



Original Article

***Aspergillus* and aspergilloses in wild and domestic animals: a global health concern with parallels to human disease**

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Abstract

The importance of aspergillosis in humans and various animal species has increased over the last decades. *Aspergillus* species are found worldwide in humans and in almost all domestic animals and birds as well as in many wild species, causing a wide range of diseases from localized infections to fatal disseminated diseases, as well as allergic responses to inhaled conidia. Some prevalent forms of animal aspergillosis are invasive fatal infections in sea fan corals, stonebrood mummification in honey bees, pulmonary and air sac infection in birds, mycotic abortion and mammary gland infections in cattle, guttural pouch mycoses in horses, sinonasal infections in dogs and cats, and invasive pulmonary and cerebral infections in marine mammals and nonhuman primates. This article represents a comprehensive overview of the most common infections reported by *Aspergillus* species and the corresponding diseases in various types of animals.

Key words: *Aspergillus*, Aspergillosis, Animals.

Introduction

Aspergillus species are saprophytic filamentous fungi that are commonly found in soil, where they thrive as sapro-

phytes, with an occasional potential to infect living hosts including plants, insects, birds, and mammals [1,2]. Aspergillosis is an umbrella term coined by Hinson, Moon,

and Plummer in 1952, covering a wide range of diseases from localized conditions to fatal disseminated infections in humans and various animals and caused by fungi belonging to the genus *Aspergillus*. Disease may also result from an allergic reaction to inhaled conidia [3–6].

In humans, *Aspergillus fumigatus* is the most common and life-threatening airborne opportunistic fungal pathogen, especially significant among immunocompromised hosts [3,7–11]. Inhalation of *A. fumigatus* spores (conidia) into the lungs may cause multiple diseases, which in humans depend on the immunological status of the host, including invasive pulmonary aspergillosis, aspergilloma, and different forms of hypersensitivity diseases such as allergic asthma, hypersensitivity pneumonitis, and allergic bronchopulmonary aspergillosis [3,12]. There is, however, increasing evidence that aspergillosis, particularly pulmonary aspergillosis, can be seen in immunocompetent patients without the classic risk factors [13,14]. However, most patients have chronic lung diseases and are critically ill. Other associated conditions include influenza, nonfungal pneumonia, chronic obstructive lung disease, immaturity, sepsis, liver failure, alcoholism, chronic granulomatous disease, and surgery. Certain focal sites, such as the sinus with possible extension to the rhinoorbital space or brain, have additional risk factors [13,14].

In animals, aspergillosis is primarily a respiratory infection that may become generalized; however, tissue predilection is highly variable among species. Of note, aspergilloses in animals are caused by *A. fumigatus* and only a few other *Aspergillus* species. Modern classification of *Aspergillus* species is by polyphasic taxonomy (i.e., taking into account all available phenotypic and genotypic data and integration in a consensus classification). At present, additional teleomorph names (i.e., names for the sexual phase of the fungus) are still in use and is summarized in Table 1 [15,16]. The most common forms of animal aspergillosis are pulmonary infections in poultry and other birds [5], mycotic abortion and mammary gland infections in cattle [17–27], guttural pouch (auditory tube diverticulum) mycosis in horses [28–36], sinonasal infections in dogs and cats [37–47], and pneumonia associated with disseminated infections in marine mammals [48–50].

Despite the potential significance of infection caused by *Aspergillus* species in animals, comprehensive comparative knowledge on these fungi is absent from the medical and veterinary literature. Therefore, the present paper highlights the most important clinical and microbiological features of these fungi in various animals and compares them with human *Aspergillus* disease. We first provide a detailed overview on the mycology and pathophysiology of *Aspergillus*, and then highlight the diseases and complications they may cause in invertebrates, cold-

and warm-blooded animals, marine mammals, and nonhuman primates. We will not review the role of *Aspergillus* species and the diseases they cause in humans but only provide key references for comparison. Furthermore, we will discuss the diagnosis and species identification, therapeutic recommendations, risk of antifungal resistance, control and prevention modalities and possible public health hazards.

Characteristics of *Aspergillus*

Most *Aspergillus* species are found in a wide variety of environments and substrates on the earth throughout the year [51]. The great majority of species are saprophytes, prevalently found in soil, decaying vegetation, and on seeds and grains. Only a few well-known species are considered to be important opportunistic pathogens in humans and animals [1,2] (Table 1). Polyphasic taxonomy has had a major impact on species concepts in the genus *Aspergillus*. The genus has been subdivided into 22 distinct sections, of which *Fumigati*, *Circumdati*, *Terrei*, *Nidulantes*, *Ornati*, *Warcupi*, *Candidi*, *Restricti*, *Usti*, *Flavipedes* and *Versicolores* contain clinically relevant species [15]. Although there are more than 200 known species in the genus, only a small number is associated with infection [52]. The first one discovered was *Aspergillus fumigatus*, described by Fresenius in 1863 from the bronchi and alveoli of a great bustard (*Otis tarda*) [53]. It now is a recognized opportunistic pulmonary pathogen of humans and animals, especially birds. Generally, high concentrations of conidia are necessary for infection. Healthy animals are able to ward off infections, and severe illness in these hosts usually results only from massive or long-term exposure [1,5].

Pathogenicity of *Aspergillus*

Mycotoxin production

Aspergillus species secrete numerous secondary metabolites into their environment, known as mycotoxins [54]. The significance of pathogenicity of *Aspergillus* in animals is increased by the production of these potent metabolites, which is thought to provide a chemical shield against competing or predatory microorganisms. Mycotoxins are one of the putative virulence factors of *Aspergillus* to suppress host immunity, thereby enhancing the infectivity of the fungus [55–57].

Mycotoxins are produced during consecutive enzyme reactions via several biochemically simple intermediary products from the primary metabolism of acetates, mevalonates, malonate, and some aminoacids. *Aspergillus* produces some of the most significant mycotoxins known including aflatoxins, gliotoxin and ochratoxin A [1]. The secondary metabolite gliotoxin has attracted the most interest in

Table 1. Schematic classification of *Aspergillus* spp. reported from animal diseases, according to teleo- or anamorph taxonomy in clinical mycology, target animals and characteristics of underlying diseases.

| Genus | Section | Species | Animals affected | Underlying disease/Predisposing factor | References |
|--------------------|-------------------|---------------------|------------------|--|--------------------------|
| <i>Aspergillus</i> | <i>Nigri</i> | <i>A. niger</i> | Honey bee | Immature stages | 125–131 |
| | | | Bird | Environmental stressors including excessive ammonia and moisture, inappropriate temperature, degraded litter, feed contamination with mycotoxins and pathogens | 5, 6 |
| | | | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 43, 206 |
| | | | Ruminant | Dairy animals in early lactation and following intense antibiotic therapy | 17, 19, 24 |
| | | | Horse | Inflammation of the intestine, Immunosuppression | 227 |
| | <i>Circumdati</i> | <i>A. ochraceus</i> | Marine Mammal | Chronic infections, immunosuppression | 239–240 |
| | | | Fish | Secondary to metabolic factors, captivity, trauma, bacterial disease, or parasites, Genetic susceptibility (cold water fish are more susceptible to mycotoxin) | 134 |
| | | | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 46 |
| | | | Honey bee | Immature stages | 125–131 |
| | | | Bird | Environmental stressors including excessive ammonia and moisture, inappropriate temperature, degraded litter, feed contamination with mycotoxins and pathogens | 5, 6 |
| <i>Aspergillus</i> | <i>Flavipedes</i> | <i>A. flavipes</i> | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 38, 96 |
| | | | Cat | Feline immunodeficiency virus, Feline parvovirus | 213 |
| | <i>Flavi</i> | <i>A. flavus</i> | Honey bee | Immature stages | 125–131 |
| | | | Bird | Environmental stressors including excessive ammonia and moisture, inappropriate temperature, degraded litter, feed contamination with mycotoxins and pathogens | 5, 6 |
| | | | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 38, 96 |
| | <i>Fumigati</i> | <i>A. fumigatus</i> | Cat | Feline immunodeficiency virus, Feline parvovirus | 213 |
| | | | Honey bee | Immature stages | 125–131 |
| | | | Bird | Environmental stressors including excessive ammonia and moisture, inappropriate temperature, degraded litter, feed contamination with mycotoxins and pathogens | 5, 6 |
| | | | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 38, 42, 46, 96, 206, 279 |
| | | | Cat | Feline immunodeficiency virus, Feline parvovirus | 208 |
| <i>Aspergillus</i> | <i>Fumigati</i> | <i>A. fumigatus</i> | Ruminant | Dairy animals in early lactation and following intense antibiotic therapy | 17, 19, 24 |

Table 1. continued

| Genus | Section | Species | Animals affected | Underlying disease/Predisposing factor | References |
|-------|-------------------|-----------------------------|------------------|--|---------------------|
| | | | Horse | Inflammation of the intestine, Immunosuppression | 227, 231 |
| | | | Marine Mammal | Chronic infections, immunosuppression | 48, 239, 240 |
| | | | Monkey | Cushing syndrome | 107 |
| | | <i>Neosartorya fischeri</i> | Cat | Feline immunodeficiency virus, Feline parvovirus | 210 |
| | | <i>Neosartorya udagawae</i> | Cat | Feline immunodeficiency virus | 211 |
| | | <i>A. viridinutans</i> | Cat | Feline immunodeficiency virus | 211 |
| | | <i>A. felis</i> | Cat, Dog | Cats with invasive fungal rhinosinusitis, Dogs with disseminated invasive aspergillosis | 210 |
| | | <i>A. lentulus</i> | Cat | Feline immunodeficiency virus, Feline parvovirus | 210 |
| | | <i>A. nidulans</i> | Bird | Environmental stressors including excessive ammonia and moisture, inappropriate temperature, degraded litter, feed contamination with mycotoxins and pathogens | 5, 6 |
| | <i>Nidulantes</i> | | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 38, 42, 46, 96, 206 |
| | | | Horse | Guttural pouch infections | 230 |
| | | <i>A. sydowii</i> | Sea fan coral | larger colonies that have lower antifungal defenses | 109 |
| | | | Sponge | — | 122 |
| | | <i>A. versicolor</i> | Dog | Injury to any of the mucous membranes, the use of catheters, administration of antibiotics and immunosuppressive drugs or diseases | 47 |
| | | | Horse | Inflammation of the intestine, Immunosuppression | 230 |
| | <i>Terrei</i> | <i>A. terreus</i> | Reptile | Immune-compromising conditions, such as husbandry deficiencies or inappropriate temperatures, humidity, or enclosure hygiene | 135 |
| | | | Bird | Environmental stressors including excessive ammonia and moisture, inappropriate temperature, degraded litter, feed contamination with mycotoxins and pathogens | 5, 6 |
| | | | Marine Mammal | Chronic infections, immunosuppression | 239, 240, 241 |

A. fumigatus because of its potent immunosuppressive and cytotoxic properties and the fact that it can be readily detected during experimental infection and in sera from patients with aspergillosis [58,59]. However, specific roles for other toxins in the pathogenesis of aspergillus disease have not been well defined.

The contribution of gliotoxin in virulence of *A. fumigatus* *in vivo* was first characterized by using mutants of two genes involved in gliotoxin biosynthesis, the transcriptional regulator *gliZ* and the non-ribosomal peptide synthetase *gliP* [60,61]. Recent studies have shown that blocking gliotoxin production had no effect on virulence in neutropenic mice [61]. However, gliotoxin did potentiate the virulence when some neutrophil function was present, raising the possibility that neutrophils are the major target of this toxin [60–62].

Reeves et al. confirmed the significance of gliotoxin for enhancing virulence of *A. fumigatus* using an insect model [63]. Therefore, it can be hypothesized that gliotoxin production by invading *Aspergillus* species can suppress immunity and thereby increase the severity of the infection. In addition, gliotoxin may reduce mucociliary function [64, 65], a property that is also found in some of the other secondary metabolites. *In vitro* experiments confirmed that culture filtrates of *A. fumigatus* that contains gliotoxin might damage human respiratory epithelial cells [64, 65]. Reduced mucociliary clearance provides an opportunity for fungal elements to reach epithelial surfaces, resulting in further damage and potentially tissue invasion.

Fungal metabolites might also impair phagocytic functions that would normally destroy conidial and hyphal forms. Gliotoxin reduces adherence and phagocytosis of fungal elements, while aflatoxin affects phagocytosis, intracellular killing and spontaneous superoxide production. Complement binding and activation of bound opsonins, which normally enhance phagocytosis, are affected too, making fungal elements less susceptible to destruction [66]. In another study, Niyo et al. demonstrated in rabbits that T2 toxin decreased phagocytosis of *A. fumigatus* conidia by alveolar macrophages, thus increasing the severity of experimental aspergillosis [56]. Khoufache et al. demonstrated that verruculogen, another mycotoxin produced by *A. fumigatus* modified the electrophysiological properties of human or porcine epithelial cells [67, 68], which might slow ciliary beating and damage epithelium to influence colonization of *A. fumigatus* in the airways.

