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Research Article

Unveiling the Public Health Impact of *Alternaria alternata* carried by Companion Animals in Northeast Portugal

Revelando o Impacto na Saúde Pública de *Alternaria alternata* associada a Animais de Companhia no Nordeste de Portugal

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ABSTRACT

The genus *Alternaria* consists of widely recognised plant pathogens responsible for several important plant diseases, and may also represent prevalent allergens for animals and humans. This study aimed to screen *Alternaria* spp. in dogs and cats in the North of Portugal. Fur samples were collected using the Mackenzie technique and were inoculated in SDA and PDA, and microscopic identification was performed using dichotomous keys. A total of 286 (83.9%) dogs and 55 (16.1%) cats were studied; 45.0% (n = 157) of the samples were from shelters, and 54.0% (n = 184) were from veterinary clinics. From the samples taken from the 341 animals, mould isolation was achieved in 303 of them. *Alternaria alternata* was isolated in 23.75% of studied animals, 22.38% in dogs and 30.91% in cats. Positive samples were analysed according to sex, species, age, origin, and clinical signs. Regarding origin, the lowest prevalence value, 17.8%, was found in the shelter, and the highest, 28.8%, in pet clinics, with these differences being statistically significant (p < 0.000). Young, old, pregnant, or immunocompromised (YOPI) owners are at high risk since there is substantial potential exposure to *A. alternata* by contact with pets and, therefore, a basis for disease transmission.

KEYWORDS: *Alternaria spp.*. Cats. Dogs. Occurrence. Portugal.

RESUMO

O género *Alternaria* é amplamente reconhecido como um conjunto de patógenos vegetais responsáveis por várias doenças importantes em plantas, podendo também representar alergénios prevalentes em animais e seres humanos. Este estudo teve como objetivo analisar a ocorrência de *Alternaria* spp. em cães e gatos no Norte de Portugal. Amostras de pelagem foram recolhidas utilizando a técnica de Mackenzie e inoculadas em SDA e PDA, sendo a identificação microscópica realizada através de chaves dicotómicas. No total, foram estudados 286 (83,9%) cães e 55 (16,1%) gatos. Das amostras, 45,0% (n = 157) eram provenientes de abrigos e 54,0% (n = 184) de Centros de Atendimento Médico-Veterinário (CAMV). Das amostras recolhidas dos 341 animais, em 303 foi possível isolar fungos. *Alternaria alternata* foi isolada em 23,75% dos animais estudados, com uma prevalência de 22,38% em cães e 30,91% em gatos. As amostras positivas foram analisadas de acordo com o sexo, espécie, idade, origem e sinais clínicos. Relativamente à origem, o valor de prevalência mais baixo (17,8%) foi observado em animais de abrigos, enquanto o valor mais elevado (28,8%) foi identificado em CAMV, sendo estas diferenças estatisticamente significativas ($p < 0{,}000$). Indivíduos jovens, idosos, grávidas ou imunocomprometidos (YOPI) apresentam um risco elevado, dado o potencial significativo de exposição a *A. alternata* através do contacto com animais de companhia, representando assim uma base para a transmissão.

PALAVRAS-CHAVE: *Alternaria spp..* Cães. Gatos. Ocorrência. Portugal.

INTRODUCTION

Since the late 20th century, fungi have been recognised as important human pathogens, especially in individuals with primary or acquired immunodeficiency, such as acquired immune deficiency syndrome (AIDS), chemotherapy, and solid organ or bone marrow transplantation (RAYENS & NORRIS 2022).

Alternaria spp. are dematiaceous hyphomycete moulds with hundreds of reported species with worldwide distribution, including many common saprophytes in soil, air, and various other habitats. Some are ubiquitous agents of decay and plant pathogens (THOMMA 2003). *Alternaria alternata* is a common species of the genus *Alternaria*, a ubiquitous filamentous Ascomycete fungus found in various environments, including soil, air, plants, and indoor spaces. While *Alternaria* spp. are primarily involved in decomposing organic matter as saprobes, they can also cause disease in humans and animals by acting as pathogens, leading to infections, toxicosis, and allergic reactions (SÁNCHEZ *et al.* 2022). The genus is frequently involved in human infection. Some species of *Alternaria* can act as pathogens, attacking plants, humans and animals such as cats and, less regularly, dogs (GABRIEL *et al.* 2016, WRIGHT et al. 2020). Evidence suggests that pets, particularly cats and dogs, may serve as carriers for *A. alternata*, harbouring the fungus on their fur and facilitating its transmission through direct contact or by dispersing fungal spores into the environment. This can increase human exposure, especially in domestic settings where close contact with pets is common (KHOSRAVI 1996, SEYEDMOUSAVI *et al.* 2018, MARTINS 2022). *Alternaria alternata* is frequently isolated from plants as both a pathogen and an endophyte and in the soil as a saprophyte (DeMERS 2022), and is detected in most (95–99%) of dust samples in homes, where infrequent cleaning and smoking indoors also contributed to higher *Alternaria* antigen levels (SALO *et al.* 2005).

While alternariosis has been described in both healthy and immunocompromised people, its prevalence has been increasing, especially in the latter, due to risk factors such as hematopoietic stem cell and solid organ transplantation, immunosuppressive medication (i.e., long-term corticosteroid treatments), diabetes, human immunodeficiency virus (HIV), and diseases that increase cortisol levels, such as Cushing syndrome (WRIGHT *et al.* 2020). This phaeohyphomycosis can manifest as various conditions such as mycosis, oculomycosis, onychomycosis, allergic rhinosinusitis, hypersensitivity pneumonitis, allergic bronchopulmonary mycosis, paranasal sinusitis (sometimes complicated with osteomyelitis), peritonitis in patients undergoing dialysis, and more rarely as fungal sepsis (SCHELL 2000, HATTAB *et al.* 2019, NOGUEIRA *et al.* 2019, LIU *et al.* 2021).

Alternaria may be found in the normal skin and conjunctiva of both animals and people and is often recognised as a contaminant in conventional laboratory mycological analyses (PESIC *et al.* 2015).

