border two areas where the swabs are smeared.

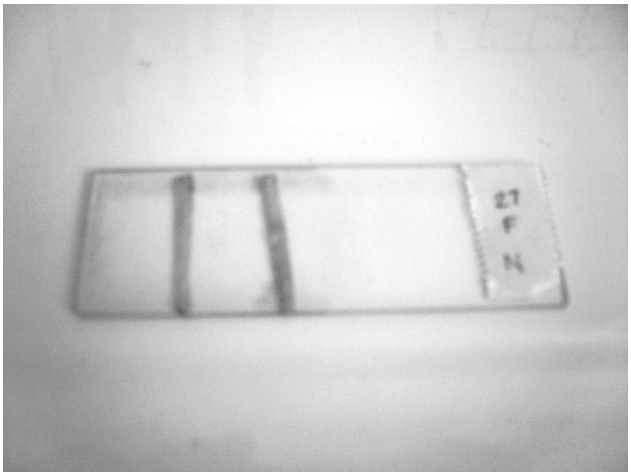

Figure 2. Sample after staining with modified Wright's stain (HemaQuik®).

Microscopic evaluation

A total of 36 microscopic fields were covered by each smear, from which *Malassezia*-like yeasts were counted. The yeast cells observed and counted were either shaped like peanuts or footprints (Figure 3), or round with small unipolar projections (Figure 4), and were identified as similar to that of the description of *Malassezia spp*. in other studies (Carlotti, 2005; Cafarchia *et al.*, 2005; Ihrke, 2008).

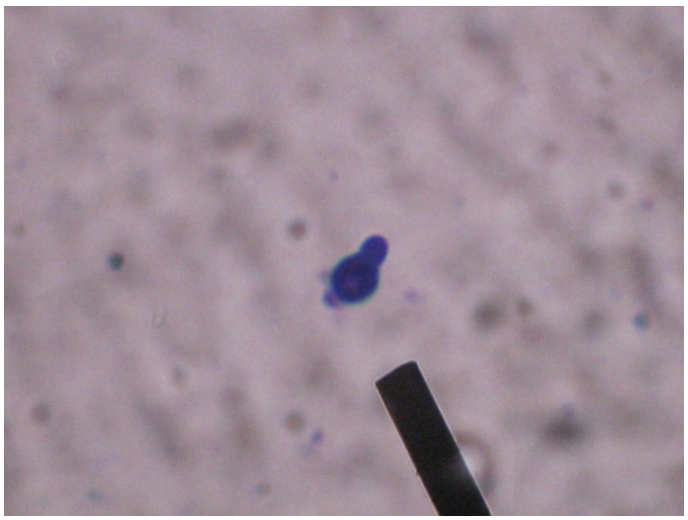

Figure 3. *Malassezia*-like budding yeast cell obtained from the right ear of a 5-year-old female mixed breed dog. Modified Wright's stain. 1000x.

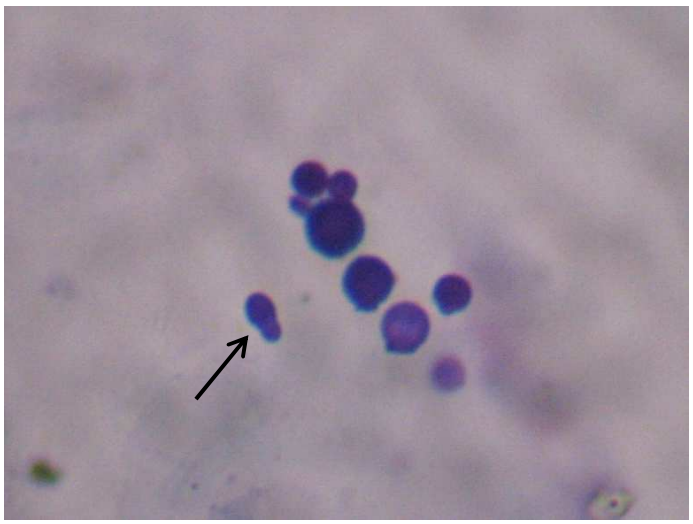

Figure 4. *Malassezia*-like yeast cells obtained from the left ear of a 1-year-and-2-month-old female Pug. A Malassezia-like yeast cell with a typical "shoe print" appearance (arrow) can be seen while the other cells in this field are round with and without small unipolar projections. Modified Wright's stain. 1000x.

Data collection and analysis

The breed, hair length (*i.e*., shorthaired or longhaired based on Animal Planet's Dog Breed Directory, 2011), sex and age of the sampled dogs were recorded and tabulated together with all counts of *Malassezia*-like yeast cells for

each sample collected from each dog. A sample from the ear was considered positive for infection if the count exceeded 36 yeast cells while a sample from any of the other sites was considered positive if the count exceeded 7 yeast cells; otherwise, the sample was regarded as negative (Macandili, 2010). Results for the frequency of *Malassezia*-like yeast infection relative to breed (*i.e*., purebred or mixed breed), hair length, age, and sex of dogs with apparently healthy skin were analyzed using Chi-square test of independence. The same statistical analysis was applied on the population size relative to breed (*i.e*., purebred or mixed breed), sex and age. Body distribution, on the other hand, was analyzed using one sample t-test.