Adaptation to vertebrate hosts

Aspergillus species possess versatile features that meet their requirements to survive under different environmental conditions and make the species a ubiquitous fungal pathogen in a wide range of hosts including humans and various

animals [51]. *Aspergillus fumigatus* conidia are dispersed more efficiently in the air than those of most other molds [51]. The slightest air current can cause conidia to disperse due to their remarkable hydrophobicity, and these airborne conidia are protected from ultraviolet irradiation due to the melanin in their cell wall [51,69].

Aspergillus fumigatus can be isolated from a wide range of environmental conditions with the optimal temperature of 37°C res (ranging between 12° and 65°C), and the pH of growth sites ranges between 2.1 and 8.8 [51]. Thermotolerance facilitates the fungus' growth, not only in decaying organic matter—its primary ecological niche—but also within the mammalian or avian respiratory tract. *Aspergillus fumigatus* commonly resides in compost, a dynamic environment that undergoes wide fluctuations in temperature as well as intense microbial activity. The ability to thrive in this habitat requires a substantial level of thermotolerance that has been speculated to contribute to virulence [70]. These properties are likely to have evolved in response to competitors within the ecological niche of the organism and are unlikely to reflect specific adaptations to counter vertebrate host defense mechanisms. In addition, the presence of numerous glycosylhydrolases [71], a group of extracellular proteinases in the *A. fumigatus* genome, attests to the ability of the fungus to grow by degradation of polysaccharides from plant cell walls and acquire nitrogen sources made available by degradation of proteinaceous substrates [72].

The physical characteristics of conidia allow *A. fumigatus* to reach and adhere to the epithelium of airways and distal parts of the respiratory tract more effectively than other fungal species with similar sized airborne spores [51]. *Aspergillus fumigatus* conidia are globose to subglobose with a size (2–3 µm in diameter with extremes up to 3.5 µm) small enough to bypass mucociliary clearance and reach the lower airways. The availability of melanin in the conidial wall and also high negatively charged sialic acid residues are also factors that protect *A. fumigatus* against host cell responses [73, 74]. Recent *in vitro* and *in vivo* studies strongly suggest that galactosaminogalactan (GAG) is a principal mediator of *A. fumigatus* virulence and plays a key role in adherence to host constituents. It was suggested that anti-GAG strategies could be useful in the therapy of invasive aspergillosis, underscoring its importance for the pathogenesis of aspergillosis [75]. In addition, specific genetic changes enhance the ability of the fungus to cause invasive disease in immunocompromised mice and appear to confer one of the following attributes: rapid germination (and growth), increased melanin content and enhanced resistance to oxidative damage [76].

Like many other infectious diseases, development of *Aspergillus* infections is dependent on prolonged interaction between the pathogen and the host. To invade animal

tissues, *Aspergillus* species rely on the coordinated expression of a multitude of genes involved in fungal growth, including conidial germination, cell wall assembly, thermotolerance, nutrient acquisition, and resistance to adverse conditions, such as oxidative stress. Many types of stress have been observed to occur during *Aspergillus* pathogenesis, causing fungal responses that overcome the stress and may be associated with increased virulence and fungal persistence [61,77]. During infection, *Aspergillus* species must tolerate and overcome diverse *in vivo* micro-environmental stress conditions including; high temperature, low pH, limited carbon and nitrogen sources, and regulate iron acquisition and gas tension (carbon dioxide and oxygen levels) [78,79]. Some of these conditions are strongly interconnected. For example, oxygen and iron availability are intimately tied with fungal virulence and response to existing therapeutics [77]. *Aspergillus* species as many other pathogenic fungi, may develop a coordinated regulatory system in response to hypoxia and iron starvation through expression of hypoxia and iron-responsive genes via cross-linked key regulators, and/or regulation of factors involved in ergosterol biosynthesis [80,81]. Moreover, several species of *Aspergillus* can grow in highly extreme environments, which facilitates their adaptation to a wide variety of host tissues [82]. For instance, *A. versicolor* and other species have been isolated from areas with high saline levels such as the Dead Sea [83–86].

Immunosuppression and predisposing factors

Infections by *Aspergillus* species represent a major cause of morbidity and mortality in immunocompromised hosts. In immunocompetent hosts, *Aspergillus* conidia that reach the alveoli are generally unable to overcome the immune defense; however in immunosuppressed patients the inability to efficiently eradicate inhaled conidia, creates an opportunity for the fungus to adapt its physiology to the altered host environment [51]. The population at risk in humans is expanding due to the increasing number of patients on hydrocortisone or other chemotherapeutic treatment resulting in severe neutropenia, patients with stem cell and solid organ transplantation, patients with immunosuppressive and myeloablative therapies for autoimmune and neoplastic disease, later stages of AIDS, and in certain hereditary immunodeficiency syndromes, such as chronic granulomatous disease, in which phagocytes fail to generate superoxide anion [6,8].

Similar to infections in humans, animals exhibiting inability to produce a normal immune response are at higher risk of infection. Aspergillosis may also occur in healthy animals under environmental immune-compromising conditions. In addition to environmental stressors, tuberculosis is a well-known underlying disease in chronic necrotizing

pulmonary aspergillosis and aspergilloma [87,88], with the clinical symptoms of aspergillosis being characterized by limited invasiveness that occurs in mildly immunocompromised animals [88–90].

In reptiles, *Aspergillus* disease may be promoted by immune-compromising conditions, such as husbandry deficiencies or inappropriate temperatures, humidity, or poor enclosure hygiene [91].

Risk factors for aspergillosis in birds seem to be of a basically different nature from those in reptiles. Both host and fungus characteristics explain the particular susceptibility of birds to *A. fumigatus* infection [92,93]. Environmental stressors may also play a role, for example, in poultry farms, where many environmental stressors may be present, including excessive ammonia and moisture, inappropriate temperature, and degraded litter. Furthermore, feed contamination with mycotoxins and/or competing pathogens may affect avian immunocompetence [94]. In wild birds it has been shown that there is a significant link between resource allocation and the costs of immunity, especially in defense against pathogens in environments where multiple factors change in time and space [95].

In dogs, a breed or gender predisposition can be recognized [96]. Factors that may predispose dogs to infection include injury to any of the mucous membranes, the use of catheters, administration of antibiotics, and immunosuppressive drugs or the presence of other diseases.

Cats stressed by underlying disease (such as viral infection) or immunosuppression are more susceptible to infection [97–99]. Viral diseases (due to Feline Immunodeficiency Virus and Feline Leukemia Virus) [6,100] may cause a severe immunodeficiency and short-term reduction of the number of neutrophils and of lymphocyte responsiveness [101]. Moreover, an inherited susceptibility are presumed to influence the incidence of aspergillosis in purebred cats of brachycephalic conformation [102].

In ruminants, dairy cows in early lactation show increased susceptibility to *Aspergillus* [17]. Other factors that seem to predispose to aspergillosis include the presence of other diseases and intense antimicrobial therapy [23].

In horses, aspergillosis can be rapidly fatal when the infection invades the lungs. In these cases, inflammation of the intestines is often a predisposing factor thought to weaken the immune system of the horse, favoring penetration and growth of opportunistic fungi [29,35,103,104].

In cetaceans the infection can be primary or secondary to any chronic infections, physiologic stress or immunosuppression [50]. Aspergillosis also may occur in various nonhuman primate species, particularly in immunocompromised hosts as a post-transplant infectious complication [105,106], or following metabolic disorders [107]. In addition, the underlying infection with Simian

Immunodeficiency Virus (SIV) could be considered a risk factor [88,108].

Diseases in invertebrates

Corals and sponges

Aspergillosis due to *A. sydowii* is a newly recognized fungal epizootic affecting sea fan corals (*Gorgonia* species) [109]. It is believed that virulence of *A. sydowii* increases with temperature, probably because the rate of pathogen development continued to increase in a temperature range where coral defenses became less potent [110]. However, it remains difficult to distinguish between the role of environment in allowing opportunistic pathogens to increase, and influence creation of a niche for new pathogenic microorganism that cause coral disease [111].

The prevalence of aspergillosis is among the highest reported for any coral disease, ranging from 8% to nearly 60% [112,113], which is considered one of the potent effectors of change in natural marine populations and communities [114,115]. Over the past decade, climate warming has caused profound and often complex changes in the prevalence and/or severity of some infectious diseases, contributing to species extinctions, in various hosts [110]. Aspergillosis disproportionately affects large sea fans [116], possibly due to larger colonies that have lower antifungal defenses [117], combined with larger targets for pathogen interception, and accumulation of more pathogen hits over longer lifetimes.

The substantial virulence of *Aspergillus* to sea fans is suggested to be due to two factors. First, given that aspergilli are terrestrial in origin, sea fans may be a naive host and thus highly susceptible to aspergillosis. Second, the density independence of aspergillosis suggests that disease dynamics are dominated by external inputs of the pathogen rather than within-population transmission. However, there is evidence of some secondary transmission among nearby sea fans in the 2–8-m range [118].

Aspergillosis in corals was first documented in 1995 near Saba the Bahamas. Subsequently it was detected throughout the Caribbean basin, including in the Florida Keys [113,119]. The pathogen responsible for the outbreak was identified from Koch's postulates as *A. sydowii* [120,121], a fungus otherwise found in soil. In another study, Kim et al. demonstrated the eradication of large sea fans due to *A. sydowii* infection in the Florida Keys, since August 1997 [109] (Fig. 1), subsequently resulting in a demographic shift to smaller colonies. The reemergence of aspergillosis was detected on small sea fans between 1999 and 2000 at Carysfort and Conch in Florida [109]. In May 1999, 80 recruits (i.e., colonies of corals ≤ 10 cm height) were detected on transects, none of which was infected. By July 2000, preva-

lence in this height class was about 11%, suggesting a shift in age-specific force of infection [109]. This shift was likely due to the input of susceptible colonies into the population rather than the appearance of more virulent strains of this pathogen, given that prevalence among larger fans actually decreased during the same time.

Ein-Gil et al. isolated a strain of *A. sydowii* from healthy marine sponges (*Spongia obscura*) collected in Bahamian inshore waters [122]. After identification on the basis of morphology, molecular markers and chemical profiling was performed as well as pathogenicity tests. These tests indicated that the strain was highly similar to the strains isolated from diseased coral and that the fungus was capable to persist in the sponge environment. This observation suggests that sponges may be a potential new reservoir of the marine pathogen, *A. sydowii*.

Honey bees

Microbiological studies on microhabitats within bee hives have shown a high diversity and abundance of *Aspergillus* species in apiaries [123]. Such close association between fungi and bees within the colony, highlights their potential to cause significant stress on the health of colonies and likewise serious disease in honey bees with weakened immune systems [123]. On the other hand, nutritional limitation, particularly lack of dandelion (*Taraxacum officinale* plants) and polyfloral pollen, can significantly increase the susceptibility of honey bee larvae when exposed to the *Aspergillus* species [124].

Aspergillus species are known to infect honey bee (*Apis mellifera*) brood, causing stonebrood disease over all larval stages [125,126]. Stonebrood is a very rare disease caused by several species of *Aspergillus*. The disease was first described by Massen (1906) and has since been found worldwide [127]. *Aspergillus flavus* has most frequently been reported causing the disease, followed by *A. fumigatus*, but also *A. niger* and other aspergilli can affect honey bees [128]. Species of *Aspergillus* producing aflatoxin have been suggested to be the primary cause of death in stonebrood-infected honey bees [129], although a negative *A. flavus* strain was observed to be equally active [127]. The adult bee is infected through the gut after conidial ingestion, although infection might be accounted through the cuticle surface during the larval stage. The conidia then remain in the larval gut until the first defecation event prior to pupation [123]. Infected brood, also called "mummies," can be seen in the combs. Conidia taken up by bee larva may hatch in the gut, growing rapidly to form a collar-like ring near the head. After death the larvae turn black and become difficult to crush, resembling small stones and hence the name stonebrood. Eventually the fungus erupts from the integument of the larvae and forms a "false skin." At this



Figure 1. Caribbean sea fan corals with aspergilliosis. Adopted from Kim et al. (REF 109) with permission of the publisher. The figure shows high and low density of disease impact. Top: Sea fans (*Gorgonia ventalina*) infected with *Aspergillus*. Bottom: Sea fan skeletons killed by aspergilliosis.

stage the larvae are covered with powdery fungal conidia [130], which are yellow, brown, green, or black depending on the species. Worker bees are unable to remove stone-brood mummies from the cells. In some cases, infected or

deceased larvae appear dry, but they do not produce visible conidia within 48 h after pathogen inoculation [127]. Aspergilliosis of Honey bees is relatively rare, suggesting that conidia are only infective when hives experience an unusual

combination of stress factors [131]. In some countries, however, stonebrood is a notifiable disease that has to be reported to the authorities if it occurs.