The identification of *Alternaria* only relies on morphological criteria: the morphology of conidia and the possibility of conidial chain formation, although there are other genera like *Ulocladium*, which seldom cause infections, that are morphologically similar to *Alternaria*. *Ulocladium* conidia are attenuated at the base, mature conidia are widely ellipsoidal, *Alternaria* conidia are rounded at the base, and mature conidia are obclavate and rostrate. Fungal culture is required for *Alternaria* identification, and these fungi grow in most conventional laboratory media. Compared to other laboratory media, where clinically significant species lose their capacity to sporulate, potato-carrot agar is a suitable culture medium for achieving excellent sporulation of isolates from clinical specimens (PASTOR & GUARRO 2008). These moulds are fast-growing colonies with dark colours ranging from grey to olive-brown. The surfaces of mature colonies may seem "downy to woolly" or fuzzy due to the presence of many hyphae. Microscopically, brown septate hyphae create septate conidiophores. Conidia can appear in chains or alone and be tiny or enormous. Interestingly, compared to those produced on agar medium, conidia in their natural habitats are bigger and more uniform, with extended tips (KUSTRZEBA-WÓJCICKA *et al.* 2014).

Alternaria alternata hypersensitivity is becoming increasingly well-known due to its role in the development and exacerbation of asthma and the induction of immunoglobulin E (IgE)-mediated respiratory disorders. Examining IgE antibodies to mould antigens validates the measure of potential allergic reactivity to mould, which may be done *in vivo* or *in vitro* (BUSH et al. 2006). *Alternaria* spores are an abundant and powerful source of allergens in the air (GABRIEL *et al.* 2016, KUSTRZEBA-WÓJCICKA *et al.* 2014).

According to the World Health Organization (WHO) and the International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-committee (2023), there are 12 allergens as results for *A. alternata*, all with airway route of exposure (aeroallergen). *A. alternata* stimulates airway epithelial cells, causing increased production of epithelial mucus and peribronchial infiltration, all of which are symptoms/signs of asthma. *Alternaria* proteolytic activity induces and activates human eosinophil degranulation, correlating with asthma and allergies' prevalence, severity, and existence (KUSTRZEBA-WÓJCICKA *et al.* 2014, GABRIEL *et al.* 2016).

The most critical *Alternaria* toxins, which usually contaminate foods during storage, are tenuazonic acid, altertoxins, altenuene, tentoxin, alternariol methyl ether, and alternariol (SIVAGNANAM *et al.* 2017, HABIB *et al.* 2021). More than 70 secondary metabolic compounds and over 30 mycotoxins, which belong to various classes based on their chemical structure, are produced by *Alternaria* spp.

The toxin tenuazonic acid is responsible for causing acute toxicity in dogs, chickens, and mice, as well as haematological disorders in humans (AWUCHI *et al.* 2021). Although most toxins show low acute toxicities, exposure to *Alternaria* mycotoxin has been reported to possess cytotoxic, carcinogenic, mutagenic, and genotoxic properties in animals and humans (PAVÓN MORENO *et al.* 2012).

When *A. alternata* was exposed to the major greenhouse gas responsible for global warming (CO_2) at concentrations of 0.05% and 0.06% (500 and 600 mmol/mol), these fungi generated nearly three times the number of spores and twice the total antigenic fungal proteins (DEMAIN *et al.* 2021, MAGYAR *et al.* 2021). Moreover, O3 exposure increases airway permeability, promotes airway inflammation, reduces lung function, and increases the likelihood of asthma exacerbation. This is especially problematic during the summer, when ground-level O3 levels are most significant due to the heat (DEMAIN *et al.* 2021).

Studies in skin microbiomes have the potential to provide insights into the critical thresholds that differentiate between normal colonisation and the onset of disease (MEASON-SMITH *et al.* 2015, DWORECKA-KASZAK *et al.* 2020).

The prevalence of *A. alternata* in the fur of pets or animals from shelters has not been found in recent literature. Many of these animals can be adopted, go to people's homes, and meet children and adults. Thus, this study aims to determine the prevalence of *A. alternata* in the fur of companion animals (dogs and cats) in shelters and clinics in northeastern Portugal.

MATERIAL AND METHODS

A structured questionnaire was used to gather comprehensive data on each animal, focusing on several key variables, including species, sex, age, breed, body condition, and clinical signs of skin health. These variables were selected for their potential association with the prevalence of *Alternaria alternata*. All resident animals in the shelters were included in the study, ensuring a comprehensive sample from these environments. In the veterinary clinics, animals were selected based on the willingness of their owners to participate during the study period. Participation was voluntary, and animals with a known history of fungal or bacterial infections or currently undergoing dermatological treatment were excluded to minimise bias. In total, 286 dogs and 85 cats were included, representing all resident dogs and cats from the shelters and those whose owners consented in the veterinary clinics.

Additionally, all animals underwent a clinical examination to rule out active infections or dermatopathies. The shelters and clinics maintained good hygiene protocols, including regular cleaning and disinfection measures to control the spread of pathogens. These facilities also followed standard animal care procedures, ensuring a clean environment for the animals. While the shelters had higher animal density than the clinics, both settings were managed under appropriate sanitation standards, which were considered in the analysis of the fungal prevalence.

During the sampling procedures, a thorough clinical examination was conducted, including a detailed inspection of all skin surfaces, with particular attention to the ears, head and body hairs. The examination aimed to detect clinical signs such as erythema, vesicles or pustules, erosion, scaling, and hyperkeratosis, from ringworm infection. Skin scrapes or hairs of each animal were collected using cotton swabs or sterilised forceps. Hair with the root end was plucked and sent to the Laboratory of Medical Microbiology, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Vila Real, Portugal.

The culture was performed in Potato Dextrose agar medium (PDA) and Sabouraud Dextrose Agar (SDA) (Oxoid®). The material was incubated for 5–$pdaff$7 days at 25°C. Each mould was subcultured in PDA for sample maintenance. The purely obtained fungal isolates were grown on PDA to observe mycological features. Colonies were subjected to lactophenol (cotton-blue) staining. The fungi were identified by their macro and microscopic morphological characteristics based on the referring identification and online tools.

Demographic categories analysed in the study included species (dogs and cats), sex (male and female), age (juvenile and adult), and origin (shelter or clinic). These variables were selected to evaluate potential differences in the prevalence of *A. alternata* across diverse animal populations. For example, age groups were defined based on life stages relevant to immune function. Sex was included as a factor due to potential hormonal influences on susceptibility to fungal infections. The origin (shelter or clinic) was considered because different living conditions may affect exposure to environmental fungi.