RESULTS AND DISCUSSION

Frequency of of *Malassezia*-like yeasts

The twenty-one (70%) purebred dogs included one Chihuahua, one Dalmatian, one Golden Retriever, one Japanese Spitz, four Labradors, one Lhasa Apso, two Malteses, one Miniature Pinscher, one Toy Poodle, three Pugs, one Sharpei, and four Shih Tzus, while the other 9 (30%) were mixed breeds. From the 30 dogs sampled, 11 (36.67%) were positive to infection: 8 (72.73%) were purebreds and 3 (27.27%) were mixed breeds. Eight (72.73%) of the 11 dogs that tested positive were purebreds while 3 (27.27%) were mixed breeds. These results agree with the study conducted by Macandili (2010) that purebreds had higher frequency of infection; however, statistical analysis showed that frequency was not related to breed.

Based on the length of hair, (*i.e*., shorthaired and longhaired), as determined by Animal Planet's Dog Breed Directory (2011) for the purebreds and as subjective observation by the authors for the mixed breed, five (45.45%) positive cases were shorthaired and 6 (54.55%) cases were longhaired. No difference was noted regarding length of hair ($P>0.05$). Five out of the 11 (45.45%) dogs tested positive were males, while 6 (54.55%) were females. No difference between male and female dogs ($P>0.05$) was noted, which agreed with the study conducted by Nardoni *et al.* (2004) and Macandili (2010).

Among the 11 *Malassezia*-like infection positive dogs, 8 (72.73%) were 1 to 5 years old, 3 (27.27%) were less than 1 year of age. The results agreed with that of the study conducted by Nardoni *et al.* (2004), Girão *et al.* (2006) and Macandili (2010) although the results were not significant ($P>0.05$).

Body distribution

Out of a total of 270 samples obtained, 14 were considered positive to *Malassezia*-like infection. Five (35.71%) samples were collected from the right ear while 4 (28.57%) were collected from the left ear. Three (21.43%) were acquired from the face, while 1 (7.14%) came from the medial surface of the arm as well as the medial surface of the thigh. The rest of the sampling sites (ventral neck, abdomen, interdigital space, and perianal area) yielded negative results. This outcome did not coincide with the study conducted by Cafarchia *et al.* (2005), wherein the perianal area of dogs with apparently healthy skin had the highest

frequency of infection. This also disagreed with the study of Kennis *et al.* (1996) on apparently healthy dogs, wherein the chin had the highest number of *Malassezia spp*. yeast cells, as well as with the study of Macandili (2010), wherein the highest occurrence was on the abdomen of dogs with skin lesions. There was no difference on the mean of body distribution of sites positive to *Malassezia*-like yeast infection (P>0.05).

Population size

Population size refers to the total number of *Malassezia*-like yeast cells counted in each sample, regardless of whether the sample was tested positive or negative. In the 270 samples evaluated, the number of *Malassezia*-like yeast cells ranged from 0 to 273. Out of the 14 dogs (46.67% of the sample population) that yielded 8-36 yeast cells, 11 (78.57%) were purebreds while 3 (21.43%) were mixed breeds. Eight dogs (26.67% of the sample population) had a range of 37-100 cells, wherein 6 (75%) were purebreds while only 2 (25%) were mixed breeds (Table). Difference in population size between males and females were insignificant, except that dogs that yielded 0-7 yeast cells were all males. In relation to age, two (50%) of the four dogs that had more than 100 yeast cells were less than one year of age while the other 50% belonged to the age group of 1-5 years old. There was no relationship between population size and breed (although there are more purebreds affected than mixed breeds), sex, and age of dogs (P>0.05), which agreed with the study of Macandili (2010). Further comparisons cannot be justified with other studies since Nardoni *et al.* (2004) and Girão *et al.* (2006) did not delve on population size while Cafarchia *et al.* (2005) did not correlate frequency, body distribution and population size with the age and sex of dogs with and without skin lesions.

Evaluation of population size per body sampling site revealed that the right ear (0-188 with a mean of 94) had the highest number of yeast cells followed by the left ear, then face and medial surface of the arm and medial surface of the thigh (Table), which did not concur with the study conducted by Kennis *et al.* (1996) wherein the chin had the highest yeast cell count. In the study of Macandili (2010),

Table. Population size of *Malassezia*-like yeasts according to body sampling site.

Body sampling site	Population size (range)	Mean
Face	0-38	19
Neck	0-3	1.5
Axilla	0-8	4
Abdomen	0-7	3.5
Thigh	0-8	4
Interdigital spaces	0-4	2
Perianal area	0-4	2
Left ear	0-147	73.5
Right ear	0-188	94

the right and left ears had the greatest population size among the sampling sites with and without lesions, respectively.

Dogs with apparently healthy skin showed high frequency and population size of *Malassezia sp*.-like yeast cells in some regions of the body such as the concave lateral surface of the left and right ears and face. The findings suggest positive cases for *Malassezia sp*.-like infections without clinical signs or gross lesions. The occurrence of such frequency, body distribution and population size may be attributed to the presence factors for growth and proliferation of the organism such as moisture and sebum of the skin and the needs of the specific species of *Malassezia spp.* yeasts. Excessive sebum and moisture conditions make the skin conducive to yeast growth, gradually leading to clinical signs such as erythema and pruritus (Carter *et al.*, 1995; Medleau, 2001; Cafarchia *et al.*, 2005; Carlotti, 2006).

The organisms identified in this study were considered as *Malassezia sp.*-like organisms based on morphology and being the most prevalent mycotic infection in the skin of dogs (Pier *et al*., 2000) since yeast culture and identification were not performed to distinguish them from other yeasts and identify the species. Candidiasis, caused by *Candida albicans*, is an uncommon skin infection, although the organism by itself is pathogenic enough, is considered a commensal inhabitant of mucosal surfaces such as in the alimentary and lower genital tracts (Carter *et al.*, 1995; Biberstein, 1999a).

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