Despite above described extensive diseases of *Aspergillus* species in honey bees and sea fan corals, aspergillosis has not been reported from any other invertebrate animal in natural conditions. This further underlines that *Aspergillus* is not a primary pathogen like *Cordyceps* or *Metarrhizium* but just infections to temporarily susceptible hosts. However, a variety of different insect species have been employed to study fungal pathogen-host interactions [132,133]. Experimental *A. fumigatus* infection has been investigated in *Drosophila melanogaster* and *Galleria mellonella* for *A. fumigatus*, but these infection models are beyond the scope of the present study.

Diseases in cold-blooded vertebrates

Fishes and amphibians

Farmed and captured fishes contribute to a significant proportion of global seafood production. In aquaculture, the problems associated with *Aspergillus* species are related to the presence of mycotoxins rather than infection of the host [134]. To our knowledge, aspergillosis has never been reported in amphibians. The low occurrence of aspergillosis in waterborne animals can be explained by the hydrophobic nature of *Aspergillus* conidia, rendering coral aspergillosis described above even more surprising.

Reptiles

In reptiles, *Aspergillus* species such as *A. fumigatus*, *A. niger* and *A. terreus* have been isolated from both cutaneous and disseminated infections [135]. Cutaneous lesions are commonly reported as a consequence of invasion to living tissues enhanced by trauma [91]. *Aspergillus* hyphal elements were isolated from the forefeet of a female musk turtle (*Sternotherus odoratus*) [136], and cutaneous and gingival lesions were observed in a mixed necrotizing dermatitis and pneumonia of crocodiles [137]. In a study by Tappe et al., *A. terreus* was isolated from edematous and necrotic lesions of two San Esteban chuckwallas (*Sauromalus varius*) [138]. Myers et al. reported a case of *Aspergillus* keratitis in an adult male free-ranging gopher tortoise (*Gopherus polyphemus*), which presented with trauma and blindness [139]. Ophthalmic examination revealed bilateral corneal ulcerations, blepharodema, blepharitis scarring and phthisis bulbi of the left globe [139].

Despite the occurrence of several cases of superficial aspergillosis in reptiles, reports of systemic aspergillosis are scarce. Suboptimal husbandry and captivity are likely major predisposing factors leading to opportunistic invasion in these animals [91]. Miller et al. reported pulmonary as-

pergillosis in two captive snakes (*Eunectes murinus*) with the postmortem evidence of multiple scattered, dark red foci on the scales and invasive lesions in the lungs [140].

Diseases in warm-blooded vertebrates

Birds

Etiology: *Aspergillus fumigatus* is considered as one of the major respiratory pathogens in birds [5,141]. Characteristics of both host and fungus explain the particular susceptibility of birds to *A. fumigatus* infection. Birds placed in environments contaminated with aerosolized conidia may show significant pathology after only a short duration of exposure. The anatomy and physiology of the avian lung-air sac system is significantly different from that of the bronchoalveolar lung of mammals. Nine air sacs function as bellows to move air through the lungs gas exchange surface [92, 93]. *Aspergillus fumigatus* conidia are small enough [51] to bypass initial physical barriers and penetrate deeply in the respiratory system [5]. Avian air sacs are particularly prone to contamination because they are submitted to an airflow that favors particle deposition. Birds have few resident macrophages to remove corpora aliena and have an epithelial surface nearly devoid of a mucociliary transport mechanism [142]. Other *Aspergillus* species like *A. flavus*, *A. niger*, *A. nidulans*, and *A. terreus* may also be isolated from cases of aspergillosis (sometimes in mixed infections) in commercial poultry but much less frequently than *A. fumigatus* [143,144]. However, in tropical countries *A. flavus* is probably more prevalent than *A. fumigatus* [145].

Epidemiology: Of note, all avian species should probably be considered susceptible. With the exception of Antarctica, fatal field cases have a worldwide distribution. In the past, *A. fumigatus* has been involved in significant die-offs of free-ranging wild birds, responsible for thousands of deaths in American crow [146,147], Tundra swans [148], Mallards [149] or Canada geese [150], and occasionally in waterfowl, gulls, and corvids following dumping of mouldy waste seeds in areas where birds feed [151]. The higher prevalence in *Anatidae* (water fowl), raptors and *Laridae* (gulls) may reflect true differences in attack rate, differences in detection likelihood in gregarious species compared to other wild birds or a higher risk of exposure [151].

Infection by *A. fumigatus* is also found in birds held in captivity including birds of prey, geese, ducks, swans, gulls, penguins, and parrots. Falconiform species at particularly high risk of developing aspergillosis include goshawks, golden eagles or Gyrfalcons [152]. Captive sphenisciforms (Magellanic and Jackass penguins) in zoological parks [153], or in wildlife rescue centers [154], appear to be extremely susceptible to aspergillosis, which may reflect

underlying husbandry deficiencies. The incidence of infection may be elevated in debilitated animals, particularly oiled birds sheltered in wildlife centers and severely impair rehabilitation success and breeding programs of endangered species [143,144,154–166]. Concomitant agents (either bacteria or parasites) and/or diseases (chronic fatigue and immune dysfunction syndrome) are regularly identified in falcons suffering from aspergillosis [161].

Economic significance of aspergillosis is most readily apparent in turkey production where disease occurs late in the growing cycle or primarily affects costly breeder toms [167]. In spontaneous outbreaks, the mortality ranged between 4.5% and 90%, with the age of diseased birds varying from 3 days to 20 weeks [144,168]. In poultry farms, mortality of acute aspergillosis tends to rise slowly [168], or increase suddenly, then peaks over a few days, and subsequently returns to the initial state [169]. In addition to direct losses related to mortality, feed conversion and growth rate associated with reduced welfare in recovering birds remain poor. Indeed, airsacculitis is a major reason for carcass condemnation at slaughter inspection [170,171].

Clinical manifestations of aspergillosis in birds depend on the infective dose, spore distribution, pre-existing diseases, and immune response of the host [5,141,161,172,173]. It is believed that impaired immunity and the inhalation of a large inoculum of conidia are important causative factors [94]. Active fungal proliferation and sporulation of *A. fumigatus* on organic material produces large amounts of small-sized conidia that are easily dispersed in air, then potentially inhaled and deposited deep in the respiratory tract. Discriminatory molecular genotyping based on multilocus microsatellite panels has demonstrated that the environment of diseased animals may be a source for *A. fumigatus* infection and that either multiple [153,174,175] or single genotype [176] linked infections could occur in confirmed cases. Susceptible hosts will develop polymorphic clinical forms in relation to either localized or disseminated lesions [141,177]. Although aspergillosis is predominantly a disease of the respiratory tract, all organs can be involved, leading to a variety of manifestations, which can be acute or chronic.

Acute aspergillosis: Acute aspergillosis is thought to result from inhaling an overwhelming number of conidia, which generally occurs in young birds, resulting in high morbidity and mortality. Acute aspergillosis may include a variety of nonspecific clinical signs: anorexia, lethargy, ruffled feathers, respiratory signs, polydipsia, polyuria, stunting, dyskinesia or sudden death. In chicks, contamination *in ovo* or during hatching, the disease, commonly known as brooder pneumonia, is highly lethal during the first ten days of life and results in major respiratory distress [178,179]. In poultry, respiratory signs include dyspnea, gasping, hyper-

pnoea with open-mouth breathing, non-productive coughing, wheezing, tail bobbing, cyanosis [144], and sometimes nasal discharge [168].

Chronic aspergillosis: Chronic aspergillosis is generally associated with immune suppression. The chronic form is sporadic. It causes less mortality and generally affects older birds, especially breeders (parent chickens that produce fertilized eggs) in poultry, presenting a compromised immune system due to poor husbandry [180]. Clinical signs of the chronic form are often nonspecific. Birds exhibit reduced level of activity, weight loss, and exercise intolerance associated with dyspnea. Involvement of the nervous system (encephalitic and meningoencephalitis lesions) causes ataxia, opisthotonos, torticollis, limb paresis, and in some cases blindness [144,168,181] (Fig. 2). Occurrence of nervous and ophthalmic complications, one week after an acute episode of aspergillosis, has been reported in a turkey flock [181]. Cloudiness of the eye with severe conjunctivitis was associated with paralysis in broiler breeders [144]. *Aspergillus* blepharitis, keratitis and keratoconjunctivitis (turbid discharge, cloudy cornea, and cheesy yellow exudates within the conjunctival sac) have been reported in numerous species [182,183]. *A. fumigatus* can colonize skin [184,185] and surgical wounds, as observed in caponized cockerels [186] and induce systemic disease [184]. Nasal aspergillosis is associated with exudative rhinitis, possibly accompanied by malformation of the nostrils, beak, and cere [187]. Paralysis, secondary to mycotic spondylitis, has been described in commercial broiler flocks [188] and pheasants [163,164]. Cases of omphalitis or articular aspergillosis of hip joints associated with *A. fumigatus* have been observed in turkeys [189,190]. Right ventricular dilatation (cor pulmonale) due to pulmonary hypertension, with or without ascites, and congestion of the lungs caused by ventricular failure occurs occasionally [191].

Mycotoxicosis: Ingestion of feedstuffs contaminated by toxic metabolites can cause mycotoxicosis in birds. The ability of *A. fumigatus* strains to produce significant levels of gliotoxin in the lung tissue of naturally [192] or experimentally [193] infected turkeys demonstrated that this immunomodulating compound [194] may be involved in the pathogenesis of aspergillosis in this host. Among the mycotoxins produced by *Aspergillus* species, aflatoxin has been responsible for rare mortality reports in free-ranging duck, geese, and crane species [195]. In the early 1960s, an unusual veterinary crisis occurred in the United Kingdom, during which approximately 100,000 turkey poults died of “turkey X disease.” The cause was revealed to be toxicity of fungal metabolites and could be attributed to aflatoxins [196]. This group of secondary metabolites is also highly toxic for ducklings and chickens provoking reduced performances and sudden mortality associated with



Figure 2. Mute swan (*Cygnus olor*) suffering from fatal aspergillosis. Adopted from courtesy of P. Arne. Top left: Severe dyspnoea with open-mouth breathing. Right: presence of granulomas and cheesy plaques in the trachea. Bottom left: Air sac completely filled by caseous necrotic material containing fungal elements.

kidney and liver lesions. Mycotoxicosis following ochratoxin or sterigmatocystin ingestion may also occur in poultry [197,198].

Dogs

Sinonasal, bronchopulmonary, and disseminated infections are the three major forms of aspergillosis in dogs. Although sinonasal aspergillosis is the most common form of as-

pergillosis diagnosed in dogs, it is an uncommon yet debilitating and often frustrating condition to treat in dogs [37]. The condition is usually seen in dolichocephalic and mesocephalic dogs and is very rare in brachycephalic dogs. German Shepherds and Rottweilers are the commonly affected breeds. Dogs of any age may be affected, but approximately 40% are 3 years or younger and 80% are 7 years or younger [96]. In several studies of dogs with chronic nasal disease, sinonasal aspergillosis occurred with a

frequency of 7 to 34% [40]. It is the second most common cause of nasal discharge in dogs after nasal neoplasia [199]. *Aspergillus fumigatus* is most frequently isolated, although various other species including *A. niger*, *A. nidulans*, and *A. flavus* have been reported. *Penicillium* species and other fungi are much less frequently detected [200].

Clinical signs in advanced disease can include nasal pain, nasal pain, ulceration or intermittent bilateral epistaxis [38, 39] (Fig. 3). Moreover, in severe cases destruction of the cribriform plate is identified.

Recently, the immunopathogenesis of canine sinonasal aspergillosis has been investigated more precisely [41,201,202], which could provide a valuable model for the equivalent human pathology [203, 204]. The mucosal inflammatory infiltrate involves a mixture of CD4⁺ and CD8⁺ T lymphocytes, IgG-secreting plasma cells, and activated macrophages and dendritic cells expressing class II molecules of the major histocompatibility complex. There is active recruitment of blood monocytes and neutrophils and up-regulation of Th1 (interleukin [IL]-12, IL-18, and interferon (IFN) γ), Th17-related (IL-23) and pro-inflammatory (IL-6, tumor necrosis factor (TNF) α) cytokine mRNA with evidence of expression of genes encoding monocyte chemo-attractant proteins 1–4. Additionally, there is significant transcription of the IL-10 gene consistent with local immunosuppression that may prevent secondary immune-mediated sequelae while permitting chronicity of the infection [41,201–204]. As a comparison, similar gene expressions were shown in sinonasal aspergillosis of humans [204]. However, recent investigation by Mercier et al. indicated that dysfunction in innate immunity, particularly in the function of pattern recognition receptors is not associated with the pathogenesis of canine sinonasal aspergillosis [205].

