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ISOLATION OF CUTANEOUS ALTERNARIAFROM DOGS AND CATS: A RETROSPECTIVE ANALYSIS

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ABSTRACT

Alternaria causes opportunistic pathogenic infections mainly in respiratory system in humans and animals. Yet, there is little information on this topic in the relevant literature. In an effort to fill this gap, the aim of this study was to describe the history and clinical findings of and mycological findings of Alternaria. In the study, 60 dogs and 7 cats were identified with the fungus. Alternaria spp. in the skin was mostly isolated in late summer and autumn. All animals had alopecia and most of them had pruritus (32 dogs and 2 cats). Paws were the foremost body part detected with lesions. The findings suggest that it would be beneficial to have these fungi in the differential diagnosis list for cats and dogs with the complaint of alopecia and pruritus, which are particularly brought in June. Further studies are required in order to better understand the effects of Alternaria on animals.

Keywords: Alternaria, cat, dog, skin

1. INTRODUCTION

Among the 50,000 to 250,000 species of fungi that have been described, fewer than 200 have been associated with human or animal diseases. Dematiaceous fungi are a large group of moulds. The presence of brown melanin or melanin-like pigments is detected in the cell walls of their conidia or hyphae, or both. *Alternaria* species, *Bipolaris* species, *Cladophialophorabantiana*, *Curvularia* species, *Exophiala* species, *Fonsecaeapedrosoi*, *Madurella* species, *Phialophora* species, *Scedosporiumprolificans*, *Scytalidiumdimidiatum*, and *Wangielladermatitidis* are the etiological agents. These organisms are saprophytic and widespread in the environment asthey can be found in soil, wood, and plant debris (Brant and Warnock, 2003; Hungerford et al., 1998). *Alternaria* is an opportunistic pathogenic mould (Pastor andGuarro, 2008). Cutaneous, subcutaneous, and corneal infections, allergic respiratory diseases –particularly in immune compromised humans–, and atopic dermatitisare being recognised worldwide, but are more common in tropical and subtropical climates(Brant and Warnock, 2003; Simon-Nobbeet al., 2008).Most cutaneous infections in humans are from Mediterranean countries(Pastor and Guarro, 2008).

The purpose of this retrospective study was to describe the history, clinical presentation, and mycological findings of Alternaria isolated from the skin of the cats and dogs.

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Immunosuppressive factors and concurrent systemic diseases, such as diabetes and Cushing's syndrome, can predispose people to fungal infections. Another goal of this study was to investigate whether immunosuppression and other diseases increased the risk of Alternaria in our study group.

2.MATERIALS AND METHODS

The electronic medical records database at our clinics between 2015 and 2017 was searched for this purpose and sixty-seven animals were detected. The criterion for inclusion was positive Alternaria spp. skin culture. Medical records were reviewed for patient signalment with regard to breed, sex and age, season of the isolation time, places of the lesions on the body, and presence of other diseases. All routine blood tests (including complete blood count (CBC), glucose, ALT, AST, ALKP, total protein, albumin, BUN, and creatinine) and skin scrapings for parasitology and mycology were done on the presentation day. Antinuclear antibodies (ANA) and blood hormone analyses were done in some patients.

Scraped scales were examined for fungal elements bydirectmicroscopyin10%potassium hydroxide. The skin samples were in oculatedon Sabouraud DextroseAgar(SDA;OxoidLtd., Basingstoke, England)with0.05%of cycloheximide and0.005%ofchloramphenicol and Dermatophyte Test Medium(DTM;HiMedia Laboratories Ltd.,Mumbai,India),and the ywere incubated at25°C for3weeks. The morphological appearance of the isolates was studied from SDA and DTM cultures by light microscopy after 10-14 days. Each isolate was described in terms of the development of secondary conidiophores, degree of branching, and conidial shape (De Hooget al., 2000; Dye et al., 2009; Kustrzeba-Wójcickaet al., 2014).

3. RESULTS

Sixty-seven animals (60 dogs and 7 cats) were selected for the study from the electronic records search. Fungal disease was detected in 340 patients in 2015, 367 patients in 2016, and 364 patients in 2017 in our clinics. Six percent of these patients were identified as *Alternaria spp*. positive in 2015, 7% in 2016, and 6% in 2017.

The most affected breeds were; Golden Retriever (n=9), Terrier (n=5), Cocker (n=4), Pekingese (n=4), Chow chow (n=3), Bulldog (n=3), and German Shepherd (n=3). In addition, there were 17mixed-breed dogs. The remaining dogs were of various races. Thirty-seven of the dogs were male. The age of the dogs ranged from less than 1 year to 12 years (mean age 4 years).

Mixed-breed cats were the most commonly affected cats among the others(6/7). The other one was an Ankara cat. Four of the cats were male. The age of the cats ranged from 1 to 14 years and the mean was 7.5 years. The distribution of the ages and sexes of animals according to years is given in Tables 1 and 2.

The distribution of the patients according to the seasons was as follows; 9 in winter, 8 in spring, 26 in summer, and 23 in autumn. Most patients were isolated with *Alternaria spp*. in June, July, and September.

The initial distribution of lesions was unknown since all patients had come from other clinics, and they were examined a few months after the first lesions. The lesions were only in one site of the body in 11 dogs and 2 cats. All animals had various degrees of alopecia. The appearance of the lesions in our patients was variable. Clinical manifestations in these animals were; hyperpigmentation (7 dogs and 1 cat), erythema (4 dogs), crusts (4 dogs and 1 cat), non-healing

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wounds (9 dogs and 1 cat), and plaques (8 dogs and 2 cats). Cutaneous nodules were detected in one dog. These nodules were painless and they were located under the abdomen. Thirty-two dogs and 2 cats had pruritus. Of these, 4 of them haddemodicosis and 1 had flea allergy.

