Title: Fungi in veterinary medicine: *Alternaria*, dermatophytes and *Malassezia* pay the bill!

Author: Luís Miguel Lourenço Martins

Affiliation: Department of Veterinary Medicine, School of Science and Technology. *MED* – Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, University of Évora. Évora, Portugal. lmlm@uevora.pt

Abstract:

Fungi kingdom comprises ubiquitous forms of life with 1.5 billion years, mostly phytopathogenic and commensal for humans and animals. However, in the presence of impaired conditions fungi may cause disease by intoxicating, infecting or sensitizing with allergy. Different genera may be implicated as etiological agents for humans and animals, with *Alternaria*, *Aspergillus*, dermatophytes like *Microsporum* and *Trichophyton*, and *Malassezia* as the commonly implicated. *Alternaria* and *Malassezia* stand as the most commonly associated to either allergy or infection, immediately followed by *Aspergillus*, while dermatophytes are usually associated to ring worm skin infection. Research in veterinary field is not much but necessary.

Keywords: Allergy; *Alternaria*; *Aspergillus*; dermatophytes; fungal allergens; immunocompetence; indoor/outdoor allergens; *Malassezia*.

<div align="center">

LITERATURE REVIEW

</div>

Introduction

Fungi are living forms with around 1.5 billion years (1). However, fossil evidence of fungi is scarce, probably because of their easily disruptible soft body nature, frequently microscopic dimension and morphology difficult to distinguish from those of other microbes (2). The majority of fungi organisms are saprophytic, lacking pathogenicity to either plants, humans and animals, but a small proportion of species may become pathogenic to plants, humans or animals, by producing toxins that affect both or by infecting or causing allergy to humans and animals. Between the genera *Alternaria*, *Mucor*, *Aspergillus*, *Fusarium* (3) as well as *Trichophyton* and *Microsporum* (4) are the fungi frequently involved in pathogeny to plants or humans and animals. Several of the species comprised in those genera are able to cause considerable economic losses to agriculture, with

relevant loss of food for consumption and serious diseases in humans and animals, especially in immunocompromised individuals (3).

Species from the Fungi kingdom can be found almost everywhere. Fungi species evolved side by side with other live beings as decomposers of the organic matter. By secreting enzymes into the surrounding environment fungal species can extract the available nutrients, mostly carbohydrate metabolites, from other organisms as they are heterotrophic. Other nutrients like proteins and lipids are also digested for fungal subsequent absorption, and at the end the environment becomes full of the leftovers from fungal digestive proteins. Successively, fungal environmental spread is made through airborne dissemination of spores, hyphas and their fragments as well as those leftovers, reaching almost everywhere (5).

Most relevant fungi in health

The ***Aspergillus*** *genus* comprises several of the most common fungal species (e.g. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans* and *Aspergillus terreus*) involved in respiratory infection, more often in birds, where it may cause large economic losses in poultry industry (6). Species from the *Aspergillus* genus are also involved in respiratory allergy like allergic bronchopulmonary aspergillosis, allergic *Aspergillus* sinusitis, IgE-mediated asthma or hypersensitivity pneumonitis (7,8). These situations may derive from primary sensitization to *Aspergillus* airborne compounds, either indoor or in an outer environment (3).

The ***Alternaria*** genus comprises several phytopathogenic species, affecting the quality of grains as well as different vegetables like tomatoes and peppers, and consequently their economic value (3). *Alternaria* species also produce several types of cytotoxic and teratogenic mycotoxins, known to block the synthesis of sphingolipid by inhibiting the rate-limiting enzyme, ceramide synthase (9) which may also compromise the integrity of the skin barrier (10). *Alternaria* is also known for its ability to cause onychomycosis, even in healthy individuals (11) but worse conditions may occur in immunocompromised individuals, where skin infections (12,11), keratomycosis (13) or sinonasal infections may be observed (14).

Sensitization to *Alternaria* fungus is also common, with species from this genus as the most frequently associated with type I hypersensitivity. The conditions have been associated with exposure in indoor and outdoor environment, mostly in warm climates (15,16).

Species from the ***Fusarium*** genus commonly grow on cereal, contaminating the grains with toxins and making it unsafe for consumption (3,17). Concerning the repercussion on human and animal health, *Fusarium* may disturb immune system either by immunotoxic impairment or sensitization with allergy. Besides the toxic effects, allergic consequences are also reported as bronchial asthma, allergic alveolitis and rhinitis, atopic conjunctivitis, organic dust toxic syndrome and chronic fatigue-like syndrome (18). There are several *Fusarium* allergens, some of them known for cross-reacting (19). *Fusarium* species are also known for their ability to infect either immunocompetent or immunocompromised individuals (20). *Fusarium solani*, for instance, contains several allergens which were found reactive with serum from patients sensitized to many fungi (21).