Bronchopulmonary aspergillosis is a rare disease in dogs [42–44]. The clinical signs are nonspecific, including depression, fever, and cough [42]. Cytological evaluation of the bronchoalveolar lavage fluid often reveals a mixed inflammatory response dominated by neutrophils and macrophages but rarely reveals the presence of fungal elements [45]. Disseminated aspergillosis in dogs is relatively infrequent, but it is a potentially fatal disease, which most often is seen in German Shepherds and is usually due to *A. terreus* and *A. deflexus*, followed in order of decreasing frequency by *A. fumigatus*, *A. niger*, and *A. flavipes* [46]. Clinical signs of disseminated aspergillosis may include lethargy, lameness, anorexia, weight loss, pyrexia, hematuria, urinary incontinence, generalized lymphadenopathy, and neurologic deficits. Lesions are frequently found in the kidneys, spleen, and vertebrae (discospondylitis). Recently, Zhang et al. reported the first case of canine aspergillosis caused by *A. versicolor* [47]. The dog suffered from

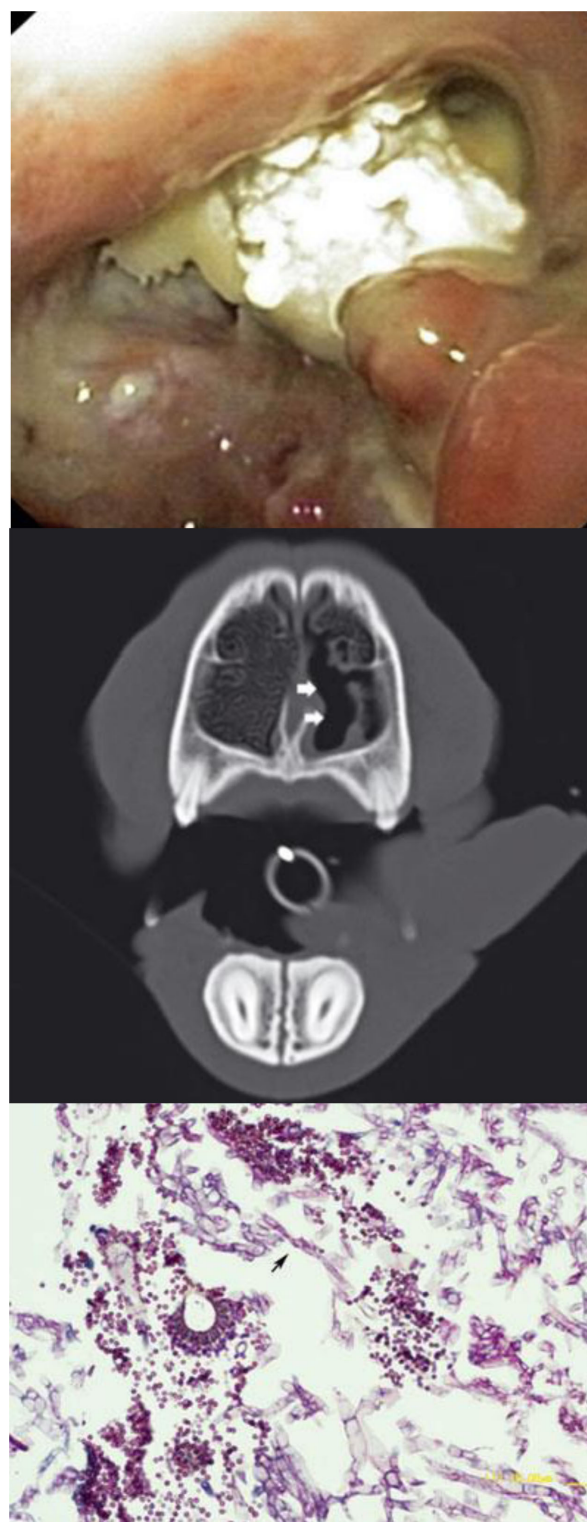


Figure 3. Nasal cavity and sinus of a dog with sino-nasal aspergillosis. Adopted from Sharman et al. (REF 37) with permission of the publisher. Top: Severe nasal turbinate destruction and fungal plaques within the nasal cavity of a dog affected by sinonasal aspergillosis. Middle: Destruction of the nasal turbinates identified on computed tomography (CT) of the nasal cavity. Bottom: Histologic section of a nasal cavity biopsy showing an *Aspergillus* spp. conidial head and many small conidia. Many septate hyphae are also seen (arrow). H&E, scale bar = 10.08 μ m.

disseminated *A. versicolor* infection presenting as diskospondylitis, osteomyelitis, and pyelonephritis [47].

Notably, immunopathogenesis of canine disseminated sinonasal aspergillosis shows significant similarities with humans [203]. The mucosal inflammatory infiltrate involves a mixture of CD4⁺ and CD8⁺ T lymphocytes, IgG⁺ plasma cells and activated macrophages and dendritic cells expressing class II molecules of the major histocompatibility complex. There is active recruitment of blood monocytes and neutrophils. Additionally, there is significant and up-regulation of Th1 (IL-12, IL-18, and IFN- γ), Th17-related (IL-23) and pro-inflammatory (IL-6, TNF α) cytokine [203]. Ootomycosis due to *Aspergillus* species has occasionally been described in dogs [206]. Dogs had previously been treated with various topical and oral antibiotics, which may have predisposed them to develop a secondary *Aspergillus* infection.

Cats

Sinonasal and sinoorbital infections are two forms of *Aspergillus* disease in cats that account for the majority of the reported cases affecting the upper respiratory tract [207, 208]. Reports on feline cases of orbital aspergillosis are on the rise possibly due to viral-induced immunodeficiencies [97–99], and orbital aspergillosis is considered an emerging disease [209]. This infection is characterized by progression of sinonasal aspergillosis to the preorbital area, which is challenging to treat and the prognosis for resolution of infection is generally poor [97–99].

Aspergillus felis has been the most frequently reported etiologic agent of sinoorbital aspergillosis in cats, followed by cryptic species of the section *Fumigati*, including: *A. udagawae* and *A. viridinutans* [99, 210]. Barrs et al. reported a novel species of *Aspergillus* section *Fumigati*, *A. felis* (with a *Neosartorya* teleomorph), isolated from domestic cats with invasive fungal rhinosinusitis, a dog with disseminated invasive aspergillosis, and a human with chronic invasive pulmonary aspergillosis [210]. *Aspergillus felis* is thermotolerant with a maximum growth temperature of $\geq 45^{\circ}\text{C}$. The species can be separated from its close relative *A. viridinutans* by its ability to grow at 45°C , and from *A. fumigatus* by its inability to grow at 50°C . The species *A. felis* can be reliably identified by ITS sequencing. In a second study, these authors also documented etiology, clinicopathological findings, and treatment outcomes in a series of 23 cats (1.5–13 years of age) with sinonasal ($n = 6$) and sinoorbital ($n = 17$) aspergillosis [207]. Cases were recruited retrospectively, and prospectively if hyphae were identified on cytological or histological examination. Polymerase chain reaction (PCR) and DNA sequencing was used to identify the fungal pathogen. Fungal culture was positive in 22 of 23 cases. In cases of sinonasal aspergillosis, the fungal

pathogen was *A. fumigatus* ($n = 4$), *Neosartorya fischeri* or *A. lentulus* ($n = 1$), or *A. felis*. ($n = 1$). However, in all cases of sinoorbital aspergillosis ($n = 17$), the fungal pathogen was identified as *spa. felis*. Cats with sinonasal aspergillosis were more likely to be infected with *A. fumigatus* and had a better prognosis than cats with sinoorbital aspergillosis (Fig. 4) [207, 210].

Orbital aspergillosis was reported by Kano et al. in a spayed female domestic short-hair cat with progressive protrusion of the left third eyelid and eyeball [211]. The cat was treated with antibacterials for a period of two months. Hematology and serum biochemistry showed no abnormal findings. Computer tomographic scan revealed a soft-tissue mass within the orbit of the left eye. Histopathologic examination of biopsy samples from this mass revealed granulomatous inflammation containing many branched *Aspergillus* hyphae [99]. Brachycephalic feline breeds seem to be at increased risk for development of nasal aspergillosis and penicilliosis [102, 212]. Ulcerative keratomycosis is common in cats and is frequently associated with feline herpes viral infection [213]. Labelle et al. examined an 8-year-old domestic short-haired cat with a 1-week history of blepharospasm and mucoid ocular discharge. Ulcerative keratitis was observed with stromal loss, stromal infiltrates, corneal edema, perilimbal vascularization and myosis. Cytology of the cornea revealed multiple dichotomously branching, septate hyphae and severe, predominantly neutrophilic inflammation. PCR of the cytological samples confirmed the presence of *A. flavus*.

Ruminants

Aspergillus species and particularly *A. fumigatus* are known worldwide to cause mycotic pneumonia, gastroenteritis, mastitis, placentitis and abortions in ruminants, especially cows [17]. *Aspergillus fumigatus* is a fairly common mold in hay and silage [18]. Healthy cows with an active immune system are resistant to opportunistic infections, but dairy cows in early lactation are more susceptible, while hemorrhagic bowel syndrome is more likely to occur in fresh cows (within 2–4 weeks since calving) [17].

Bronchopulmonary aspergillosis: *Aspergillus* pneumonia is a fatal disease in ruminants that may progress rapidly [23]. Clinical signs of disease include pyrexia, rapid, shallow and stertorous respiration, nasal discharge, and a moist cough. The lungs are firm, heavy, and mottled and do not collapse. In subacute to chronic mycotic pneumonia, the lungs contain multiple discrete granulomas, and the disease grossly resembles tuberculosis [19–21]. Overall, histologic examination of the lungs in pulmonary aspergillosis indicates abundant hyphae and high numbers of associated oxalate crystals [19]. The mechanism of oxalate crystal

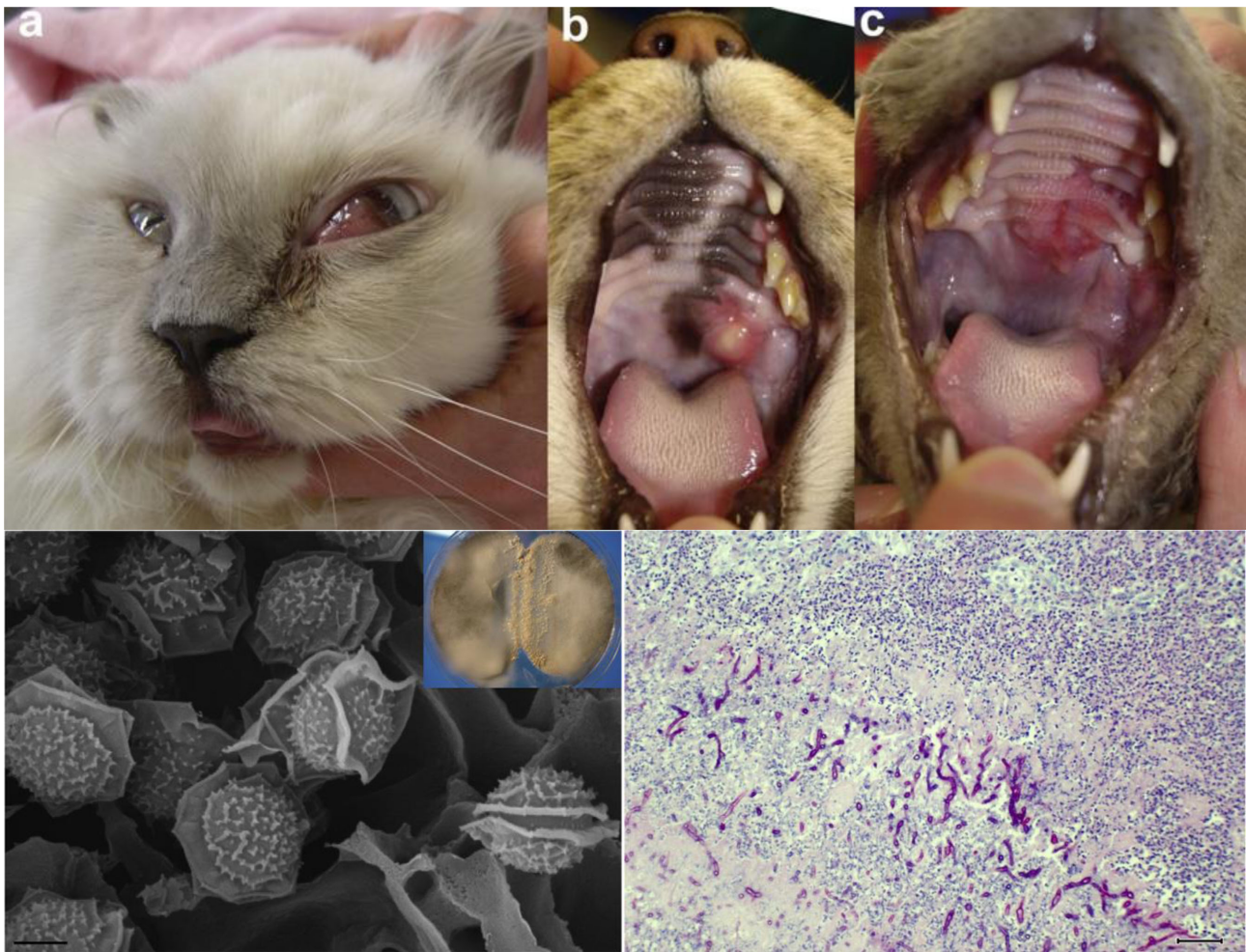


Figure 4. Eye and orbital cavity of a cat with sino-orbital aspergillosis. Adopted from Barrs et al. (REF 210) with permission of the publisher. Top: a. Exophthalmos, b. mass in the left pterygopalatine fossa and c. ulceration of the hard palate. Bottom left: Cleistothecia (small spherical structures) at the colony junction in paired cultures of *Neosartorya* spp. isolates from two cases (inset). *Neosartorya* spp. ascospores with roughened side walls and two axial crests. Scanning electron micrograph (Zeiss EVO LS15). Scale bar = 2 μ m. Bottom right: Periodic acid-Schiff-stained section of frontal sinus epithelium from a cat with invasive sino-orbital aspergillosis. Fungal hyphae are present deep within the sinus epithelium, demonstrating the invasive nature of this mycosis. Scale bar = 60 μ m.

production is not known, but several *Aspergillus* species, particularly *A. niger* are known to produce oxalic acid via the tricarboxylic acid cycle. Oxalic acid exerts a toxic effect on tissues and causes marked tissue necrosis. The crystals themselves may also cause severe localized tissue damage, which can be extensive enough to cause fatal pulmonary hemorrhage [214]. In humans, there is also a direct association between *A. niger* infection and oxalate crystal deposition in tissue [214–216].