Differences in fungi prevalence among demographic categories were compared with a chi-square (χ^2) ($p<0.05$) for statistical significance. In prevalence, 95% confidence intervals (CI) were calculated. Statistical analyses were performed using descriptive and analytical statistics with a 95% confidence interval (CI). Chi-squared (χ^2) tests were applied to compare demographic variables and the prevalence of *A. alternata* infection. All analyses were conducted using the Statistical Package for the Social Sciences (SPSS®, International Business Machines Corporation [IBM], Armonk, New York [NY], United States of America [USA]) version 25.0 for Windows. A *p*-value less than 0.05 was considered statistically significant.

The fungus was then examined under a microscope by staining with lactophenol cotton blue. The fungal characteristics, including size, shape, and arrangement of microconidia and macroconidia, were observed under 100× and 400× magnification.

This study was approved by the ethics committee of UTAD, Portugal (Doc6-CE-UTAD-2022).

RESULTS

A total of 286 (83.9%) dogs and 55 (16.1%) cats were studied; 45.0% (n = 157) of the samples were from shelters, and 54.0% (n = 184) were from clinics. From the samples taken from the 341 animals, mould isolation was achieved in 303.

In culture (Figure 1), the surface appeared initially grey-white but gradually turned greyish-black with a light border. The reverse of the colonies showed a dark brown colour. In optical microscopy (Figure 2), colonies revealed septate brown hyphae and erect, brown, multicellular conidiophores that produced unbranched, club-shaped conidial chains. The conidia presented a round base, a short, cylindrical beak, and muriform septation. The microbiological characteristics observed led to the identification of the fungus as *A. alternata*.

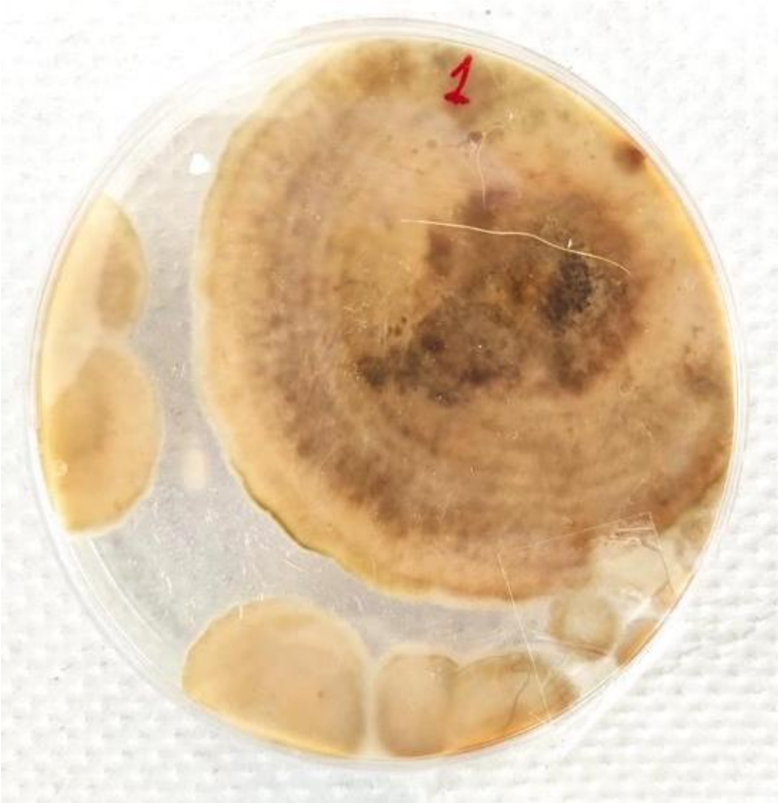

Figure 1. *Alternaria* spp. PDA culture.

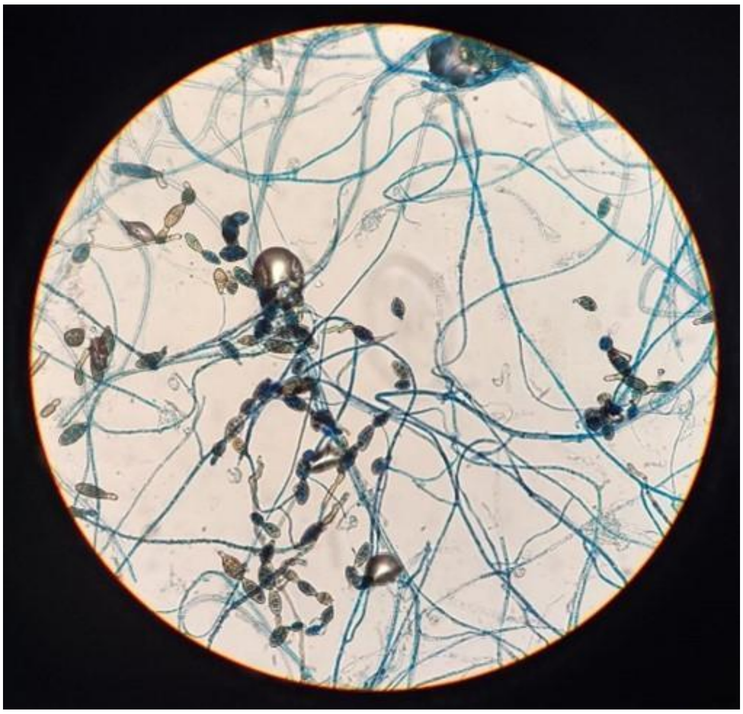

Figure 2. *Alternaria alternata* stained with Lactophenol Cotton Blue observed at 400x magnification.

Alternaria alternata was isolated in 81/341 of the studied animals. The prevalence of *A. alternata* was 23.8% (*n* = 81; 95% CI: 19.3-28.6%).

Positive samples, based on sex, species, age, origin, and clinical signs, are examined in Table 1. The prevalence values among males and females were 23.42% (95% CI: 17.06–30.80%) and 24.04% (95% CI: 18.05–30.90%), respectively (*p* = 0.892) (Table 1).

Table 1. Screening for *A. alternata* in dogs and cats from Portugal.