Curvularia is also a relevant genus comprising at least 40 saprophytic species, but only a few of those are known for their capacity to become phytopathogenic, also producing several mycotoxins with cytotoxic activity as curvulins and brefeldins (3). Brefeldin A is in fact also used for that property, as a blocker of the intracellular transport of cytokines for different immunological studies (22). Besides, *Curvularia lunata* was reported able to cause eye and skin infection upon trauma (23) as well as onychomycosis, skin ulcerations and subcutaneous mycetoma (24). *Curvularia* may also sensitize human individuals, causing especially respiratory signs (25) and showed marked cross-reactivity with *Alternaria alternata* and *Epicoccum nigrum* (26,27). Dogs were also reported with either infection by *Curvularia* fungi (28) or allergy upon sensitization to their allergens (29).

Cladosporium is a ubiquitous genus and can be isolated from different materials like organic matter, soil, straw, textiles and even ink. It may damage fresh vegetables and fruits, producing great economic losses (3). Infection by *Cladosporium* fungi have been reported in several species like humans, dogs (30), horses (31) and cats (32,33). Furthermore, allergic conditions associated with *Cladosporium* are currently referred in humans (34,3) and dogs (35).

Mucor and ***Rhizopus*** are two other genera belonging to the Mucorales group, comprising pathogens of plants (3) that may also affect humans, mainly immunosuppressed individuals (36) as well as allergic individuals, either humans (15) or animals (29,37,38).

Other relevant fungal group of diseases is Dermatophytosis. This zoonosis is mainly caused by fungi from the genera ***Microsporum*** and ***Trichophyton*** and is rather common among humans and animals attending dermatological consultation, frequently affecting immunocompetent

individuals. Prevention and treatment of dermatophytosis rely in good sanitation and hygiene as well as specific treatment. It is frequently called the ringworm disease for its round-shaped skin lesions and besides the etiotropic therapy, vaccination with first generation live attenuated preparations has allowed successful control and even eradication, when large numbers of cattle and fur-bearing animals were affected (39).

Fungi as sources of allergens

Allergy to *Dermatophyte* fungi has been also reported, with several *Trichophyton* allergens, already identified and evidence of *Trichophyton*-related IgE-mediated asthma in humans. In an individual, the same antigens who do not elicit immediate hypersensitivity may nevertheless trigger delayed-type hypersensitivity. Based on the observation of acute *vs* chronic skin infection, delayed responses appear to confer protection, while immediate ones don´t. Amino acid sequence identity of *Trichophyton* allergens suggest a dual role of these proteins in fungal pathogenesis and for allergic etiopathogeny. Some T-cell epitopes have been mainly associated to delayed hypersensitivity, which may result useful for the development of rather efficacious peptide vaccines, allowing better control of *Trichophyton* infection and related allergy (40).

Regarding sensitization and possible subsequent allergy, fungal spores are between the first substances found as sensitizing for humans, following contact in indoor or outdoor environments. Sensitization to fungal species commonly figures above 5% in general population but reaches higher rates in atopic individuals. Exposure to fungal allergens may occur by contacting intact spores and mycelia or with their fragments. Spores in germination are known for presenting a wider allergen frame. Studies on the *Alternaria* genus, probably the most studied from all allergenic fungi, have been very helpful in terms of the effect of common long-term low-level fungal exposure. In any case, fungal exposure does not mean sensitization or any other pathology as it has been showed when the exposed population does not include atopic individuals. In fact, indoor fungal exposure and respiratory disease is frequently associated with atopic predisposition (15) or immune compromising conditions (3).

Sensitization to *Alternaria* has been estimated in 7%, while 6% to *Aspergillus* (41) but considering the occurrence of subclinical sensitization, those figures may be underestimated. Furthermore, sensitization to fungi is a considerable cross-reactive condition. Hence, contact with primary sensitization to a limited number of fungal species could result in sensitization to a wide variety of other fungal species as it was suggested when 6565 individuals with positive IgE in, at least, one

fungal test, were tested with a larger battery of fungal species, showing positivity for all in 1208 cases (42). In fact, fungal proteins sharing homologous structural elements and similar functions showed marked cross-reactivity (43,44). Fungal structure-derived particles may become aerosolized in concentrations 300-500 times greater than spores (45), which may potentiate contact leading to possible sensitization.

So far, in Allergome – allergen database (http://www.allergome.org/) there are 1024 registrations for "fungi" out of 7535 entries. Approximately half of those respect to *Alternaria* with 309 and *Aspergillus* with 195, with respectively 186 and 142 allergens, including isoforms (46). This makes *Alternaria* the more relevant fungal genus in allergy, by far. It is the fungus with more humans sensitized to and the highest association with asthma deaths (47).

Fungi may present the highest concentration of airborne allergen particles but there is evidence that increased exposure to indoor microbial diversity, including fungi, may represent a protective issue regarding the occurrence of atopy (15) which falls into the paradigm of the hygiene hypothesis (46). Regarding the prevalence of airborne fungi, in northern regions the amount of fungal spores per cubic meter of outdoor air is usually low during spring, rising by rainfall and temperature until a peak during Autumn (around 50000/cubic meter of air) while in southern regions levels tend to stay more constant, around tens of thousands, varying according environmental humidity (49). Regarding the indoor concentration of fungi spores, it usually correlates with outdoor figures, despite major genera like *Chaetomium* and *Stachybotrys* not correlating with outdoor concentrations. Furthermore, major genera associated with indoor environment as *Aspergillus* and *Penicillium* also do not correlate much with outdoors as *Cladosporium* and *Alternaria* do (50).

