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Short Communication / *Kısa Bilimsel Çalışma*

First isolation of *Alternaria alternata* from a dog in Turkey

Meriç Lütfi AVSEVER[1], Süleyha HİLMİOĞLU POLAT[2], İlker ÇAMKERTEN[1], Adil AKSOY[1]

[1]Aksaray University, Eskil Vocation of High School, Laboratory and Veterinary Sciences, Aksaray; [2]Ege University, Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey.

Summary: *Alternaria alternata* is a fungus species which can infect animals and people as well as being commonly found in nature. On the other hand, animals with *Alternaria* infection can infect other animals and people by spreading a high amount of fungal spores. In this work, *Alternaria alternata* was detected for the first time in Turkey from the skin lesions of a dog, an antifungal susceptiblity testing was carried out and treatment with itracanozole to which the agent showed susceptibility was accomplished. The aim of this work was to report the *Alternaria* infection in dogs in Turkey for the first time, to draw attention to the zoonotic dimension of this disease and to emphasize the importance of antifungal susceptibility tests.

Keywords: *Alternaria alternata,* anti-fungal susceptibility test, dog.

Türkiye'deki bir köpekte ilk *Alternaria alternata* izolasyonu

Özet: Bir mantar türü olan *Alternaria alternata* doğada yaygın olarak bulunmakla birlikte insan ve hayvanları enfekte edebilmektedir. Diğer taraftan *Alternaria* enfeksiyonu geçiren hayvanlar mantar sporlarını yoğun bir şekilde etrafa bulaştırıp, diğer hayvanları ve insanları enfekte edebilir. Bu çalışmada; Türkiye'de bir köpeğin deri lezyonlarından ilk kez *Alternaria alternata* infeksiyonu tespit edilmiş, etkene karşı antifungal duyarlılık testi uygulanmış ve duyarlı tespit edilen itraconazole etken maddesi ile tedavi gerçekleştirilmiştir. Çalışmanın amacı, köpeklerde *Alternaria* enfeksiyonunu ülkemizde ilk kez bildirmek, hastalığın zoonotik yönüne dikkat çekmek ve antifungal duyarlılık testlerinin önemini vurgulamaktır.

Anahtar sözcükler: *Alternaria alternata,* anti-fungal duyarlılık testi, köpek.

Skin lesions in dogs may develop as a result of allergic, immunologic, hormonal diseases, cancers and nutritional defects as well as parasitic, fungal and bacterial infections (13). Dermatophytes which are keratinophyllic fungi are the cause of dermatomycoses in humans and animals. Dermatophytes such as *Microsporum* and *Trichophyton* spp.; yeasts such as *Candida* spp., *Malassezia pachydermatis* are the most common causes of dermatomycoses in dogs. Also, molds such as *Alternaria* spp., *Rhizopus* spp., *Trichoderma* spp., *Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp. can be found as saprophytes on skin as well as agents of dermatomycoses (13, 15, 20, 22).

There are more than 80 species in the *Alternaria* genus. They are commonly found in water, plants and food as well as on the skins of animals and humans as saprophytes (12). They can also be the agent for dermatomoycoses (2, 8). Although Alternariosis is commonly reported as skin Alternariosis in humans, it is also reported to be the cause of hypersensitivity, pneumonitis, bronchial asthma, allergic sinusitis, rhinitis and disseminated infection (4, 8, 21). Animals with *Alternaria* infection can contaminate their surroundings with fungal spores and may infect other animals and people (16, 19).

Alternariosis is most commonly reported from cats. In cats, infection is mostly seen as slow growing painless nodules on the face, nose and less commonly on other parts of the body (11, 14, 18). Alternariosisis less commonly reported from dogs as purulant ulcerative lesions with scabs and erythrema located in different parts of the body (6, 23). Alternariosis was very rarely reported from other animals (3, 5). No reports from Turkey on *Alternaria* infection in dogs were found.

In this work, a ten year old dog kept in a house was presented to the laboratory with severe pruritus and redness on outer skin of auricle, ventral abdomen and joints. In the examinations, lesions with irregular borders and erythrema, scabbing and skin thickening were seen. Lesions were evaluated by parasitologists for mange but were found to be negative.

Skin scrapings and hair samples were taken from the lesions. From a portion of these samples, smears were prepared with 20% KOH and were examined under light

microscope. Thick walled, septate fungal hyphae were detected in the impression smears. From the remaining samples inoculations were made on Blood Agar (LabM) and Sabouraud Dextrose Agar (SDA) (Oxoid). Blood agar was incubated under 37ºC one week and SDA plates were incubated in 25ºC for 3 weeks. While there was no growth on Blood Agar, on SDA pale grey colonies with aerial mycelia were grown on the fifth day.

The surface of colonies were initially grey white and then became greyish black with a light border, the reverse of the colonies were dark brown. Lacto-phenol cotton blue mount preparations were prepared from the colonies and septate brown hyphae, and erect, brown, multicellular conidiophores, producing unbranched, club shaped, conidial chains were seen. The conidia had a round base, a short cylindrical beak and muriform septation (Figure 1). With these morphological characteristics the fungus was identified as *A. alternata*.