Of note, concurrent tuberculosis and aspergillosis has been reported in cattle with underlying mycotic lymphadenitis [90]. In another report, aspergillosis was associated with underlying paratuberculosis, cholangiocarcinoma and peritonitis in a goat [89].

Gastrointestinal aspergillosis: In cows, the gastrointestinal tract, and almost exclusively the omasum, is the primary site of mycotic lesions caused by *A. fumigatus* [23]. A sur-

vey conducted in Denmark revealed the presence of mycotic lesions in the forestomachs of 6% of 932 cattle necropsied between 1986 and 1992 [217]. Common findings in these animals were intense treatment with broad-spectrum antimicrobial drugs, lack of rumen contraction, and diarrhea and/or melena. Experimental infections in pregnant cows and mice as well as histopathological examination of tissue from spontaneously infected cattle suggested that placentitis and pneumonia are secondary infections resulting from hematogenous spread of the fungus from primary gastrointestinal lesions [22,218,219]. In addition, *Aspergillus* species have recently been proposed as pathogenic agents associated with mycotic hemorrhagic bowel syndromes in dairy cattle [17].

In humans, local invasive aspergillosis of the gastrointestinal system is quite rare, with the small intestines most commonly being involved [220–222]. Gastric involvement

is assumed to develop following chemotherapy and high-dose steroid use. However, there are some reports that patients did not have any history of predisposing drug usage or immune suppressive condition [220].

Mycotic mastitis: The reported incidence of mycotic mastitis in cows caused by *A. fumigatus* has increased, although the number of reported cases is lower than in small ruminants [24–26]. Sporadic cases of *Aspergillus* mastitis were described in dairy sheep subsequent to the antibiotic treatment of animals before parturition [24–26, 223].

Fungal placentitis: Fungal placentitis due to *Aspergillus* species is an important cause of abortion in cattle, which generally occurs as an uncomplicated infection in the third trimester of pregnancy [27]. However, early abortion (from the third month of gestation) is also possible. Concomitant abortions may be detected in the same herd due to massive environmental contamination by *Aspergillus* conidia. Following abortion, placental retention is frequently observed. In cattle, placentitis is secondary to the dissemination of *Aspergillus* in the entire body. Nevertheless, no clinical signs are seen before the abortion. Moreover, affected cows may have a normal gestation the next year and the mycotic infection of the placenta should not be considered a poor prognostic signs for fecundity. Rarely cows may present with pneumonia or endometritis a few weeks after abortion. Among 369 cases, *A. fumigatus* was identified as single agent in 64% of cases, while two different species were detected in 87% [27].

Although the pathogenesis of mycotic placentitis is thought to involve hematogenous spread of fungal elements from foci of infection in either the respiratory or digestive tracts, experimental model of infection have failed to confirm this route of infection [22]. Sarfati et al. investigated the route of infection by genotyping *A. fumigatus* isolates from a cow with disseminated aspergillosis, cows with single aspergillosis lesions, calves that had aborted due to bovine aspergillosis, mothers of those calves, and cattle without aspergillosis [22]. The authors demonstrated that the portal of entry was the gastrointestinal tract and that the infection of aborted calves was due to maternally derived isolates that had possibly crossed the placental barrier. Cattle from the same farm that were slaughtered on the same day harbored isolates with the same genotype.

Mycotoxiosis: *Aspergillus* secretory products such as gliotoxin and tremorgens are toxic to cattle. *A. fumigatus*-contaminated silage was found to contain fumigaclavine A and C and several fumitremorgins [18]. Cattle consuming this silage demonstrated signs of generalized deterioration, protein deficiency, malnutrition, diarrhea, irritability, abnormal behavior, and occasionally death. When contaminated hay was fed to goats and rats, retarded

growth and histopathological changes in liver and kidney were observed. Gliotoxin was shown to affect rumen fermentation, reducing digestibility [224].

Moreover, a neurological syndrome in dairy cattle associated with consumption of contaminated foodstuffs by strains of *A. clavatus* has been described [225,226]. *Aspergillus clavatus* is known to produce several tremorgenic metabolites such as patulin and clavatul that are selectively neurotoxic to animals [225,226].

Horses

Aspergillus species primarily cause guttural pouch (a pair of air chambers in the neck just behind the skull and below the ears of the horses) infections and pneumonia in horses [28,227]. Equine aspergillosis is considered a rare but life-threatening infection, with a prevalence ranging from 0.5% to 17% in some European studies [29]. Predisposing factors usually include enteritis [35], prolonged administration of antibiotics, immunosuppressive state of the host, and the presence of endocrinopathies and/or neoplasia [103]. Accordingly, disease usually occurs when host-debilitating conditions favor the penetration and growth of *Aspergillus* fungi [29], but there does not appear to be a breed or gender predisposition [104]. Immunosuppression due to debilitating disease (like salmonellosis) may also predispose horses to aspergillosis [29,104].

Pulmonary aspergillosis in horses may present with mild respiratory signs, tachypnea associated with adventitious lung or pleural sounds and fever [30–32]. Because invasive pulmonary aspergillosis can be difficult to diagnose, veterinarians should be aware of clinical and epidemiologic settings in which this disease might develop [30,35].

In horses, the role of fungi in diseases of the guttural pouch was first suspected at the end of the 19th century, but definitive proof was provided only in 1968 [228]. Typical lesions are characterized by clearly demarcated, yellow-brown, necrotic tissue firmly adherent to the surface of the medial compartment of one guttural pouch [33,34] (Fig. 5). As long as the underlying structures (vessels and nerves) are not affected, the infection remains asymptomatic. The erosion of the internal carotid or maxillary artery leads to the sudden development of profuse epistaxis in a horse at rest. Within a few days, fatal hemorrhage occurs in 34% to 60% of cases. Inflammation of the cranial nerves leads to the development of dysphagia (with nasal discharge), laryngeal hemiplegia, facial paresis or Horner's syndrome. Lane et al. reported that 10% of horses presenting with unilateral or bilateral discharge were affected by guttural pouch aspergillosis [229]. Ludwig et al. reviewed the etiological agents in 21 horses with guttural pouch mycosis in France between 1998 and 2002, underlining the role

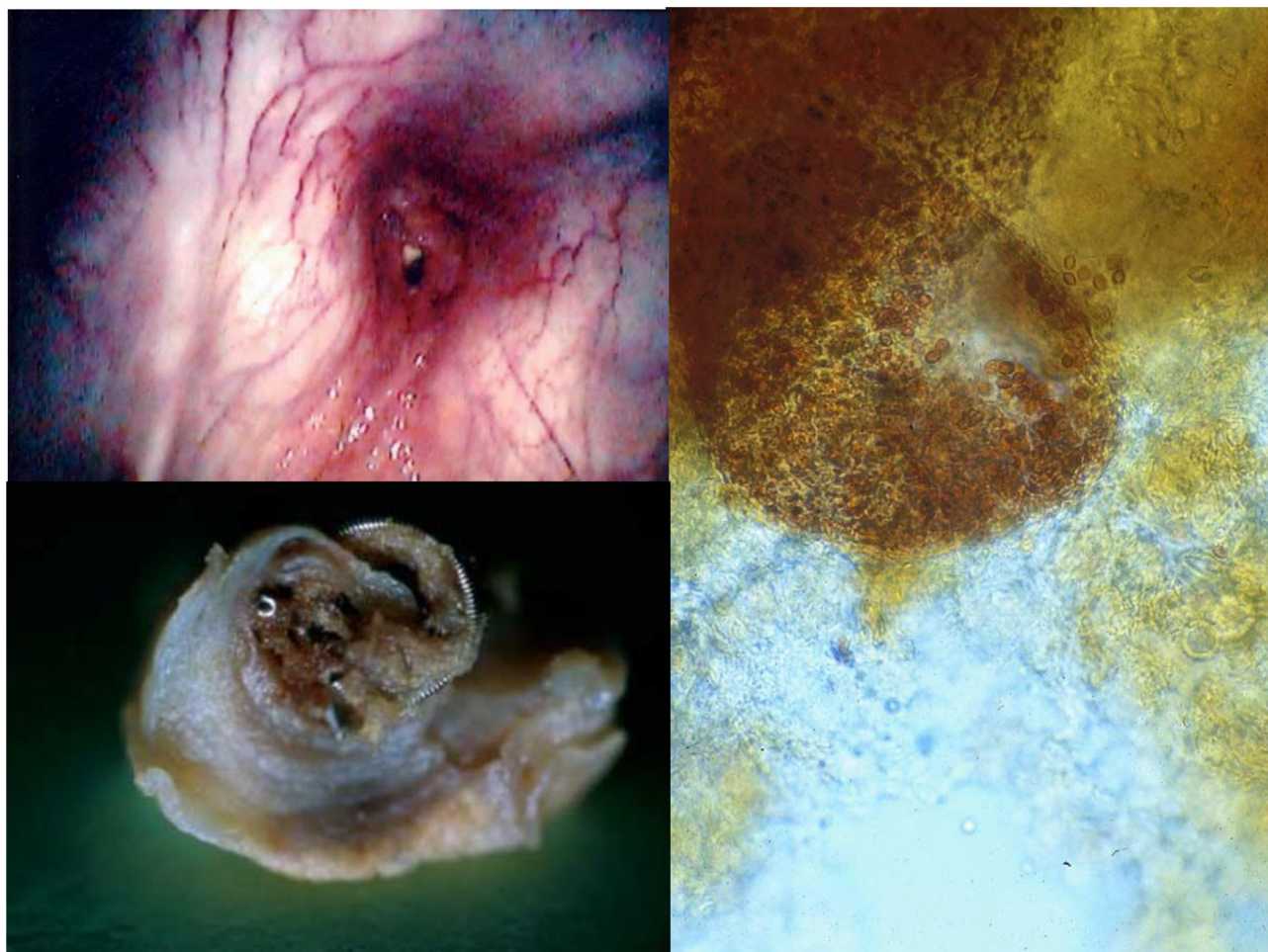


Figure 5. Guttural poach in a horse with *Aspergillus* infection. Adopted from Ludwig et al. (REF 230) and Lepage et al. (REF 104) with permission of the publishers. Top left: An endoscopic view of the pharynx of a two year-old female pony, showing a fistula in communication with the guttural pouches. Bottom left: Macroscopic view of a section of the proximal aspect of an embolized internal carotid artery one week after occlusion. Right: Direct examination of the biopsy sample showing a cleistothecia of *Aspergillus* spp.

of *Aspergillus* species [230]. Biopsies were taken from the lesions caused by guttural pouch mycosis during endoscopic examination. In 87% of the cases, direct examination was consistent with fungal infections, but 43% of cultures remained negative. Among the fungi detected were *A. fumigatus* (in three cases), *A. versicolor* (in two cases, together with other fungi), *A. nidulans* and *A. niger* (one case each), while in six cases the *Aspergillus* could not be identified to the species level.

Nasal aspergillosis is another uncommon presentation of disease in horses with a wide range of clinical signs, characterized by dyspnea and nasal discharge [36]. Des Lions et al. reported a case of mycotic mass in the frontal sinus of a 7-year-old French saddle gelding due to *A. fumigatus* [231]. Direct endoscopic examination revealed a dorsal lobular mass and a small amount of purulent exudate within the dorsal conchal sinus. Histopathology of the mass revealed regular branching, septate fungal hyphae consistent with *Aspergillus*.