Lesions were in the paws (10 dog and 1 cat), on the chin (4 cat), under the abdomen (7 dog and 1 cat), on the back (5 dog), and around the eyes (3 cats). The remaining lesions were scattered in various parts of the body.

CBC and serum biochemistry panel were within normal levels in 63 of the patients. 24 of the patients were with another disease. These diseases were demodicosis (9%), auto-immune skin disorders (7%), pyoderma (6%), sarcoptes (2%), hormonal skin disorders (2%), flea allergy (2%), and leishmaniosis (2%).

No fungal elements were observed by direct microscopic examination of scales in potassium hydroxide preparations. On SDA, the fungal coloniesreacheda diameter of 18to19mmin10-14days at 25°C. The colonies were grayish white, turning greenish black, or olive brown with light border. Flat were downy to woolly with short grayish aerial hyphae. There verse of the colonies was brown to black .On DTM, the fungal colonies did not change the agar plate's colour. Theisolates were examined form icroscopicmorphology by using lactophenol cottonbluestain. Conidiophores were erect, brown, multicelled, and were producing conidia in sympodial order. Conidia formed chains or occurred singly, and were divided by transverse and longitudinal septations. They usually had a club-shaped configuration, were brown, smooth-walled or verruculose, with rounded base, and beaked apex. The strain was morphologically identified as *Alternaria spp.*

4.DISCUSSION

Phaeohyphomycoses are pigmented fungi and found worldwide in a variety of climates and environments. Alternariais one of them and, is a common soil saprophyte or plant pathogen (Dedola et al., 2010).This study presents the largest reported collection of *Alternaria spp.* in the skin of dogs and cats, to the authors' knowledge. Alternaria cases have been detected mostly in cats and rarely in dogs in the literature (Mckay et al., 2001). In this study, the authors have identified 60 dogs and 7 cats with Alternaria spp. in the mycological cultures.

Alternariaalternata is the most frequently isolated species as the causative agent; however, identification of the species is not often performed (Simon-Nobbeet al., 2008),since the microscopic differences between Alternaria species are not significant, and the character of every species varies depending on the conditions of growth (Dye et al., 2009). The isolation of Alternaria spp. have been done more correctly with techniques such as PCR; however, only microscopic isolation techniques were used in our routine examinations. Further studies should be conducted for the species to be distinguished.

It was found that the infections were more frequent in males than females in human patients(Pastor &Guarro, 2008). Dye et al.(2009), reported in their article that all 9 cats were male. In our study 62% of the animals were male. Of these 4 of them were cats.

All cats had outdoor access and Alternaria species are known as a common contaminant of soil and plants, which explains why these cats had the infection.

Because of the increases in spore release of Alternariaby the decreases in humidity in the air, itreaches greatest abundance in late summer and autumn(Mueller et al., 2001). Similar to Mueller et al. study (2001),Alternaria was isolated mostly in June, July, and September in our study.

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Alternaria species mainly causes cutaneous lesions that require surgical debridement and prolonged antifungal treatment in humans with significant underlying disease. In a study conducted by Mcatee et al. (2017),113 dogs receiving cyclosporine were searched for opportunistic cutaneous fungal infections. Of these, 15 of the dogs were diagnosed with infections, and 10 of 15 (67%) had a fungal infection by *Paraconiothyrium s*pp., *Bipolaris*spp., *Cladosporium* spp., *Alternaria* spp., or *Curvularia*spp. (Mcatee et al., 2017). In 24 of the cases, Alternaria infection occurred in the presence of other diseases and non-antifungal drug therapy. Serum glucose levels were within the limits in all our patients. None of them were on corticosteroid therapy before the examinations in our clinics. However, five of the dogs were ANA positive and one dog had a Cushing's disease. Similar to Mckay's et al.(2001) conclusions, immunosuppression and diabetes were not felt to be predisposing factorsin our study.

Infection may result from skin trauma. Therefore, arms and legs are the predisposed sites. Mckay et al.(2001) found that, lesions tended to occur on the facial area or extremities in the cats. 16% of the animals had lesions on their paws and 6% of them had on their chins in our study. Our numberswaslower compared to Mckay'sstudy(2001). The reason was likely to bethat all patients came to our clinics a few months after the initial lesions.

Pruritus is an interesting finding in this study. All reports related to the animals indicatednon-pruritic lesions. However, a huge number of the patients in this study had pruritus except for 5 patients.

Outerbridgeet al.(1995) and Mckay et al.(2001), had one cat with a nodule in their case reports similar to human studies. Parallel to this finding, only one dog in our study had a painless cutaneous nodule.

In conclusion, exposure rates of cutaneous *Alternaria spp.* were high in dog population than in cats in our study. Although Alternaria species-related disease is rare in animals, Alternaria infections should be considered in the differential diagnosis of any animal with non-healing and slowly progressive lesions.

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Table 1: Sex of the animals with *Alternaria spp.*

	2015	**2016**	**2017**
Cat	1 Female	1 Female	1 Female
	1 Male	2 Male	1 Male
Dog	10 Female	8 Female	5 Female
	10 Male	13 Male	14 Male

Table 2: Age of the animals with *Alternaria spp.*

	2015	**2016**	**2017**
>1	2	-	3
1<X<7	15	20	10
>7	5	4	8