Regarding mold, or even house-dust mites and insect allergens for animals, especially dogs and horses, there are not many reports and major allergens may also differ from those to humans. Despite the evidence of sensitization to mold allergens in dogs, leading to atopic dermatitis, the reported rate of sensitization is diverse between studies, which may result from low level of standardization of allergen extracts, resulting in poor specificity of the assays (51).

In equine, recurrent airway obstruction (RAO) has been associated with exposure to moldy hay. Despite the occurrence of sensitization to fungi and challenging with moldy hay or even mold extracts leading to the aggravation of clinical signs, only mechanisms apart from fungi-specific IgE have been implicated in the pathogenesis of RAO. However, basophil histamine releasing test

upon stimulation with fungal allergens showed higher in horses with RAO than in healthy individuals (52). In fact, increased *Aspergillus fumigatus* specific IgE and IgG responses were found in bronchoalveolar lavage fluid of RAO-affected horses, following *in vitro* provocation with fungal extracts (53). Despite no difference in specific IgE to fungal extracts had been reported between healthy and affected horses, specific IgE to recombinant allergens like Alt a 1 and Asp f 7, 8 and 9 was mostly detected in bronchoalveolar lavage and serum from RAO-affected individuals. Specific IgG to *Aspergillus fumigatus* extract has been detected in both healthy and RAO-affected individuals, but later ones were found with higher IgG levels to Asp f 8 (54,55). Despite the lack of knowledge about which proteins are major allergens for horses, significant differences in specific IgE against Asp f 7 were found between RAO-affected and healthy individuals. In one study by Scharrenberg et al (2010)(56) those differences were extended to the offspring of one stallion but not to the other´s, suggesting a genetic predisposition to sensitization and allergy. In fact, genetic evaluation identified different quantitative trait loci associated with that phenotype (56). Relevant mold allergome for dogs or cats has not been clarified yet and recombinant mold allergens have not been used for diagnostic purposes in these species. For horses there are already a few mold allergens identified but a significant rate of recognition is not still established, in order to point the major allergens (51).

Regarding the nature of immune response against antigenic structures it is necessary to have in mind that all body epithelial barriers represent ecosystems in which xenobiotics as the microbiota (bacteria, fungi and viruses) find nutritive conditions to multiply. These surfaces are consequently highly populated by those, producing several metabolites which influence host immune system, inducing either tolerance or triggering defensive mechanisms as sensitization, leading sometimes to allergy. Healthy immunity relies in a good equilibrium between microbiota and host defense system, simultaneously preventing invasion by pathogens and avoiding host overreaction. For this purpose two opposite pathways – immune activation by microbial metabolites and immune regulatory processes – constantly stand in a tiny equilibrium (57). With regard to animal atopic dermatitis, defects in the lipid and protein constitution of skin, aggravated by inflammation, may contribute to the impairment of the barrier function, favoring the penetration of allergens in depth, with stimulation of the immune response, which configures the outside/inside way from the etiopathogenic paradigm outside/inside – inside/outside. On the other way, if a marked genetic predisposition to develop a Th2 kind of immune response is present, even a low epidermal

penetration of allergens may trigger sensitization with subsequent allergy, configuring the inside/outside way of the paradigm (10).

The higher concentration of immune resources is found in the gastrointestinal tract, where a rich mix of commonly commensal bacteria, archaea, fungi and viruses is found. Therefore, its role for the host health/disease equation is crucial, however poorly understood (58). Human gastrointestinal tract is recognized as the first barrier towards food-derived contaminants, including a large variety of xenobiotics. Gastrointestinal tract immune system must face all the related challenge to keep the mucosal barrier up, supporting its structural integrity (59). Ironically, despite mycotoxin action, possibly affecting immune response, *Alternaria alternata* toxins may also contribute to the function of the epithelial barrier, by activating the aryl hydrocarbon receptor pathway (60).

For diagnostic skin testing commercial whole-allergen extracts usually vary in the content of major and minor allergens, compromising the reproducibility of the results. There have been produced molecular allergens (natural purified or recombinant) for nearly all relevant allergen sources, like pollens, mites, fungi, *Hymenoptera* venom and food, which can be used for diagnosis (61).

Malassezia, a complex big issue in animal allergy

Regarding fungi, the most frequent species found in human and animal skin belong to the *Malassezia* genus, a lipophilic group of yeasts (61) comprising 18 species (62). Not many phenotyping-based tests are available to identify different *Malassezia* species, also frequently allowing several overlaps. Current identification of *Malassezia* yeasts has been possible by molecular methods as sequencing of D1/D2 domain of the large subunit of the rRNA gene, ITS, IGS, CHS2 and ß-tubulin genes, allowing the identification of genotypes possibly associated to host-adaptation virulence. Multiplex PCR and MALDI-TOF mass spectrometry are also recognized methods allowing the identification of *Malassezia* species from skin or in culture, respectively (62). In the Allergome – allergen database (http://www.allergome.org/ (46) there are 54 registrations for "*Malassezia*" out of 7535 entries, including 11 species already related to sensitization and 42 allergens, including isoforms. Some of these allergens may be responsible for pro-inflammatory immune response by interacting with dendritic or T cells, probably through Toll-like receptor 2. Specific IgE to *Malassezia sympodialis* allergen 11 (Mala s 11) a Mn superoxid dismutase, is correlated with the severity of atopic dermatitis, supposedly by inducing the release of several pro-inflammatory cytokines like interleukin (IL)-6, IL-8, IL- 12p70 and TNF-α, by dendritic cells.