In horses, keratomycosis is a relatively common disease, particularly in warm climates, usually following a corneal injury by plant material. There are several clinical presentations varying from superficial punctate lesions that disrupt the tear film and colonize the epithelium, to anterior stromal plaques that consist of mats of fungal elements and necrotic debris, or severe melting ulcers to intrastromal corneal abscesses [232–234]. Blepharospasm, photophobia, and lacrimation are consistent features of the involved cases. Many different fungi have been isolated from the conjunctival sac of horses, including *Aspergillus* species [232]. The identity of species concerned may change with geography and climate. Prolonged topical application of antibiotics may cause a shift in the normal conjunctival flora, which may enhance the incidence of fungal keratomycosis. Also corticosteroids promote fungal growth. Small corneal ulcers may heal spontaneously, trapping the organisms deep within the stroma, and result in the development of a stromal abscess. However, large stromal ulcers may fail to heal

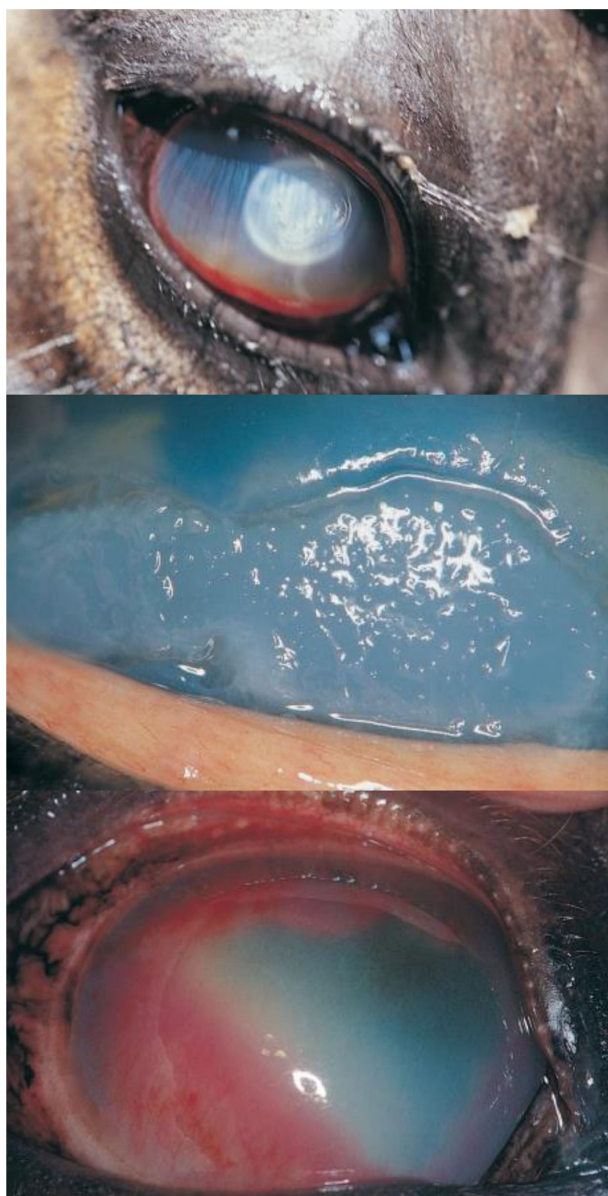


Figure 6. Eye of a horse with ulcerative fungal keratomycosis due to *Aspergillus* infection. Adopted from Sansom et al. (REF 232) with permission of the publisher. Top: The “cake frosting” appearance. Middle: A large central area of corneal ulceration with furrowing Bottom: Stromal abscessation with intense corneal vascularization.

and may become slowly progressive [235]. In the study of Sansom et al., the six cases described could be classified into three of these categories: corneal ulceration with a surrounding furrow, corneal ulceration with a “cake frosting” appearance, and corneal abscessation [232] (Fig. 6).

In humans, *Aspergillus* species are among major fungal pathogens that cause ocular morbidity and blindness [236,237]. Mycotic keratitis and mycotic endophthalmitis are the most common clinical manifestations of these infections in humans [236,237].



Figure 7. Brain of a northern bottlenose whale (*Hyperoodon ampullatus*) with encephalitis due to *Aspergillus fumigatus*. Adopted from Dagleish et al. (REF 240) with permission of the publisher. Top: There are roughly circular, poorly circumscribed areas of hemorrhage (black) in coronal sections of the cerebrum (top left) and midbrain (bottom), both anterior views, and a sagittal section of the cerebellar vermis (top right). Scale bar = 1 cm. Bottom: Histological preparation of the cerebral cortex: Thrombosed blood vessel and surrounding thick cuff composed of polymorphonuclear neutrophils, hemorrhage and fibrin (arrow) and fungal hyphae in the neuropil (arrowhead) are visible. Haematoxylin and eosin. Scale bar = 100 μ m. Inset: Histological preparation of cerebral cortex showing the presence of septate dichotomously branching fungal hyphae, typical of *Aspergillus fumigatus*, in the neuropil. Grocott-Gomori methenamine silver. Scale bar = 50 μ m.

Marine mammals

In marine mammals, aspergillosis, although considered rare, is reported with increasing frequency [48–50]. *Aspergillus* diseases are critically important among the fatal infectious diseases in marine mammals as primary infection or secondary to any chronic infectious process and may be indicative of underlying immunosuppression [50].

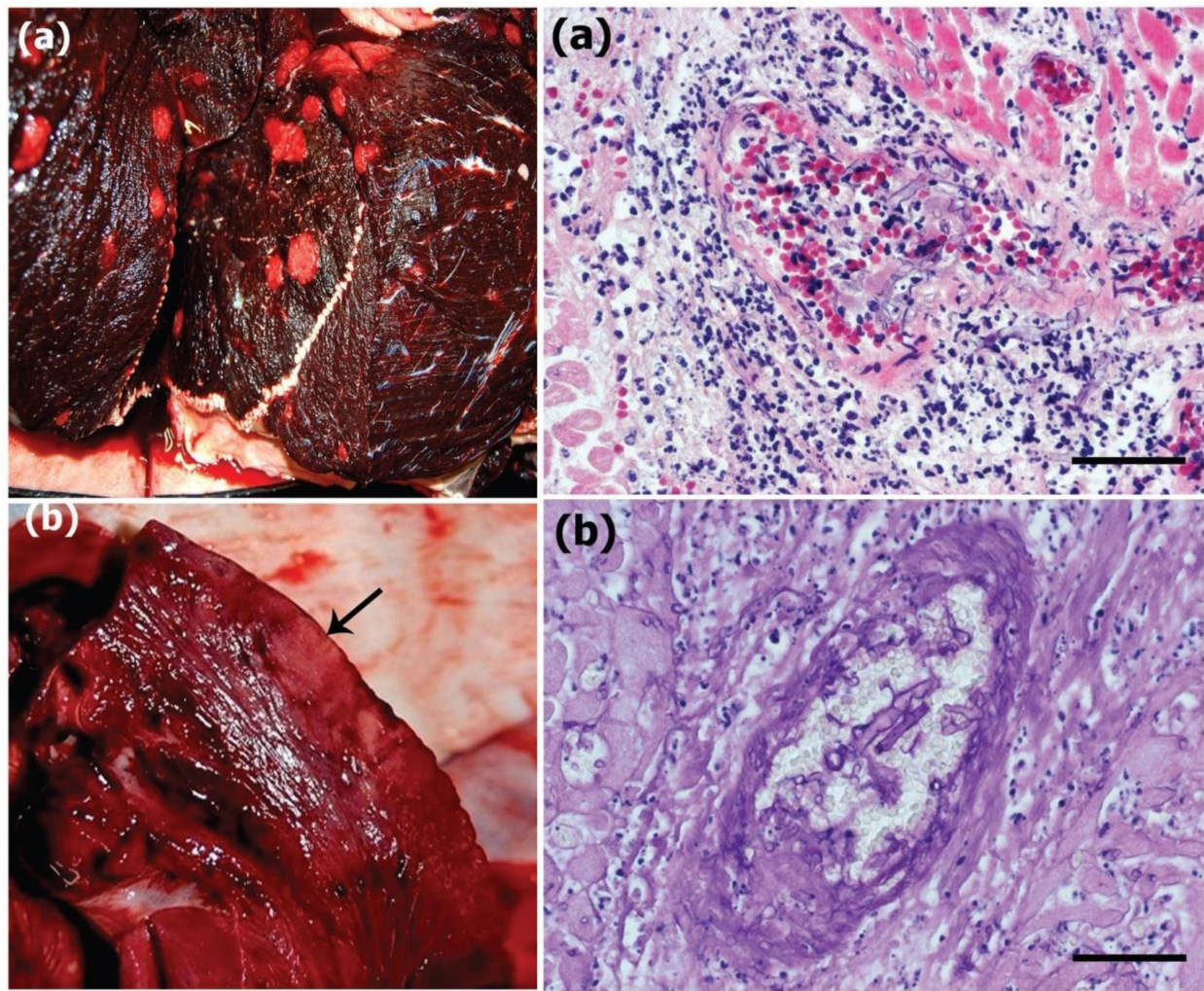


Figure 8. Muscle and heart of a killer whale (*Orcinus orca*) with disseminated mycoses caused by dual infection with *Mucor* and *Aspergillus* species. Adopted from Abdo et al. (REF 50) with permission of the publisher. Top left: Multifocal well-demarcated, light tan, and soft foci were present in various muscles in the caudal thorax and abdominal areas. Bottom left: Two tan nodules (arrow), 1 cm × 4 cm, bulged slightly from the surface in the left ventricle 7 cm from the apex. Top right: Frequent mycotic embolisms in small blood vessels. Hematoxylin and eosin stain. Scale bar = 40 μ m. Bottom right: Necrotizing thromboarteritis due to fungal invasion of the arterial wall. Periodic acid-Schiff reaction. Scale bar = 20 μ m.

Pulmonary mycotic infections due to *A. fumigatus* or less frequently *A. niger* or *A. terreus* occur in cetaceans [238, 239]. Other organs may also be affected [48, 50, 240]. Dagleish et al. reported a case of severe mycotic encephalitis with the gastrointestinal involvement caused by *A. fumigatus* in a stranded northern bottlenose whale (*Hyperoodon ampullatus*) [48]. Gross *postmortem* examination revealed that the right lung was congested. The meninges of the brain were congested and a single focal, roughly circular area of hemorrhage approximately 2 cm in diameter was present immediately beneath the leptomeninges of the left cerebral hemisphere. Sectioning of the brain revealed multiple roughly circular focal hemorrhagic lesions up to 3 cm in diameter with poorly defined margins throughout both the gray and white matter. Most were in the cerebrum but lesions were also present in the midbrain and the base of

the cerebellum (Fig. 7). In another study by Abdo et al., disseminated mycoses due to dual infection with *Mucor* and *Aspergillus* species was reported in a killer whale (*Orcinus orca*) [50]. At necropsy, multiple, light tan foci of necrosis were found in the skeletal and cardiac muscles, and lung consolidation (Fig. 8). Aspergillosis also has been reported from otitis media and interna in a stranded harbor porpoise (*Phocoena phocoena*) caused by *A. terreus* [241]. Severe fungal infestation by *A. terreus* was documented in the otic region, but not in any other site of the body. Adjacent to the promontorium, massive accumulation of fibrinous secretion and infiltration of clusters of inflammatory cells were present. Newly formed cysts and vessels replaced the round window membrane location, reminiscent of granulation tissue. Complete absence of sensory cells of the Organ of Corti characterized a further severe phenomenon,