Variable	Animals (*n*)	Positive (*n*)	Prevalence	95% CI (%)	*p* value
Sex					0.892
Male	158	37	23.4	17.1–30.8	
Female	183	44	24.0	18.1–30.9	
Species					0.184
Dogs	286	64	22.4	17.7–27.7	
Cats	55	17	30.9	19.2–44.8	
Age					0.994
Juvenil	118	28	23.7	16.4–32.4	
Adult	223	53	23.8	18.3–29.9	
Origin					
Shelter	157	28	17.8	12.2–24.7	< 0.000
Pet clinics	184	53	28.8	22.4–35.9	
Lesions on the skin					0.872
Presence	28	7	25.0	10.7–44.9	
Absence	313	74	23.6	19.0–28.8	
Total	341	81	23.8	19.3–28.6	

This mould was detected in 64/286 dogs and 17/55 cats. Regarding demographic variables, the prevalence of *A. alternata* was higher in cats (30.9%) compared to dogs (22.4%), though this difference was not statistically significant (p = 0.184). Age did not show a significant impact, with similar prevalence rates between juveniles (23.7%) and adults (23.8%) (p = 0.994). Additionally, the origin of the animals played a significant role, with a higher prevalence in animals from clinics (28.8%) compared to shelters (17.8%) (*p* < 0.000). These differences highlight the importance of considering environmental factors, such as hygiene protocols and population density, in the analysis. There was no significant difference in positivity results between lesions in the skin related to the presence (25.0%; 95% CI: 10.7–44.9) and absence (23.6%; 95% CI: 19.0–28.8) in the studied species (*p* = 0.872) (Table 1).

Prevalence of Alternaria alternata in dogs

Alternaria alternata was isolated from 64/286 (22.4%; 95% CI: 17.7-27.7%) dogs.

Positive samples in dogs according to sex, age, breed, origin, and clinical signs examined are presented in Table 2. The prevalence values among males and females were 22.3% (95% CI: 15.7–30.1) and 22.5% (95% CI: 16.0–30.1), respectively (*p* = 0.976) (Table 2).

Alternaria alternata was detected in 18/90 juveniles and 46/196 adults. The prevalence in juveniles (20.0%; 95% CI: 12.3–29.8) was lower than in adults (23.5%; 95% CI: 17.7–30.0), but the difference was not statistically significant (*p* = 0.510). Regarding breed, the prevalence found in mongrel dogs was 22.7% (95% CI: 17.8–28.2), and in other breeds was 17.7% (95% CI: 3.8–43.4), but the difference was not statistically significant (*p* = 0.620). Regarding origin, the lowest value of prevalence

(13.9%; 95% CI: 8.3-21.4%) was found in the shelter, and the highest (28.7%; 95% CI: 21.9-36.2%), in pet clinics (Table 2), and the difference was statistically significant (*p* = 0.003).

There was no significant difference in positivity results between lesions in the skin related to the presence (30.0%; 95% CI: 11.9–54.3%) and absence (21.8%; 95% CI: 17.0–27.3%) in the studied species (*p* = 0.412) (Table 2).

Table 2. Individual *A. alternata* prevalence in dogs in Portugal (n = 286).

Variable	Animals (*n*)	Positive (*n*)	Prevalence	95% CI (%)	*p* value
Sex					0.976
Male	139	31	22.3	15.7–30.1	
Female	147	33	22.5	16.0–30.1	
Age					0.510
Juvenile	90	18	20.0	12.3–29.8	
Adult	196	46	23.5	17.7–30.0	
Breed					0.620
SRD	269	61	22.7	17.8–28.2	
Other	17	3	17.7	3.8–43.4	
Origin					0.003
Shelter	122	17	13.9	8.3–21.4	
Pet clinics	164	47	28.7	21.9–36.2	
Lesions on the skin					0.412
Presence	20	6	30.0	11.9–54.3	
Absence	266	58	21.8	17.0–27.3	
Total	286	64	22.4	17.7–27.7	

Prevalence of Alternaria alternata in cats

In cats, *Alternaria alternata* was isolated in 17/55 (30.9%; 95% CI: 19.2–44.8) of the studied animals.

Positive samples in cats according to sex, age, breed, origin, and clinical signs examined are presented in Table 3. The prevalence values among males and females were 31.58% (95% CI: 12.6–56.6) and 30.6% (95% CI: 16.4–48.1), respectively (*p* = 0.938) (Table 3).

Table 3. Individual *A. alternata* prevalence in cats in Portugal (n = 55).

Variable	Animals (*n*)	Positive (*n*)	Prevalence	95% CI (%)	*p* value
Sex					0.938
Male	19	6	31.6	12.6–56.6	
Female	36	11	30.6	16.4–48.1	
Age					0.431
Young	28	10	35.7	18.6–55.9	
Adult	27	7	25.9	11.1–46.3	
Breed					0.655
European shorthair	14	5	35.7	12.8–64.9	
Other	41	12	29.3	16.1–45.5	
Origin					0.912
Shelter	35	11	31.4	16.9–49.3	
Pet clinics	20	6	30.0	11.9–54.3	
Lesions on the skin					0.191
Yes	8	1	12.5	0.0–52.7	
No	47	16	34.0	20.9–49.3	
Total	55	17	30.9	19.2–44.8	

Alternaria alternata was detected in 10/28 juveniles and 7/27 adults. The prevalence in juveniles (35.7%; 95% CI: 18.6–55.9) was higher than in adults (25.9%; 95% CI: 11.1–46.3), but the difference was not statistically significant (*p* = 0.431). Regarding breed, the prevalence found in European shorthair was 35.7% (95% CI: 12.8–64.9%), and in other breeds was 29.3% (95% CI: 16.1–45.5%), but; the difference was not statistically significant (*p* = 0.655). There was no significant difference in positivity results among origins related to shelter (31.4%; 95% CI: 16.9–49.3) and pet clinics (30.0%; 95% CI: 11.9–54.3) in the studied species (*p* = 0.912).

Regarding clinical signs in the skin, the lowest value of prevalence (12.5%; 95% CI: 0.0–52.7) was found in the presence of lesions, and the highest (34.0%; 95% CI: 20.9–49.3) in the absence of skin lesions (Table 3), but these were not statistically significant differences (*p* = 0.191).