Mala s 11 is also known for its capacity to activate autoreactive T cells and cross-reactivity with *Aspergillus fumigatus* (Asp f) 6, electing the detection of specific IgE to Asp f 6 as a possible marker for autoreactivity in atopic dermatitis. An allergen (MGL_1304) from *Malassezia globosa* was found able to activate mast cells, leading to degranulation and inducing basophils to release IL-4, a trigger interleukin in the pathway to IgE synthesis (61).

In a study by Di Tommaso *et al.* (2021)(63) all (n = 45) dogs subjected to sera determination in an indoor allergen species panel showed positive for at least one house-dust or storage mite, 12 for at least one mold species (1 to *Malassezia*), 11 to *Malassezia* and 1 to flea saliva. In fact, *Malassezia* had already demonstrated to trigger hypersensitivity response in atopic dogs (64). As referred by Di Tommaso *et al.* (2021)(63) Serum Allergen-Specific IgE Test (SAT) to detect specific IgE to *Malassezia* revealed a range of positivity between 0% and 60%. In another recent study, evaluating both intradermal tests (IDT) and SAT to *Malassezia*, there was 24% positivity in IDT, whereas no positivity was found in SAT (65). Another study reported a percentage of positivity of 35% in IDT (66).

A retrospective study of 111 allergic dogs (60 males and 51 females; 33 from predisposed breeds; 74% indoor and 25.2% outdoor; 59% with mainly seborrheic disruptive skin barrier) living in the inland region of Londrina, Brazil, revealed 49.6% patients with *Malassezia* overgrowth associated dermatitis, mostly with atopic dermatitis (67). A similar study performed also in Brazil, in the region of São Paulo, with 84 allergic dogs (45 males and 39 females; 31 from predisposed breeds; 77.4% indoor and 22.6% outdoor; 69% with atopic dermatitis) revealed 58.3% patients with *Malassezia* overgrowth associated dermatitis (68). In a veterinary allergy outpatient consultation in Évora, Portugal, from 90 allergic dogs 14.4% presented positive IDT to *Malassezia*, 6.7% of them simultaneously to other fungi, whereas in SAT, from 77 allergic dogs 16.9% presented with specific IgE to *Malassezia*, 5.2% of them to other fungi species, simultaneously (69).

With regard to *Malassezia pachydermatis*, it is a commensal inhabitant of canine and feline skin and mucosae, but there are also other species like *Malassezia nana* (more associated to outer ear) and *Malassezia slooffiae* (more frequent in the claw fold). Despite this commensal frame without causing lesion, in presence of different factors like host´s innate and adaptive immune defenses and expression of cell wall and secreted virulence factors, *Malassezia* populations may overgrow, causing more or less severe inflammation, in a complex homeostatic equilibrium. This immune-

mediated hyperreactivity configures the _Malassezia_ dermatitis condition (62) which is rather common in dog allergic patients, even without confirmed hypersensitivity to _Malassezia_ (70).

When that highly demanding homeostatic equilibrium is disrupted, namely with excessive sebum production, diminished sebum quality, moisture accumulation, disrupted epidermal surface or concurrent dermatitis, conditions may become favorable for _Malassezia_ overgrowth. Cutaneous inflammation with altered sebum production may frequently occur in conditions as skin allergies like atopy, food hypersensitivity and flea allergy, keratinization disorders with seborrhea, pyoderma, endocrinopathies like hyperadrenocorticism, hypothyroidism, diabetes mellitus, metabolic diseases like zinc-responsive dermatosis and superficial necrolytic dermatitis, and cutaneous or internal neoplasia, creating the adequate skin microenvironment for _Malassezia pachydermatis_ overgrowth (71).

Testing hypersensitivity to _Malassezia_ is commonly performed through intradermal and serology, although the lack of standardization of the available extracts turns it somehow unrealiable (62).

There are several dog breeds, namely American Cocker Spaniel, Australian Silky Terriers, Basset Hound, Boxers, Dachshund, English Poodle, Setter, Shih Tzu and West Highland White Terrier, showing increased risk of _Malassezia_ overgrowth with consequent dermatitis. Among cats, Devon Rex and Sphynx are also considered for their predisposition. Concurrent diseases, mostly allergic but other skin or endocrine pathologies, constitute a risk factor for dog and cat _Malassezia_ dermatitis (62). Cats suffering from visceral paraneoplastic syndromes are also frequently referenced for _Malassezia_ overgrowth-derived complications (72).