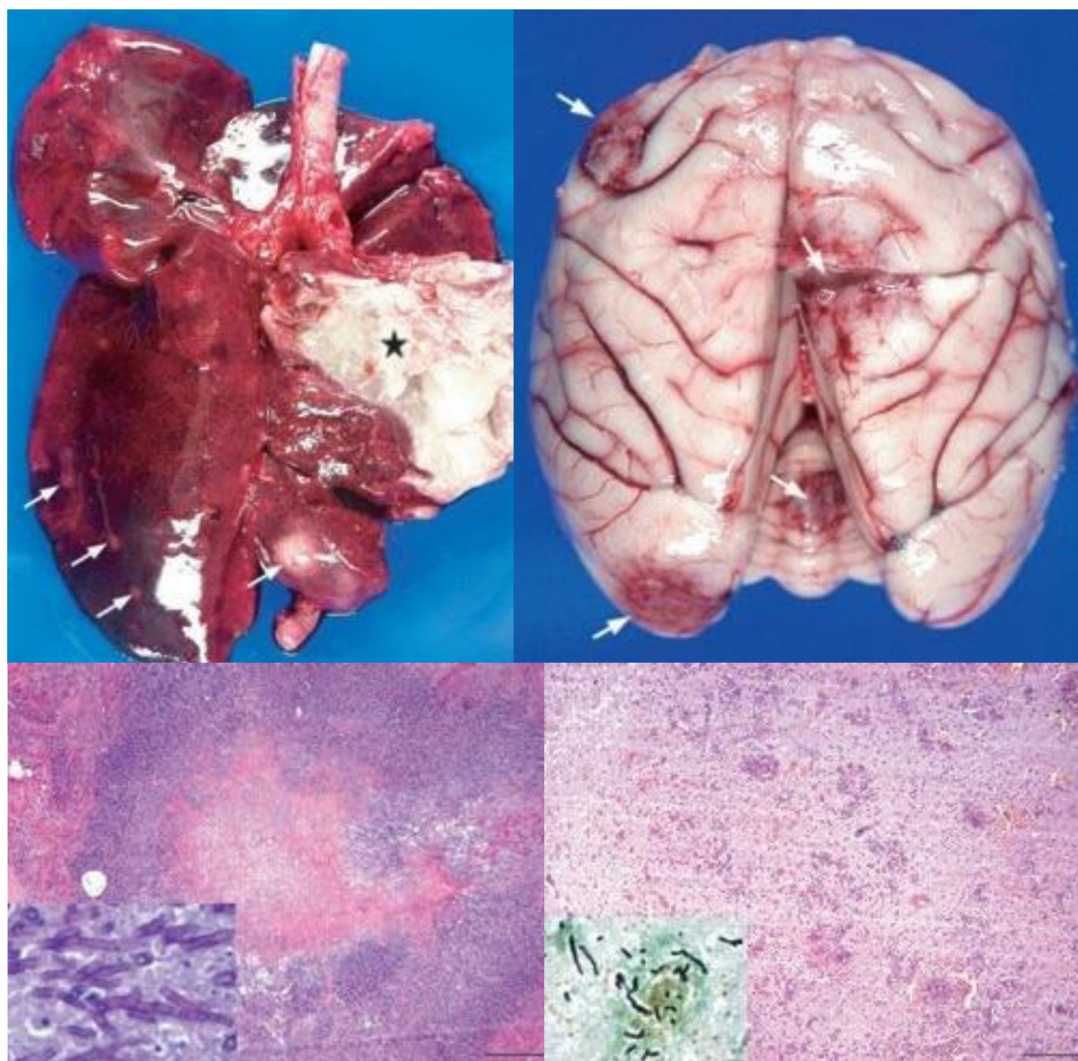


Figure 9. Lung and brain of a putty-nosed monkey (*Cercopithecus nictitans*) with invasive aspergillosis. Adopted from Jurczynski et al. (REF107) with permission of the publisher. - Necropsy findings of invasive aspergillosis: Top left: Lung with right-sided severe compression atelectasis and marked diffuse fibrino-suppurative pleuropneumonia (asterisk). Left lung shows marked congestion and multifocal abscesses of different size (arrows). Top right: Brain with diffuse meningeal hyperemia, multifocal suppurative meningitis, and multiple necro-hemorrhagic foci on the cerebral and cerebellar cortex (arrows). -Representative histopathological findings with marked invasive aspergillosis and hyperglucocorticism: Bottom left: Lung parenchyma showing extensive necrosis admixed with abundant fungal hyphae surrounded by numerous degenerate inflammatory cells, intraalveolar edema and fibrin (HE stain, scale bar 200 μ m). Inset: morphologic detail of *Aspergillus fumigatus* hyphae [periodic acid-Schiff (PAS)]. Bottom right: Brain section with multifocal thrombosis and necrotizing vasculitis throughout the neutrophil associated with moderate hemorrhage and gliosis (HE stain, scale bar 200 μ m). Inset: Microthrombus with angioinvasive fungal hyphae (Grocott silver stain, scale bar 20 μ m).

which possibly led to the animal's poor nutritional status and stranding.

Nonhuman primates

Aspergillosis may occur in various non-human primate species, particularly in immunocompromised hosts [105]. Jurczynski et al. reported a case of spontaneous adrenocortical hyperglucocorticism predisposing to systemic aspergillosis with pulmonary and cerebral manifestation in a 18-year-old captive female putty-nosed monkey (*Cercopithecus nictitans*) [107]. The monkey was euthanized be-

cause of perforating thoracic trauma induced by group members and subsequent development of neurological signs. Complete necropsy and histopathological examination of formalin-fixed tissue samples was carried out. Lung parenchyma showing extensive necrosis admixed with abundant fungal hyphae surrounded by numerous degenerate inflammatory cells, intraalveolar edema and fibrin (Fig. 9).

Environmental exposure to *Aspergillus* species is an important source of infection in non-human primates such as rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and baboons (*Papio* spp.), which are

extensively used in research models of solid organ transplantation [88,105,108,242]. *Aspergillus* infection occurs in this group of experimental animals within 1 and 6 months after transplantation, mainly presenting as tracheobronchitis (necrosis, ulceration, and pseudomembranes in lung-transplant anastomosis), invasive pulmonary disease (dry cough and dyspnea, low-grade fever, hemoptysis) or disseminated aspergillosis [243]. Therefore, appropriate housing and maintenance of non-human primates undergoing transplantation is important for minimizing exposure to sources of *Aspergillus* spp. [244].

Similar to ruminants, when diagnosing pulmonary aspergillosis in primates, the possibility of concurrent involvement of tuberculosis should be considered [88]. In a very old study details are reported of nine cases of aspergillosis and a further nine associated with tuberculosis in captive (Old World) monkeys housed at the London zoological park [245]. The lungs were the main organs involved, but miliary nodules were occasionally found throughout the viscera.

Diagnosis and species identification

The diagnosis of *Aspergillus* diseases in animals is not straightforward and relies on multiple modalities, as no single diagnostic procedure has 100% sensitivity and specificity. Therefore, in practice, a combination of procedures will generally be employed including clinical signs, culture, histology, serology, imaging and molecular techniques. In many cases practitioners need understanding of the immune status of the host and the resultant risk of allergic, local or potentially invasive disease, in order to make an appropriate diagnosis and management plan [246].

Gross pathologic features

In general, the clinical signs of aspergillosis in animals might be specific or non-specific. The signs observed in a honey bee colony affected by *Aspergillus* species are similar to those caused by the specialized pathogen *Ascosphaera apis*, which is responsible for a specific condition named “chalkbrood.” *Aspergillus* diseases in reptiles are often underdiagnosed because lesions and clinical signs are similar to bacterial diseases.

In birds, lungs and air sacs are the main target organs; however, *Aspergillus* infections may disseminate into brain, eyes, gastrointestinal tract, liver, spleen, heart, kidneys, skin, and bursa of Fabricius [177,188]. Cacciottolo et al. defined two forms of avian aspergillosis: a deep nodular form located in organs with non-aerated parenchyma and a superficial, diffuse form associated with serosae in lungs [247]. Gross pathologic features include yellow nod-

ules that vary in size and texture or plaque lesions in the respiratory passages, lungs, air sacs, or membranes of body cavities. Fur-like growth of *Aspergillus* may be found on the thickened walls of air sacs. When fungal fructification occurs on aerated membranes, the color of plaques may turn to green. Some birds with bronchopulmonary aspergillosis have nodular lesions in the lungs or develop acute pneumonia accompanied by serosanguineous fluid in the pleural cavity, and fibrinous pleuritis.

Diagnostics remain challenging in horses because of similarities with other respiratory diseases (e.g., retropharyngeal lymph node infection, pharyngeal lymphoid hyperplasia, pharyngeal polyps, pneumonia, viral and bacterial infections) [248].

Culture, histology, serology, imaging, and molecular techniques

Aspergillus species are common environmental airborne contaminants; therefore, a positive culture from a nonsterile specimen is not proof of infection. However, the detection of *Aspergillus* in cultures is likely to be of diagnostic importance.

Aspergillosis in honey bees cannot be diagnosed by its gross signs and positive identification is required following the cultivation of the fungal pathogen in the laboratory by subsequent microscopic or molecular examination [127]. It should be realized that *Aspergillus* species are osmotolerant and will also saprobially present in several habitats in the colony.

In reptiles, definitive diagnosis is made with cytology, histology and culture [139], but for superficial locations *in situ* DNA hybridization would be recommendable in order to exclude isolation of contaminants.

In birds, combination of diagnostic methods might help practitioners to diagnose aspergillosis in various avian species [249–254], including serology, cytology, fungal culture, histopathology, radiography, endoscopy, and computed tomography [5]. Caseous nodules or plaques, and massive granulomas with necrotic cores surrounded by macrophages, lymphocytes, large foreign-body giant cells and finally by an outer fibrous capsule can be detected on histopathology [247]. In valuable species such as raptors, falcons, parrots and penguins, measurement of *Aspergillus* antibody and antigen titers in blood was shown to be directly relevant to clinical management of the infection [255]. In addition, studies on turkey and fowl flocks have shown that measurement of *Aspergillus* antibodies and galactomannan antigenemia, in contrast with beta-glucan, may help to diagnose respiratory or disseminated aspergillosis [6,256]. However, the success of these

techniques in appropriate diagnostics of the disease depends on the species tested and the onset of the infection [255].

Disseminated aspergillosis in dogs can generally be diagnosed by a combined approach using clinical signs, imaging techniques (radiography and computed tomography), and laboratory findings including serology, cytology, mycology, and histopathology. Since disseminated mycoses caused by other fungi, particularly *Penicillium* species, may mimic disseminated aspergillosis, identification of the infecting fungus by culture is necessary to confirm the clinical diagnosis [46]. Conflicting opinions exist regarding the diagnosis and treatment of sinonasal aspergillosis in dogs [37]. Although clinical findings and course of disease are indicative for the infection, a combined CT imaging or radiography, rhinoscopy/sinoscopy, histopathology, cytology, serology, and culture are recommended for definitive diagnosis. Other common causes of chronic nasal disease including neoplasia, nasal foreign bodies, rhinitis secondary to dental disease and idiopathic lymphoplasmacytic rhinitis should be excluded.

Sinonasal and sinoorbital aspergillosis in cats is diagnosed, similarly to dogs, with CT imaging and rhinoscopy assessing the extent of the disease. Fungal culture may lead to false negative or positive results eventually leading to isolation of contaminants and must be used in conjunction with other diagnostic tests such as serology. Furthermore, hyperglobulinemia, possibly explained by chronic antigenic stimulation, is the most common biochemical abnormality in cats with sinonasal and sinoorbital aspergillosis [207].

Of note, the sensitivity of serological tests for detection of *Aspergillus*-specific antibodies in aspergillosis depends on the systemic immunocompetence of the host [102]. An enzyme-linked immunosorbent assay (ELISA) to detect *Aspergillus* fungal cell wall antigen, galactomannan (GM), in serum (Platelia *Aspergillus* EIA, Bio-Rad) has a sensitivity of up to 90% in immunocompromised patients, including neutropenic human patients with pulmonary aspergillosis and dogs with disseminated invasive aspergillosis [102,257,258]. However, the sensitivity of this test is <30% in nonneutropenic human patients with aspergillosis, in immunocompetent dogs with sinonasal aspergillosis and in cats with upper respiratory tract aspergillosis [102,259–261].

The presence of oxalate crystals can be considered as a potentially important diagnostic aid in the detection of pulmonary aspergillosis in ruminants [19]. Similarly, in humans, there is an association between oxalate crystal production and *A. niger* or, less frequently, *A. fumigatus* infection [214]. The presence of calcium oxalate crystals in cytologic or histologic preparations of discharges or tissues thus generally suggests the possibility of an underlying infection with *Aspergillus*, in partic-

ular with *A. niger*. For the diagnosis of *Aspergillus* abortion, fungal elements (hyphae) should be seen in association with placentitis, fetal dermatitis, or pneumonia. Garcia et al. attempted to evaluate molecular and immunological techniques for the diagnosis of mammary aspergillosis in ewes [223]. The authors examined sera from 20 infected animals and demonstrated that anti-*Aspergillus* antibodies could be detected in all positive serum samples, whereas antigens were detected in only 11 of the infected animals. The relationship between antibody titers and antigenemia was not straightforward. Control sera (n = 20) were all negative for anti-*Aspergillus* antibodies but antigens could be detected in sera from two animals.

Diagnosis of upper airway aspergillosis and guttural pouch disease in horses is based on clinical course and endoscopic examination [28,230]. However, definitive diagnosis requires culture of *Aspergillus* from clinical samples and histological detection of hyphae in tissue [248]. In addition, serological tests have been shown to be of value in the diagnosis of horse aspergillosis [262].

Species identification

There is no single method (morphological, physiological or molecular) that can be used to identify all approximately 250 *Aspergillus* species. Taxonomy is generally based on a multilocus approach, but this requires skills and equipment that are not present in most diagnostic laboratories [263]. A two-step approach has been suggested for identification of *Aspergillus* in the clinical setting. The general barcoding marker rDNA ITS can be used for inter-section level identification, and subsequently partial β -tubulin for identification of individual species within the sections [264]. In a recent study, 97% of etiological agents of canine sinonasal aspergillosis were identified as *A. fumigatus* by PCR and sequencing of the ITS and partial β -tubulin genes [200].

Management recommendations

Treatment

To date, there is no effective treatment against mycotoxicoses in animals. Topical antifungal agents such azoles and supportive therapy are recommended in the treatment of *Aspergillus* keratitis and dermatitis in reptiles [135,139]. However, frequently extended courses of treatment are necessary to achieve satisfactory outcome [131,139]. Table 2 summarizes the recommended dosages of antifungals currently used in animals against aspergillosis.