DISCUSSION

The results of this study demonstrate a significantly higher prevalence of *A. alternata* (23.8%) in companion animals compared to previous reports in the literature, which typically indicate a prevalence of approximately 13%. This finding is noteworthy, suggesting that certain local environmental conditions or animal management practices in Northeast Portugal may have contributed to this elevated prevalence.

Concerning fungal allergies, *Alternaria* and *Aspergillus* are likely the most important genera of moulds affecting both humans and animals (MARTINS 2022). Although Basidiomycota is responsible for most aerosol spores, Ascomycota, which accounts for only 4% of airborne fungi, is significantly associated with allergic rhinitis and asthma (PATEL *et al.* 2018). The dominant allergenic fungal genera, ranked in order of frequency, are *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp., and *Alternaria* spp. These fungal spores have been observed to exacerbate symptoms in individuals diagnosed with asthma, particularly children (HUGHES *et al.* 2022). There was no distinction between urban and rural areas in Portugal regarding the abundance of fungal types (OLIVEIRA *et al.* 2010). *Alternaria alternata* is accepted as the most critical fungal species causing sensitisation, especially in the Mediterranean area (QUESADA 2022).

According to the revised literature, very few epidemiological studies cover the comparison of *A. alternata* in the fur of pets according to demographic variables, complicating the comparison of results. The demographic variables analysed in this study provide important insights into the distribution of *A. alternata*. The higher prevalence in cats than dogs may be linked to differences in grooming behaviour or immune responses between species. Similarly, while age did not significantly affect prevalence, other studies suggest that younger animals may be more susceptible to fungal infections due to an immature immune system. The statistically significant difference in prevalence between animals from clinics and shelters underscores the impact of living conditions on fungal exposure, with clinics likely benefiting from more controlled environments and individual veterinary care.

Alternaria spp. has long been identified as part of the normal fungal mycobiota of dogs (PHILPOT & BERRY 1984) and cats (MORIELLO & DeBOER 1991). The prevalence of *A. alternata* was high, indicating that pet owners or adopters may be at potential risk from this allergen.

A study conducted by DWORECKA-KASZAK (2013) in Poland in animals with clinical signs found that *A. alternata* was present in 13.86% of the animals sampled.

In 2017, *A. alternata* infection was reported for the first time from the skin lesions of a dog in Turkey (AVSEVER *et al.* 2017). In the present study, *A. alternata* was also detected in the skin lesions of a female dog from a shelter.

Our study found a prevalence of *A. alternata* of 23.8%, significantly higher than that reported in previous studies. This discrepancy could be attributed to several factors. First, the specific geographic region where the study was conducted (Northeast Portugal) may have environmental conditions that favour the growth and dissemination of *A. alternata*. Additionally, the differences in hygiene protocols, animal density, and the types of animals studied (shelters versus clinics) could also have contributed to the higher prevalence observed.

Furthermore, previous studies may have used different sampling techniques or targeted different populations, which could explain the variation in results. Our findings highlight the need for further research into regional and environmental factors that could influence the presence of fungal pathogens in companion animals. The high prevalence of *Alternaria alternata* identified in this study reflects the need for enhanced control strategies, similar to those applied for other zoonotic agents, such as Leishmania infantum, *Ehrlichia canis*, and *Rickettsia conorii*, where differences in seroprevalence between shelter and domestic animals highlight the influence of environmental and management conditions (AFONSO *et al.* 2023; 2024c).

These findings underline the importance of evaluating not only pathogen presence but also the role of population density and hygiene protocols, as evidenced in dermatophyte carriage studies conducted in Northern Portugal (AFONSO *et al.* 2024b). Furthermore, the risk of opportunistic infections is particularly concerning in immunocompromised animals, such as those infected with Feline Immunodeficiency Virus (FIV) and Feline Leukaemia Virus (FeLV), which demonstrate higher susceptibility to secondary fungal infections (AFONSO *et al.* 2024a). This reinforces the importance of robust environmental control measures, especially in high-density settings like shelters. Adopting a One Health approach is crucial for managing zoonotic risks, integrating public health, veterinary medicine, and environmental monitoring to mitigate exposure to pathogens in companion animals and humans (AFONSO *et al.* 2023; 2024a; 2024b; 2024c; 2025).

In the present study, 30.9% of the cats under investigation were isolated *A. alternata*. The mould was isolated in 31.4% of animals from shelters and 30.0% of cats that attended the clinics. Cats are becoming increasingly popular as pets and companion animals. Every year, thousands of mixed cats in Europe are abandoned and available for adoption at a low cost from animal shelters. It is also fashionable to purchase expensive purebred cats from breeding units. In both situations, animals are acquired from communities and may transmit diseases to humans, whether visible or not (SEYEDMOUSAVI *et al.* 2018). Indeed, *Alternaria* spp. was one of the most saprophytic fungal isolates from the hairs of stray cats in Iran (KHOSRAVI 1996).

In six cats in the United Kingdom, *A. alternata* was culture-confirmed nasal infection (DYE *et al.* 2009).

In a study conducted in Portugal, nine out of 21 horses with a clinical history of allergy showed positive results for intradermal tests (IDT) for *A. alternata*. (LEOCÁDIO *et al.* 2019). Dermatomycoses caused by *Alternaria tenuissima* were reported by TYCZKOWSKA-SIEROŃ & GŁOWACKA (2013) in two Shetland ponies and a Beagle dog (TYCZKOWSKA-SIEROŃ & GŁOWACKA 2013). Equine dermatitis with cutaneous fungal granulomas has been described in international veterinary literature for many years, with several authors noting the presence of *Alternaria* fungi in skin nodule biopsies (GENOVESE *et al.* 2001, DICKEN *et al.* 2004, VALENTINE *et al.* 2006).

The diversity of airborne spores fluctuates based on local meteorological and geographical factors. Still, specific genera commonly associated with allergies, including *Alternaria* spp., *Aspergillus* spp., *Cladosporium* spp., and *Penicillium* spp., are widespread (HUGHES *et al.* 2022). While our study focused on the prevalence of *A. alternata* in companion animals, we did not directly assess the role of these environmental variables. The geographic region where the study was conducted, Northeast Portugal, may have ecological characteristics that influence the dissemination of fungal spores. Future studies should include monitoring airborne spore diversity and environmental conditions to better understand their impact on fungal pathogens' prevalence in animals.