Clinical, dermatitis due to _Malassezia_ overgrowth usually presents as pruritic ceruminous otitis externa and kerato-sebaceous scale. Skin develops erythematous lesions, especially on folded areas, which constitute a common risk factor for localized disease, either in dogs or cats, where intertriginous dermatitis will develop (72). Regarding zoonotic risk it is low, especially to immunocompetent people as shown by the scarcity of _Malassezia_-derived conditions in humans and by PCR assessment. Anyway, good hand hygiene by individuals contacting mostly with dogs and cats presenting _Malassezia_ overgrowth should be considered (62).

Main conclusions

Fungi are ubiquitous forms of life, mostly phytopathogenic and commensal in humans and animals. However, in the presence of impaired conditions, especially concerning skin and mucosal

barrier or immune competence, fungi may cause disease by infecting/intoxicating or inducing sensitization with allergy, in the presence of genetic predisposition. Severity of fungal diseases may vary from mild to severe life-threatening. Environmental conditions like humidity and temperature are well-known supporters for fungi populations' growth and the amount and continuity of microbial pressure are also known to play a not good role over the effectiveness of animal defenses. Towards allergy to fungi, *Alternaria* and *Aspergillus* are probably the major implied genera of molds for humans and animals. However, in animals, *Malassazia* genus comprises a group of yeasts with a remarkable responsibility in dermatological inflammatory conditions, mainly in allergic patients, where skin barrier is found frequently impaired. In the presence of this frame, several direct diagnosis methods are currently used as well as more state of the art molecular biology ones, allowing more precise specific results. Prophylaxis should rely on eviction measures, to avoid the contact with fungi, in which environmental measures to reduce their population stands very helpful. Reestablishment of the impaired skin, mucosa and immune conditions should also be attended. Environmental rehabilitation, surfaces cleaning and disinfection, skin and mucosa antisepsis and topical or systemic antifungal medication are widely useful directed measures to fight the fungal problem. Specific immunotherapy to fungi is also an on-development resource as a possible useful co-measure in fungal disease control.

<div align="center">**BACK MATTER**</div>

Conflicts of interest:

The author declares no conflict of interest.

References:

1. Wang, D.Y.; Kumar, S.; Hedges, S.B. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proc. Royal Soc. B* **1999**, 266, 163-171.

2. Donoghue, M.J.; Cracraft, J. In *Assembling the tree of life*. Oxford University Press, Ed.; Oxford (Oxfordshire), UK, 2004; 187.

3. De Lucca, A.J. Harmful fungi in both Agriculture and Medicine. *Rev Iberoam Micol* **2007**, 24, 3-13.

4. Moriello, K.A.; Coyner, K.; Paterson, S.; Mignon B. Diagnosis and treatment of dermatophytosis in dogs and cats. Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. *Vet Dermatol* **2017**, 28, 266-268.

5. Yafetto, L.; Carroll, L.; Cui, Y.; Davis, D.J.; Fischer, M.W.; Henterly, A.C.; Kessler, J.D.; Kilroy, H.A.; Shidler, J.B.; Stolze-Rybczynski, J.L.; Sugawara, Z.; Money, N.P. The fastest flights in nature: highspeed spore discharge mechanisms among fungi. *PLoS One* **2008**, 3, e3237.

6. Akan, M.; Hazirogiu, R. ; Ihan, Z.; Sareyyupogiu, B.; Tunca, R.; A case of aspergiiiosis in a broiier breeder flock. *Avian Dis* **2002**, 46, 497-501.

7. Maurya, V.; Gugnami H.C.; Sarnia, P.U.; Madan, T.; Shah, A. Sensitization to *Aspergiiius* antigens and occurrence of allergic bronchopulmonary aspergillosis in patients with asthma. *Chest* **2005**, 127, 1252-1259.

8. Shah, A.; Panjabi, O. Allergic bronchopulmonary aspergillosis: a review of a disease with a worldwide distribution. *J Asthma* **2002**, 39, 273-289.

9. Van der Westhuizen, L.; Shephard, G.S.; Snyman, S.D.; Abel, S.; Swanevelder, S.; Gelderblom, W.O. Inhibition of sphingolipid biosynthesis in rat primary hepatocyte cultures by fumonisin B1 and other structurally related compounds. Food Chem Toxicol **1998**, 36, 497-503.

10. Paixão, A.; Caldeira, J.; Leocádio, J.; Martins, L. The Importance of Skin Barrier Integrity for the Prevention of Veterinary Allergy. *Rev Port Imunoalergologia* 2022, *in press*.

11. Romano, C.; Paccagnini, E.; Difonzo, D. Onychomycosis caused by *Alternaria* spp. in Tuscany from 1985 to 1999. *Mycoses* **2001**, 44, 73-76.

12. Romero, C.; Vanzi, L.; Massi, D.; Difonzo, E.M. Subcutaneous alternariosis. *Mycoses* **2005**, 48, 408-412.

13. Zahra, L.V.; Mallia, D.; Hardie, J.G.; Bezzina, A.; Fenech, T. Case report: Keratomycosis due to *Alternaria alternata* in a diabetic patient. *Mycoses* **2002**, 45, 512-514.