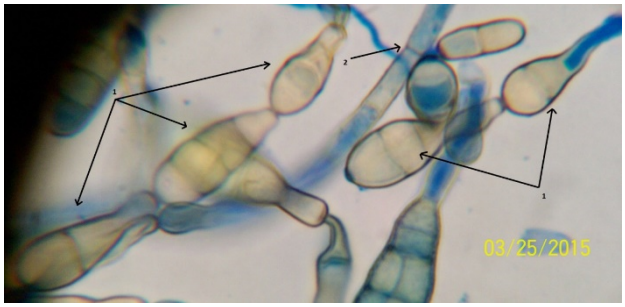

Figure 1. The appearence of slide of *A. alternaria* from a colony grown on SDA plate and stained with lactophenol blue under light microscopy (x40): Conidia specific to *A. alternata* (arrow 1), (the conidia of *Alternaria alternata* with a round base, a short, cylindrical beak) and muriform septation (arrow 2).
Şekil 1. SDA plaklarda üreyen *A. alternaria* kolonisinden laktofenol pamuk mavisi ile boyanan preparatın ışık mikroskopundaki görüntüsü (x40): *A. alternata*'ya özgü konidyumlar (ok 1) *Alternaria alternata*'nın enine ve boyuna bölmeli ve hife tutunan kısmı yuvarlak tabanlı, kısa, silindirik gaga şeklinde olan konidyumları (ok 2).

To ensure the diagnosis, skin samples were taken twice with two day intervals and *A. alternata* was isolated from these samples as well. Antifungal susceptibility test was performed for the isolate on RPMI 1640 medium by E test (AB biodisc) method. The MICs were as follows: Itraconazole, 0.19 µg/mL; voriconazole, 0.19 µg/mL; posaconazole, 0.125 µg/mL (Figure 2). Within the 3 antifungals (itraconazole, voriconazole, posaconazole) to which the agent was susceptible to; itraconazole was chosen for therapy due to its lesser toxicity. Treatment with itraconazole (oral solution) 10 mg/kg was daily started and given for two weeks with marked improvement of the lesions. Then itraconazole was reduced to 5 mg/kg/day and was administered for further two months. At the end of therapy control specimens were taken, and no growth was observed on the cultures.

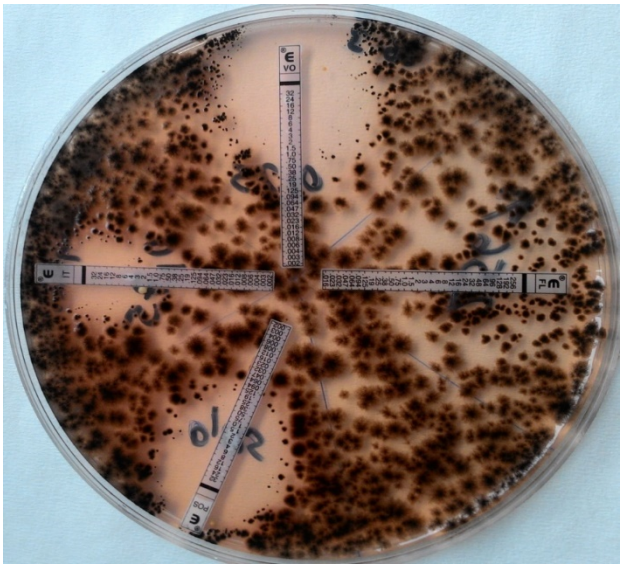

Figure 2. Antifungal susceptibility testing, MIC values. Itraconazole, 0.19 ug/ml; Voriconazole, 0.19 ug/ ml; Posaconazole 0.125 ug/ml; Fluconazole, 256 ug/ml.
Şekil 2. Antifungal duyarlılık testi, MIK değerleri. Itraconazole, 0.19 ug/ml; Voriconazole, 0.19 ug/ ml; Posaconazole 0.125 ug/ml; Fluconazole, 256 ug/ml.

Despite the world-wide distribution of the genus *Alternaria* and its ubiquitous presence in the environment, its infections are rare (15). Most of the reported human cases occured either in patients with severe underlying disease or in those receiving immunosuppressive therapy (1, 2). Only rarely healthy patients are affected (24). In animals, most cases are associated with long term exposure to soil and garbage, a penetrating trauma, or subsequent to a course of steroid therapy. The infection usually manifests as a single poorly circumscribed cutaneous nodules or plaques, redness of the skin, crusts, erosions or ulcerations (3, 6, 9, 17). In the present case, the lesions detected on the skin of the auricle, ventral abdomen and on the joints, as reddness, scaling and thickening of the skin, with severe prurutis. No compatible systemic signs were detected, and there was no underlying disease or immunosuppression. Hence, the lesions could be attributed to a multiple minor traumatic injuries, during grooming or hunting outside.

For the diagnosis of fungal infections, fungal hyphae have to be seen and the fungal agent has to be cultivated in the microbiologic examinations of skin and biopsy samples. As biopsy would have required general anaesthesia, this was not carried out, as the owner of the dog rejected the biopsy. To confirm that *Alternaria* was the aetiologic agent in this case, three consequent specimens were taken from the same lesions and the microscopic findings were the same as for the first specimen, and *A. alternata* was isolated from each of the specimens.

Diseases caused by melanised fungi are difficult to treat, both in humans and animals. There have been reports of many recurrences of the infections due to *Alternaria*, especially in cases with underlying diseases (9, 10). Azoles are the most popular treatment choice despite the necessity of surgery in some cases. Itroconazole is the preferred drug within the azoles due to its low toxicity (7, 8, 24).

In this case, itraconazole therapy was applied and was considered to be succesful as the lesions disappered after the treatment and no fungal agents were isolated from the skin scrapings from the same regions. No significant side effects were observed in the dog during treatment and this corresponds with the reports stating that itraconazole is less toxic than others.

As a result in this presentation, *Alternaria alternata* infection was detected for the first time from a dog in Turkey, antifungal susceptibility testing was carried out and treatment with itracanozole was accomplished.

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Adress for correspondence:
Dr. Meriç Lütfi AVSEVER
Aksaray University, Eskil Vocation of High School,
Laboratory and Veterinary Sciences,
68800, Eskil, Aksaray, Turkey.
Gsm: +90 554 700 61 70
Email: lutfiavsever@gmail.com