Active asthma symptoms in humans are linked to the presence of *A. alternata* in homes across the United States of America (USA) (SALO *et al.* 2006).

Alternaria alternata is a significant contributor to fungal allergies, which often result in severe hypersensitivity reactions in the airways, negatively impacting the health and quality of life of susceptible individuals. Although traditionally associated with childhood asthma, its prevalence is increasing among adults. Understanding the mechanisms by which this mould induces allergic symptoms and triggers lung inflammation before the onset of asthma is essential (HERNANDEZ-RAMIREZ *et al.* 2021).

Despite ongoing efforts to identify *Alternaria* as a cause of respiratory allergic inflammation, questions remain regarding the specific effects of its allergenic components, particularly its primary allergen, Alt a 1. A thorough comprehension of the immunological mechanisms triggered by this mould is crucial to improving diagnosis and developing effective treatment formulations. Consequently, *A. alternata* is one of the most common allergens implicated in asthma (GABRIEL *et al.* 2016). In 2019, asthma accounted for 21.6 million disability-adjusted life years (DALYs), equivalent to 20.8% of the total DALYs attributed to chronic respiratory disease, ranging from 17.1 to 27.0 million. Asthma death rates were highest in low and middle socio-demographic index (SDI) countries. In contrast, the prevalence of asthma was higher in high-SDI countries (GBD 2019 DISEASES AND INJURIES COLLABORATORS 2020).

Among adults worldwide, the prevalence rates of doctor-diagnosed asthma, clinical/treated asthma, and wheezing were 4.3%, 4.5%, and 8.6%, respectively. The prevalence of asthma wheezing was 13.7% in adolescents (13-14 years) and 11.6% in children (6-7 years) (ASHER *et al.* 2020). These findings highlight the urgent need for effective prevention and management strategies to address the growing burden of asthma ("THE GLOBAL ASTHMA REPORT 2022," 2022).

Furthermore, asthma and related respiratory diseases are a significant concern in veterinary medicine, affecting various animal species. Feline asthma, for example, is a common respiratory disease affecting cats, with an estimated prevalence of 1-5% in the general cat population (Trzil 2020). Canine asthma has also been reported to occur naturally, although this condition is less commonly diagnosed and infrequently, so that detailed studies have not yet been conducted (CHAPMAN 2008). In horses, substantial evidence supports the association between exposure to environmental dust and the pathophysiology of mild and severe Equine Asthma; however, the possible involvement of infectious agents, such as bacteria and viruses, remains unclear (COUETIL *et al.* 2020).

Unlike other allergens, such as house dust mites, cat dander, or grass pollen, which only contain allergenic protein, fungi have the added ability to actively germinate and infect the host skin or attempt to colonise the respiratory tract. As a result, fungi may significantly impact individuals by triggering host defences against pathogens and producing non-allergen toxins and enzymes that may contribute to allergy development (DENNING 2006). Potential fungal triggers for human asthma include *Curvularia*, *Helminthosporium*, *Alternaria*, *Puccinia*, *Epicoccum*, and *Fusarium*. Evidence has begun to emerge demonstrating a solid association between fungal sensitisation and the severity of asthma (DENNING 2006).

Allergens are natural substances that can trigger IgE-mediated allergic reactions in humans. Among these, *Alternaria* spp. are known to cause type 1 hypersensitivity reactions, leading to various respiratory illnesses. The indoor environment is a significant source of allergens, with the bakery, poultry, granary, and sugar industries being the primary contributors. Numerous fungi, including *Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, *Epicoccum*, *Pullularia*, *Ganoderma*, *Trichoderma*, and *Stemphylium*, are recognised as common allergens (KUMAR *et al.* 2021). Across-sectional study involving 1,132 adults with asthma found that sensitisation to *A. alternata* (odds ratio [OR]: 2.03) is a significant risk factor for severe asthma (both 2.34) in various European countries, as well as in Australia, New Zealand, and Portland (Oregon, USA) (ZUREIK 2002).

Individuals with severe asthma who had been admitted to the hospital with acute asthma at least twice showed a higher likelihood of being positive in skin tests for at least one of the moulds, such as A. fumigatus, Penicillium notatum, C. herbarum or *A. alternata*, or for the yeast Candida albicans (76%) compared to individuals with moderate or mild asthma (16-19%; $p<0.0001$) (O'DRISCOLL *et al.* 2005).

Alternaria is the primary allergen responsible for the development of asthma in children raised in semiarid environments, and skin test responses at the age of 6 years are more strongly associated with asthma than those at the age of 11 years (HALONEN *et al.* 1997). This evidence has continued accumulating in recent years, supporting the link between fungal exposure and asthma exacerbations (HUGHES *et al.* 2022). Despite concerns about hygiene and a general lack of awareness of the associated zoonotic risks and how to protect against them, an increasing number of pets are being allowed in the bedroom or even in bed and having intimate contact with their owners or personal utensils (DO VALE *et al.* 2020 e 2021, MORGADO *et al.* 2022, ZANEN *et al.* 2022).

It is not only sleeping with pets that exposes owners to any pathogens that they might be carrying. Exposure can occur simply by allowing them into the home and petting them. There needs to be better consciousness of how physical contact and fur can be potential transmission pathways (DO VALE *et al.* 2021). This means that, in addition to all infectious agents, and according to the results of this study, there is significant potential exposure to *A. alternata*. These behaviours can create a basis for disease transmission from pets, even in the presence of serious threats such as fungal, bacterial or viral infection. This is particularly risky for YOPI owners, who are at a greater risk of developing infections (DO VALE *et al.* 2021, ZANEN *et al.* 2022).

Our findings highlight the importance of considering environmental and host-related factors in the epidemiology of fungal infections. Given the zoonotic potential of *A alternata*, especially in YOPI individuals, these results have significant public health implications. Further research should focus on identifying specific environmental conditions favouring fungal growth and determining the best strategies to mitigate these risks in companion animals and human populations.