14. Morrison, V.A.; Mc Glave, P.B. Mucormycosis in the BMT population. Bone Marrow Transplant **1993**, 11, 383-388.

15. Barnes C. Fungi and Atopy. *Clin Rev Allergy Immunol* **2019**, 57, 439-448.

16. Čelakovská, J.; Bukač, J.; Ettler, K.; Vaneckova, J.; Ettlerova, K.; Krejsek, J. Sensitisation to outdoor and indoor fungi in atopic dermatitis patients and the relation to the occurrence of food allergy to peanuts and walnuts. *Mycoses* **2018**, 61, 698-703.

17. Saberi-Riseh, R.; Javan-Nikkhah, M.; Heidarian, R.; Hosseini, S.; Soleimani, P. Detection of fungal infectious agent of wheat grains in store-pits of Markazi province, Iran. *Commun Agric Appl Biol Sci* **2004**, 69, 541-554.

18. Lacey, J.; Dutkiewicz J. Bioaerosols and occupational lung disease. *J Aerosol Sci* **1994**, 25, 1371-1404.

19. Verma, J.; Singh, B.P.; Sridhara, S.; Gaur, S.N.; Arora, N. Purification and characterization of a cross-reactive 45-kD major allergen of *Fusarium solani. Int Arch Allergy Immunol* **2003**, 130, 193-199.

20. Muhammed, M.; Anagnostou, T.; Desalermos, A.; Kourkoumpetis, T.K.; Carneiro, H.A.; Glavis-Bloom, J.; Coleman, J.J.; Mylonakis, E. Fusarium infection report of 26 cases and review of 97 cases from the literature. *Medicine* **2013**, 92, 305-316.

21. O'Neil, O.E.; McOants, M.L.; Salvaggio, J.E.; Lehrer, S.B. *Fusarium solani:* prevalence of skin reactivity and antigenic allergenic analysis. *J Allergy Clin Immunol* **1986**, 77, 842-849.

22. Martins, L.; Pires, E.; Inácio, F. CD4 T-Cell Cytokine Response to Mite Recombinant Tropomyosin in Mite, Snail and Shrimp Allergic Patients. *Internet J Asthma Allergy Immunology **2009, 7, 1.*

23. Wilhernus, K.R.; Jones, D.B. *Curvularia* keratitis. *Tr Am Ophthalmol Soc* **2001**, 99: 111-132.

24. Fernandez, M.; Noyola, D.E., Rossman, S.N.; Edwards, M.S. Cutaneous phaeohyphomycosis caused by *Curvularia lunata* and a review of *Curvularia* infections in pediatrics. *Pediatr Infect Dis J* **1999**, 18: 727-731.

25. Gupta,R.; Singh, B.P.; Sridhara, S.; Gaur, S.N.; Chaudhary, V.K.; Arora, N. Allergens of *Curvularia lunata* during cultivation in different media. J Allergy Clin Immunol, **1999**, 4, 857-862.

26. Gupta, R.; Singh, B.P.; Sridhara, S.; Gaur, S.N.; Kunar, R.; Chaudhary, V.K.; Arora, N. Identification of cross-reactive proteins amongst different *Curvuiaria* species. *Int Arch Allergy Immunol* **2002**, 127, 38-46.

27. Gupta, R.; Singh, B.P.; Sridhara, S.; Gaur, S.N.; Kumar, R.; Ghaudhary, V.K.; Arora, N. Allergenic cross-reactivity of *Curvularia lunata* with other airborne fungal species. *Allergy* **2002**, 57, 636-640.

28. Strzok, E.; Siepker, C.; Armwood, A.; Howerth, E.; Smith, J.; Banovic, F. Successful treatment of cutaneous *Curvularia geniculata, Nocardia niigatensis*, and viral papillomatosis in a dog during the therapeutic management of immune-mediated hemolytic anemia. *Front Vet Sci*, **2019**, 6:249.

29. Kang, M.; Kim, H.; Jang, H.; Park, H. Sensitization rates of causative allergens for dogs with atopic dermatitis: detection of canine allergen-specific IgE. *J Vet Med Sci* **2014**, 15, 545-550.

30. Spano, M.; Zuliani, D.; Peano, A.; Bertazzolo, W. *Cladosporium cladosporioides*-complex infection in a mixed-breed dog. *Vet Clin Pathol* **2018**, 47, 150-153.

31. Dauvillier, J.; Ter Woort, F.; van Erck-Westergren, E. Fungi in respiratory samples of horses with inflammatory airway disease. *J Vet Intern Med* **2019**, 33, 968-975.

32. Jarrah, S.A.; Zanetti, C.C.; Maruyama, F.H. ; Ito, A.T.; Rosa, J.M.; E.M. Colodel, E.M.; Lima, S.R.; Nakazato, L.; Dutra, V. Cladosporium cladosporioides isolated from a cat with squamous cell carcinoma. *Arq Bras Med Vet Zootec*, **2017**, 69, 377-380.