CONCLUSION

The results of this study demonstrate a considerable prevalence of *Alternaria alternata* in dogs and cats in Northeast Portugal, significantly higher than previously reported in the literature. This difference may be associated with specific environmental factors in the region, animal management practices, and differences in hygienic-sanitary conditions between shelters and veterinary clinics. The significantly higher prevalence in animals from clinics (28.8%) compared to those from shelters (17.8%) may reflect distinct environmental conditions, population density, and the more efficient and rigorous application of hygiene protocols in shelters.

The relevance of *A. alternata* as a potential agent of respiratory allergies and fungal infections in humans, particularly in YOPI, underlines the need for strategies to mitigate the risk of exposure. Direct contact with companion animals carrying this fungus may represent a relevant transmission route, especially in domestic settings where proximity between humans and animals is frequent. These findings are consistent with the literature, which describes *A. alternata* as an essential allergen in indoor environments and a relevant agent in respiratory diseases.

Given the ability of this fungus to act as a significant allergen and potential pathogen, and considering its high prevalence in companion animals in the studied region, it is crucial to raise awareness among pet owners and veterinary health professionals regarding the implementation of good hygiene practices, environmental control, and periodic monitoring of animals. Furthermore, future research should explore the specific ecological conditions that favour the proliferation of *A. alternata* in companion animals and identify risk factors associated with the increased prevalence in urban and rural environments.

These findings reinforce the importance of the One Health approach in managing risks associated with fungal zoonoses, promoting the integration of public health measures, animal welfare, and environmental control to minimise the impact of *A. alternata* on human and animal health.

NOTES

AUTHOR CONTRIBUTIONS

Conceptualisation, methodology, and formal analysis**, PA, HQ, LC and ACC**; software and validation**, PA, HQ, LC and ACC;** investigation**, PA, DS, AFV and MM**; resources and data curation**, PA, HQ, LC and ACC**; writing-original draft preparation**, PA**; writing-review and editing**, PA, DS, AFV, MM, HQ, LC and ACC**; visualisation**, PA, HQ, LC and ACC**; supervision, **HQ, LC and ACC**; project administration, **PA, HQ, LC and ACC**; funding acquisition, **HQ, LC and ACC**. All authors have read and agreed to the published version of the manuscript.

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INSTITUTIONAL REVIEW BOARD STATEMENT

This study was approved by the ethics committee of UTAD, Portugal (Doc6-CE-UTAD-2022).

INFORMED CONSENT STATEMENT

Not applicable because this study did not involve humans.

DATA AVAILABILITY STATEMENT

The data can be made available upon request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

AFONSO P et al. 2023. Leishmania seroprevalence in dogs: comparing shelter and domestic communities. Animals. 13: 2352.

AFONSO P et al. 2024a. Whisking away the veil: exploring feline immunodeficiency virus and feline leukaemia virus prevalence among shelter cats in Portugal. Veterinarska Stanica. 55: 503–513.

AFONSO P et al. 2024b. Furry hosts and fungal guests: investigating dermatophyte carriage in shelter and clinic cats and dogs of northern Portugal. Veterinarski Glasnik. 78: 28–46.

AFONSO P et al. 2024c. Ehrlichia canis and Rickettsia conorii infections in shelter dogs: seropositivity and implications for public health. Pathogens. 13: 129.

AFONSO P et al. 2025. Health Sentinels: Canine Parvovirus and Coronavirus in Portuguese Shelter Dogs. Veterinarska Stanica. 56: 39–56.

ASHER MI et al. 2020. Trends in worldwide asthma prevalence. Eur Respir J. 56: 1-14

AVSEVER ML et al. 2017. First isolation of *Alternaria alternata* from a dog in Turkey. Ankara Üniv Vet Fak Derg. 64: 137–139.

AWUCHI CG et al. 2021. Mycotoxins affecting animals, foods, humans, and plants: types, occurrence, toxicities, action mechanisms, prevention, and detoxification strategies—a revisit. Foods 10: 1-48.

BUSH RK et al. 2006. The medical effects of mold exposure. Journal of Allergy and Clinical Immunology. 117: 326–333.

CHAPMAN RW. 2008. Canine models of asthma and COPD. Pulmonary Pharmacology & Therapeutics. 21: 731–742.

COUETIL L et al. 2020. Equine asthma: current understanding and future directions. Frontiers in Veterinary Science. 7: 1-21.

DEMAIN JG et al. 2021. The impact of climate change on the pollen allergy and sporulation of allergic fungi. Current Treatment Options in Allergy. 8: 60–73.

DeMERS M. 2022. *Alternaria alternata* as endophyte and pathogen. Microbiology 168: 1-15.

DENNING DW. 2006. The link between fungi and severe asthma: a summary of the evidence. European Respiratory Journal 27: 615–626.

DICKEN M et al. 2010. Cutaneous fungal granulomas due to *Alternaria* spp. infection in a horse in New Zealand. N Z Vet J. 58: 319–320.

DO VALE B et al. 2020. Systematic review on infection and disease caused by *Thelazia callipaeda* in Europe: 2001–2020. Parasite 27: 1-13.

DO VALE B et al. 2021. A cross-sectional study of knowledge on ownership, zoonoses and practices among pet owners in northern Portugal. Animals 11: 1-19.

DWORECKA-KASZAK B. 2013. Fungi from *Alternaria* genus–a new skin pathogen. Magazyn Wet. 22: 654–656.

DWORECKA-KASZAK B et al. 2020. Occurrence of various pathogenic and opportunistic fungi in skin diseases of domestic animals: a retrospective study. BMC Veterinary Research. 16: 1-8.

DYE C et al. 2009. *Alternaria* species infection in nine domestic cats. Journal of Feline Medicine and Surgery 11: 332–336.

GABRIEL MF et al. 2016. *Alternaria alternata* allergens: Markers of exposure, phylogeny and risk of fungi-induced respiratory allergy. Environment International 89–90: 71–80.

GBD 2019 DISEASES AND INJURIES COLLABORATORS. 2020. Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet 396: 1204-1222.

GENOVESE LM et al. 2001. Cutaneous nodular phaeohyphomycosis in five horses associated with *Alternaria alternata* infection. Vet Rec. 148: 55–56.

HABIB W et al. 2021. Mycotoxin profile and phylogeny of pathogenic *Alternaria* species isolated from symptomatic tomato plants in Lebanon. Toxins 13: 1-14.

HALONEN M et al. 1997. *Alternaria* as a major allergen for asthma in children raised in a desert environment. American Journal of Respiratory and Critical Care Medicine. 155: 1356–1361.

HATTAB Z et al. 2019. *Alternaria alternata* infection causing rhinosinusitis and orbital involvement in an immunocompetent patient. New Microbes and New Infections 32: 1-2.

HERNANDEZ-RAMIREZ G et al. 2021. *Alternaria* as an inducer of allergic sensitization. Journal of Fungi 7: 1-14.

HUGHES KM et al. 2022. Impact of fungal spores on asthma prevalence and hospitalization. International Journal of Molecular Sciences 23: 1-12.

KHOSRAVI AR. 1996. Fungal flora of the hair coat of stray cats in Iran. Mycoses 39: 241–243.

KUMAR P et al. 2021. Biological contaminants in the indoor air environment and their impacts on human health. Air Quality, Atmosphere & Health 14: 1723–1736.

KUSTRZEBA-WÓJCICKA I et al. 2014. *Alternaria alternata* and its allergens: a comprehensive review. Clinical Reviews in Allergy & Immunolog. 47: 354–365.

LEOCÁDIO JAG et al. 2019. Horse sensitization and allergy to mold, pollen, dust and storage mites, and culicoides in a horse population from southern Portugal. Lisboa: EAACI. (Thematic Poster Session 59).

LIU J et al. 2021. Clinical relevance of *Alternaria alternata* sensitization in patients within type 2-high and type 2-low asthma. International Immunopharmacology 101: 1-7.

MAGYAR D et al. 2021. Impact of global megatrends on the spread of microscopic fungi in the Pannonian Biogeographical Region. Fungal Biology Reviews 37: 71–88.

MARTINS LML. 2022. Allergy to fungi in veterinary medicine: *Alternaria*, Dermatophytes and *Malassezia* pay the bill! Journal of Fungi 8: 1-13.

MEASON-SMITH C et al. 2015. What is living on your dog's skin? Characterization of the canine cutaneous mycobiota and fungal dysbiosis in canine allergic dermatitis. FEMS Microbiology Ecology 91: 1-12.

MORGADO ACT et al. 2022. First report of human *Thelazia callipaeda* infection in Portugal. Acta Tropica. 231: 1-3.

MORIELLO KA & DEBOER DJ. 1991. Fungal flora of the coat of pet cats. American Journal of Veterinary Researc 52: 602–606.

NOGUEIRA R et al. 2019. Hypersensitivity pneumonitis: Antigen diversity and disease implications. Pulmonology 25: 97–108.

O'DRISCOLL BR et al. 2005. Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions. BMC Pulmonary Medicine 5: 1-10.

OLIVEIRA M et al. 2010. Outdoor allergenic fungal spores: comparison between an urban and a rural area in northern Portugal. Journal of Investigational Allergology & Clinical Immunology 20: 117–128.

PASTOR FJ & GUARRO J. 2008. *Alternaria* infections: laboratory diagnosis and relevant clinical features. Clinical Microbiology and Infection 14: 734–746.

PATEL TY et al. 2018. Variation in airborne fungal spore concentrations among five monitoring locations in a desert urban environment. Environmental Monitoring and Assessment 190: 1-10.

PAVÓN MORENO MÁ et al. 2012. The importance of genus *Alternaria* in mycotoxins production and human diseases. Nutricion Hospitalaria 27: 1772–1781.

PESIC Z et al. 2015. *Alternaria*-associated fungus ball of orbit nose and paranasal sinuses: case report of a rare clinical entity. Mycopathologia 180: 99–103.

PHILPOT CM & BERRY AP. 1984. The normal fungal flora of dogs. Mycopathologia. 87: 155–157.

QUESADA JM. 2022. Special issue: *Alternaria alternata*: should we still consider this fungus as a minor brother in the etiology of respiratory allergy? Journal of Fungi 8: 1-3.

RAYENS E & NORRIS KA. 2022. Prevalence and healthcare burden of fungal infections in the United States, 2018. Open Forum Infectious Diseases 9: 1-12.

SALO P et al. 2006. Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. Journal of Allergy and Clinical Immunology 118: 892–898.

SALO PM et al. 2005. Dustborne *Alternaria alternata* antigens in US homes: results from the national survey of lead and allergens in housing. Journal of Allergy and Clinical Immunology 116: 623–629.

SÁNCHEZ P et al. 2022. Fungal allergen and mold allergy diagnosis: role and relevance of *Alternaria alternata* Alt a 1 protein family. Journal of Fungi 8: 1-11.

SCHELL WA. 2000. Unusual fungal pathogens in fungal rhinosinusitis. Otolaryngologic Clinics of North America 33: 367–373.

SEYEDMOUSAVI S et al. 2018. Fungal infections in animals: a patchwork of different situations. Medical Mycology 56: 165-187.

SIVAGNANAM K et al. 2017. Rapid screening of *Alternaria* mycotoxins using MALDI-TOF mass spectrometry. Journal of the Science of Food and Agriculture 97: 357–361.

THE GLOBAL ASTHMA REPORT 2022. 2022. The International Journal of Tuberculosis and Lung Disease 26: 1–104.

THOMMA BPHJ. 2003. *Alternaria* spp.: from general saprophyte to specific parasite. Molecular Plant Pathology 4: 225–236.

TRZIL JE. 2020. Feline asthma. Veterinary Clinics of North America: Small Animal Practice 50: 375–391.

TYCZKOWSKA-SIEROŃ E & GŁOWACKA A. 2013. *Alternaria tenuissima* as a cause of dermatological infections in pets - Case report. Mikologia Lekarska 20: 116–118.

VALENTINE BA et al. 2006. Equine cutaneous fungal granuloma: a study of 44 lesions from 34 horses. Vet Dermatol. 17: 266–272.

World Health Organization (WHO)/ International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-committee. 2023. Allergen nomenclature. http://allergen.org/index.php

WRIGHT GR et al. 2020. A case of multifocal cutaneous alternariosis in a patient with hairy cell leukemia. Official Journal of the Association of Medical Microbiology and Infectious Disease Canada 5: 251–255.

ZANEN LA et al. 2022. Zoonotic risks of sleeping with pets. Pathogens 11: 1-10.
ZUREIK M. 2002. Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. BMJ 325: 411–411.