33. Mariani, C.L.; Platt, S.R.; Scase, T.J.; Howerth, E.W.; Chrisman, C.L.; Clemmons, R.M. Cerebral phaeohyphomycosis caused by Cladosporium spp. in two domestic shorthair cats. *J Am Anim Hosp Assoc* **2002**, 38, 225-230.

34. Ozdemir, O. Molds and Respiratory Allergy - Part 1. *MOJ Immunol* **2015**, 2, 00045.

35. Meason-Smith, C.; Diesel, A.; Patterson, A.P.; Older, C.E.; Mansell, J.M.; Suchodolski, J.S.; Hoffmann, A.R. What is living on your dog's skin? Characterization of the canine cutaneous mycobiota and fungal dysbiosis in canine allergic dermatitis. *FEMS Microbiol Ecol* **2015**, 91, fiv139.

36. Stone, N.; Gupta, N.; Schwartz I. Mucormycosis: time to address this deadly fungal infection. *Microbe* **2021**, 2, e343-344.

37. Cafarchia C.; Figueredo L.A.; Otranto D. Fungal diseases of horses. *Vet Microbiol* **2013**, 167, 215-234.

38. Youn, H.Y.; Kang, H.S.; Bhang, D.H.; Kim, M.K.; Hwang, C.Y.; Han, H.R. Allergens causing atopic diseases in canine. *J Vet Sci* **2002**, 3, 335-341.

39. Lund, A.; DeBoer, D.J. Immunoprophylaxis of dermatophytosis in animals. *Mycopathologia* **2008**, 166, 407-424.

40. Woodfolk, J. Allergy and dermatophytes. *Clin Microbiol Rev* **2005**, 18, 30-43.

41. Salo, P.M.; Arbes, S.J. Jr.; Jaramillo. R.; Calatroni, A.; Weir, C.H.; Sever, M.L.; Hoppin, J.A.; Rose, K.M.; Liu, A.H.; Gergen, P.J.; Mitchell, H.E.; Zeldin, D.C. Prevalence of allergic sensitization in the United States: results from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. *J Allergy Clin Immunol* **2014**, 134, 350-359.

42. Amado, M.; Portnoy, J.M.; Barnes, C. *J Allergy Clin Immunol* **2014**, 133, Suppl 2, AB92.

43. Williams, P.B.; Barnes, C.S.; Portnoy, J.M. Environmental allergens workgroup. Innate and adaptive immune response to fungal products and allergens. *J Allergy Clin Immunol Pract* **2016**, 4, 386-395.

44. Crameri, R.; Weichel, M.; Fluckiger, S.; Glaser, A.G.; Rhyner, C. Fungal allergies: a yet unsolved problem. *Chem Immunol Allergy* **2006**, 91, 121-133.

45. Green, B.J.; Tovey, E.R.; Sercombe, J.K.; Blachere, F.M.; Beezhold, D.H.; Schmechel, D. Airborne fungal fragments and allergenicity. *Med Mycol* **2006**, 44, Suppl 1, S245-S255.

46. Allergome – The Platform for Allergen Knowledge. Available online: https://www.allergome.org/ (assessed on 20 January 2022).

47. O'Hollaren, M.T.; Yunginger, J.W.; Offord, K.P.; Somers, M.J.; O'Connell, E.J.; Ballard, D.J.; Sachs, M.I. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* **1991**, 324, 359-363.

48. Pfefferle, P.I.; Keber, C.U.; Cohen, R.M.; Garn, H. The Hygiene hypothesis – Learning from but not living in the past. Front Immunol **2021**, 12, 635935.

49. Portnoy, J.M.; Barnes, C.S.; Kennedy, K. Importance of mold allergy in asthma. *Curr Allergy Asthma Rep* **2008**, 8, 71-78.

50. Jara, D.; Portnoy, J.; Dhar, M.; Barnes, C. Relation of indoor and outdoor airborne fungal spore levels in the Kansas City metropolitan area. *Allergy Asthma Proc* **2017**, 38, 130-135.

51. Mueller, R.S.; Janda, J.; Jensen-Jarolim, E.; Rhyner, C; Marti, E. Allergens in veterinary medicine. *Allergy* **2016**, 71, 27-35.

52. Dirscherl, P.; Grabner, A.; Buschmann, H. Responsiveness of basophil granulocytes of horses suffering from chronic obstructive pulmonary disease to various allergens. *Vet Immunol Immunopathol* **1993**,38, 217-227.

53. Schmallenbach, K.H.; Rahman, I.; Sasse, H.H.; Dixon, P.M.; Halliwell, R.E.; McGorum, B.C.; Crameri, R.; Miller, H.R. Studies on pulmonary and systemic Aspergillus fumigatus-specific IgE and IgG antibodies in horses affected with chronic obstructive pulmonary disease (COPD). *Vet Immunol Immunopathol* **1998**, 66, 245-256.

54. Kunzle, F.; Gerber, V.; Van Der Haegen, A.; Wampfler, B.; Straub, R.; Marti, E. IgE-bearing cells in bronchoalveolar lavage fluid and allergen-specific IgE levels in sera from RAO-affected horses. *J Vet Med A Physiol Pathol Clin Me*d **2007**, 54, 40-47.

55. Eder, C.; Crameri, R.; Mayer, C.; Eicher, R.; Straub, R.; Gerber, H.; Lazary, S.; Marti, E. Allergen-specific IgE levels against crude mould and storage mite extracts and recombinant mould allergens in sera from horses affected with chronic bronchitis. *Vet Immunol Immunopathol* **2000**, 73, 241-253.

56. Scharrenberg, A.; Gerber, V.; Swinburne, J.E.; Wilson, A.D.; Klukowska-Rotzler, J.; Laumen, E.; Marti, E. IgE, IgGa, IgGb and IgG(T) serum antibody levels in offspring of two sires affected with equine recurrent airway obstruction. *Anim Genet* **2010**, 41, Suppl 2, 131-137.

57. Tizard, I.R.; Sydney, W.J. The microbiota regulates immunity and immunologic diseases in dogs and cats. *Vet Clin North Am Small Anim Pract* **2017**, 48, 307-322.

58. Tamburini, S.; Shen, N.; Wu, H.C.; Clemente, J.C. The microbiome in early life: implications for health outcomes. *Nat Med* **2016**, 22, 713-22.

59. Hohenbichler, J.; Spindler, V.; Pahlke, G.; Rychlik, M.; Del Favero, G.; Marko, D. Immunomodulatory potential of combined *Alternaria alternata* mycotoxins in non-

cancerous epithelial colon cells. Proceedings of the 56th Congress of the European Societies of Toxicology (EUROTOX 2021). Toxicology of the next generation. Virtual Congress, 27th September-1st October 2021. *Abstracts/Toxicology Letters 350S (2021)* P14-11.

60. Hohenbichler, J.; Aichinger, G.; Rychlik, M.; Del Favero, G.; Marko, D. *Alternaria alternata* Toxins Synergistically Activate the Aryl Hydrocarbon Receptor Pathway *in vitro*. Biomolecules, **2020**, 10, 1018.

61. Matricardi, P.M.; et al. EAACI Molecular Allergology User's Guide. *Pediatr Allergy* Immunol **2016**, 27, Suppl 23, 1-250.

62. Bond, R.; Morris, D.O.; Guillot, J.; Bensignor, E.J.; Robson, D.; Mason, K.V.; Kano, R.; Hill, P.B. Biology, diagnosis and treatment of Malassezia dermatitis in dogs and cats. Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. *Vet Dermatol* **2020**, 31, 27, e4.

63. Di Tommaso, M.; Luciani, A.; Crisi, P.E.; Beschi, M.; Rosi, P.; Rocconi, F.; Miglio, A. Detection of serum allergen-specific IgE in atopic dogs tested in northern Italy: Preliminary study. *Animals* **2021**, 11, 358.

64. Farver, K.; Morris, D.O.; Shofer, F.; Esch, B. Humoral Measurement of type-1 hypersensitivity reactions to a commercial Malassezia allergen. *Vet Dermatol* **2005**, 16, 261-268.

65. Han, C.; Chan,W.Y.; Hill, P.B. Prevalence of positive reactions in intradermal and IgE serological allergy tests in dogs from south Australia, and the subsequent outcome of allergen-specific immunotherapy. *Aust Vet J* **2020**, 98, 17-25.

66. Furiani, N.; Scarampella, F.; Noli, C.; Ordeix, L. A retrospective study of 486 intradermal tests performed on atopic dogs in Northern Italy. *Veterinaria* (Cremona) **2009**, 23, 41-46.

67. Cecci G.M.; Cardoso, M.L.; Bento, O.; Martins, L.M. Allergy approach to a dog population from a veterinary dermatology consultation at the tropical inland city of Londrina, Paraná, Brazil. Proceedings of the European Academy of Allergy and Clinical Immunology (EAACI) Congress. June 1-5, 2019, Lisbon, Portugal. Thematic Poster Session 59 – Allergen extracts and molecules for veterinary allergology. P1658.

68. Esteves de Campos, I.; Ferreiro Pinto, C.; Munhoz Severino, A.C.; Bento, O.; Antunes, C.; Costa, A.R.; Martins LM (2019). Dermatological and allergy approach to a dog population from a veterinary consultation at the tropical coastal city of São Paulo, Brazil. Proceedings of the European Academy of Allergy and Clinical Immunology (EAACI) Congress. June 1-5, 2019, Lisbon, Portugal. Thematic Poster Session 59 – Allergen extracts and molecules for veterinary allergology. P1656.

69. Martins, L.M. (University of Évora, Évora, Portugal). Results from veterinary immunoallergology outpatient consultation, 2022 (unpublished work).

70. Martins, L.; Bento, O.; Inacio, F. Veterinary allergy diagnosis: past, present and future perspectives. *Allergo Journal Int* **2016**, 25, 20-32**.**

71. Balappanavar, B.R.; Vasanth, M.S. Clinico-diagnostic and therapeutic management of canine malasseziosis. *Intas Polivet* **2013**, 14, 353-357.

72. Guillot, J.; Bond, R. *Malassezia* yeasts in veterinary dermatology: An updated overview. *Front Cell Infect Microbiol* **2020**, 10, 79.