Treatment of aspergillosis in poultry farms is virtually impossible and no vaccines are available. In domestic as well as in captive birds, several management strategies against aspergillosis have been suggested. The majority of these

Table 2. Recommended dosages of antifungals for use in animals against aspergillosis.

| Drug | Animal species | Dosages |
|----------------------|----------------|---|
| Amphotericin B (AmB) | Birds | Conventional AmB : –IV: 1.5 mg/kg, q8h, 3 to 5 days –Nebulization: 1 mg/kg q24 h, 10 to 14 days |
| | Dogs | –Conventional AmB: 0.5 mg/kg IV q48 (Slow infusion) to a cumulative dose of 4–8 mg/kg –Liposomal AmB: 3 mg/kg/day IV, at a rate of more than 90–120 mg/kg, 3 times a week, up until 12 treatments |
| | Cats | –Conventional AmB: 0.25 mg/kg IV q48 (Slow infusion) to a cumulative dose of 4–8 mg/kg –Liposomal AmB: 1 mg/kg/day IV, at a rate of more than 90–120 mg/kg, 3 times a week, up until 12 treatments |
| | Horses | Conventional AmB: 0.3 mg/kg IV for 3 consecutive days, and repeat after 24–48 h drug-free interval |
| | Ruminants | – |
| Itraconazole | Birds | Treatment: 5 to 15 mg/kg, q12h with food for 7 to 21 days, or 10 mg/kg q24 h for 3 weeks Prevention: 10 mg/kg q24 h for 10 days, or 20 mg/kg q24 h, or 15 to 25 mg/kg/day, for 1 week |
| | Dogs | 2.5 mg/kg q12h or 5 mg/kg q24h PO (give with food), for 15 to 30 days |
| | Cats | 2.5 mg/kg q12h, or 5 mg/kg q24h PO (give with food), for 15 to 30 days |
| | Horses | 2.5 mg/kg q12, or 5 mg/kg q24h PO |
| | Ruminants | – |
| Voriconazole | Birds | 10–18 mg/kg q12h |
| | Dogs | 4–5 mg/kg q12h PO |
| | Cats | 4–5 mg/kg q12h PO |
| | Horses | –2–4 mg/kg q24h, or 3 mg/kg q24h PO –1.5 mg/kg q24h IV |
| | Ruminants | – |
| Posaconazole | Birds | – |
| | Dogs | 5–10 mg/kg q12–24h |
| | Cats | 5 mg/kg q24h |
| | Horses | – |
| | Ruminants | – |
| Enilconazole | Birds | –Nebulization: 0.1 ml/kg for 30 min q24h (5 days on/2 days off) –Disinfection of environment: flush with solutions as recommended for use in poultry houses |
| | Dogs | 10 mg/kg q12h instilled into nasal sinus for 14 days (10% solution diluted 50/50 with water) |
| | Cats | 10 mg/kg q12h instilled into nasal sinus for 14 days (10% solution diluted 50/50 with water) |
| | Horses | –Wash lesions with 0.2% solution 4 times at 3–4 days intervals –Treatment: Infuse in nasal catheter a 2% solution every 12 hours (25–100 ml) |
| | Ruminants | Wash lesions with 0.2% solution 4 times at 3–4 days intervals |

protocols rely on topical or systemic administration of single or combined antifungal agents including amphotericin B, 5-fluorocytosine, azole derivatives (clotrimazole, enilconazole, fluconazole, itraconazole, miconazole, ketoconazole, or voriconazole) and terbinafine. When possible, surgical removal of granulomas in combination with systemic treatment may increase the probability of recovery [154].

In dogs, treatment of disseminated aspergillosis is difficult, and the prognosis is usually unfavorable. Long term treatment with oral itraconazole (up to 3 years), voriconazole or posaconazole may clear the infection and prolong survival, but are very expensive [47,265]. Retrospective studies indicated that the disease is refractory to conventional amphotericin B treatment [46,266]. Although the causes of therapeutic failure are multifactorial, delayed initiation of treatment is certainly a major reason, as the dogs are usually presented in advanced stages of the infection.

Despite similarities between human chronic, erosive rhinosinusitis and canine sinonasal aspergillosis, the treatment of these two conditions is different. In humans, endoscopic

surgery to remove fungal plaques is curative without topical therapy or ongoing medical management with antifungal agents [267]. However, treatment of dogs remains challenging and topical administration of azoles is recommended after endoscopic debridement of fungal plaques. Azoles are the most widely used antifungal agents in canine sinonasal aspergillosis [37]. In addition, topical therapy using indwelling surgically placed catheters resolves a large percentage of the cases; however, side effects associated with this treatment regimen include subcutaneous emphysema, excessive salivation, anorexia, and catheter removal necessitating another anesthetic event to replace the infusion catheters. Currently, the most popular approach is infusion of the nasal cavity and paranasal sinuses with azoles (enilconazole or miconazole). Infusion catheters are placed bilaterally using either a non-invasive or a surgical technique (Fig. 3). Depending on the severity of the disease only a single treatment may be required, whereas in advanced cases multiple treatment courses may be necessary [6,37].

In ruminants, the treatment of respiratory aspergillosis should be aimed at the elimination of predisposing factors and supportive treatment. Administration of antifungal drugs is usually impossible due to the cost of the products and the potential residues in meat and milk [22].

For guttural pouch aspergillosis in horses, medical treatment alone has been reported as having variable success rates and, currently, is not recommended without concurrent surgical therapy [268]. The preferred approach is to surgically obliterate the affected artery or arteries to prevent further, possibly fatal, hemorrhage. Since the 1970s, ligation of the affected artery close to its origin has been used [269]. Subsequently, occlusion of the affected artery by a balloon-tipped catheter was the treatment of choice. Other methods include transarterial coil embolization, or embolization using a detachable, self-sealing latex balloon [270]. Recently, Delfs et al. reported successful treatment of epistaxis caused by guttural pouch mycosis by placing transarterial nitinol vascular plugs into the affected arteries [271].

Prevention and control

In honey bees, several preventive strategies are available to minimize the effects of stonebrood disease, such as: reduction of the volume of the brood chamber for the overwintering, enlargement of the colony entrance to aid ventilation, replacement of old combs, heat treatment of the wax and requeening of affected colonies [127].

To prevent invasive infection in reptiles, treatment should be based on eliminating predisposing factors such as improper husbandry, and appropriate samples should be taken for culture and susceptibility testing and selec-

tion of antifungal agents. Adequate supportive therapy, including fluid therapy and nutritional support, is indicated in animals with clinical signs of anorexia, lethargy, and systemic illness. Ultimately, preventing introduction of potential pathogens into an established collection, developing specific diagnostic tests, and vaccines will help to protect the reptiles from the most commonly encountered pathogens [91,272,273].

In poultry farms, dust and moldy litter or feed should be avoided. Bedding, like feeders, should be kept dry and clean in order to limit fungal development. Control of relative humidity through appropriate ventilation should be verified to prevent wet litter. Spraying of fungistatic agents like thiabendazole, nystatin, or copper sulphate contributes to decreased fungal contamination of beddings. Enilconazole is available in special formulations for decontamination of the environment as part of a strategic program [274]. It may be sprayed, fogged, or nebulized to treat surfaces or indoor volume in farm building and hatcheries. Finally, effects of stressors like beak trimming and high stocking densities should be minimized [5].

With regards to mycotoxin and mycotoxicoses in dairy cattle, management strategies to reduce mycotoxin loads in animal feed are required. Good silage management can reduce the incidence of mycotoxins. Increasing dietary levels of nutrients such as protein, energy, and antioxidants may be advisable. In some situations, poultry responds to water-soluble vitamins or to specific minerals, but data are lacking for cattle. Acidic diets seem to exacerbate effects of mycotoxins, and therefore adequate dietary fiber and buffers are recommended. Because mycotoxins reduce feed consumption, feeding management to encourage intake can be helpful. Dry cows, springing heifers and calves should receive the cleanest possible feed. Transition rations, which is switch of feeding programs from high in forage and fiber to high in grain and protein with less long fiber, can reduce stress in fresh cows and reduce effects of mycotoxins. Strategic use of mold inhibitors can be beneficial. In addition, mycotoxin binders have been effective experimentally in partially reducing toxicoses [275–277], but at this time, the FDA has approved no products for that indication.

The risk of antifungal resistance

In humans, the management of aspergillosis has become more complicated due to the emergence of acquired azole resistance in *A. fumigatus* [278], which commonly involves changes in the *Cyp51A* gene, the target for azole antifungals [279, 280]. Efforts are underway to explore alternative treatment modalities [281–284]. This is important because resistance to medical triazoles may be associated with resistance selection to azole fungicides in the environment [285].

In humans azole-resistant *Aspergillus* disease is observed in patients without previous azole therapy, indicating that hosts inhale both azole-susceptible and azole-resistant *A. fumigatus* conidia [286]. A fungicide-driven route of resistance selection in *A. fumigatus* may have implications for the management of aspergillosis in animals. Indeed, avian azole-resistant *A. fumigatus* has been reported in Belgium and the Netherlands where azole resistance is widespread both in clinical and environmental isolates [287]. To assess the potential risk of azole-resistance in avian farms where azole compounds are used for the control of avian mycoses, Wang et al. conducted a drug susceptibility study including *A. fumigatus* isolates from birds and avian farms in France and Southern China [288]. A total of 175 isolates were analyzed: 57 isolates were collected in France in avian farms where chemoprophylaxis with parconazole was performed; 51 isolates were collected in Southern China in avian farms without chemoprophylaxis, and 67 control isolates were taken from a collection. No resistant isolate was detected and the distribution of MICs was similar for isolates collected in all farms.

Recent changes in the taxonomy of *Aspergillus* have major implications for our understanding of drug susceptibility profiles [289]. New sibling species of *A. fumigatus* exhibit *in vitro* susceptibility profiles that differ significantly from that of *A. fumigatus*. While acquired resistance is an emerging problem in *A. fumigatus* [278,290], other *Aspergillus* species may be intrinsically resistant to, for example, amphotericin B and azoles [289,291]. MICs of *A. flavus* clinical isolates to amphotericin B are consistently two-fold dilution steps higher than those of *A. fumigatus* [292]. Using Clinical Laboratory Standards Institute methodology [293], *A. nidulans* was shown to have MIC values of 1–2 mg/l of amphotericin B, which is higher than commonly observed with *A. fumigatus* [294]. Itraconazole and voriconazole cross-resistance and variable susceptibilities against caspofungin were observed *in vitro* against *A. felis*, another, possibly intrinsically resistant sibling of the *A. fumigatus* species complex [210,295,296]. In the section *Usti*, azoles are not active against *A. calidoustus* with MICs of ≥ 8 mg/l, while also other classes of antifungal drugs also appear less active compared to *A. fumigatus*. For instance, MICs of amphotericin B were shown to be 1–2 mg/l, which is relatively high [297]. Resistance of *A. terreus* to amphotericin B is well known [298]. Based on susceptibility to azoles, three different susceptibility patterns were distinguished in the black aspergilli (section *Nigri*). Some isolates showed low azole MICs, others high MICs, and a third group showed an uncommon paradoxical effect. However, these groups did not coincide with species boundaries, making it difficult to interpret as an intrinsic or acquired property [299].

Public health considerations

In principle, lesions where conidial heads can be present (e.g., air sacs in birds or nasal cavities in dogs) may release conidia into the environment that could be inhaled by susceptible humans and thus possess some zoonotic potential. However, in animal tissue, *Aspergillus* usually develop without producing conidial heads. Even if some conidia are finally released, their number is limited in comparison with the quantity of conidia resulting from the development of *Aspergillus* species in the environment. As a consequence, animal aspergillosis should not be considered as a zoonotic disease.

The high density of environmental *Aspergillus* conidia is a significant risk factor for developing invasive infection in hematological and immunocompromised patients [300]. Therefore, risk factors associated with the occurrence of *Aspergillus* conidia in highly colonized environments such as bird farms should be accurately monitored. Cafarchia et al. investigated the epidemiology of *Aspergillus* species in laying hen farms, their concentration in the environment and the occurrence of associated symptoms in birds and workers [301]. Although high concentrations of airborne *Aspergillus* conidia did not cause disease in birds, a significant relationship was observed between occurrence of these fungi and human colonization [301], indicating a potential risk of infection for human health.

Mycotoxins are also a major concern for public health. Aflatoxins are carcinogenic if inhaled or ingested, and therefore precautions need to be taken when stonebrood occurs in honey bees to protect beekeepers and consumers. Given the frequent consumption of milk and dairy products particularly by infants, mycotoxins are an issue of considerable importance to public health.

Concluding remarks

Our study shows that *Aspergillus* species are capable of causing different clinical diseases in a wide range of living organisms. In mammals and birds, *A. fumigatus* remains the most frequently causative fungal agent. Contamination usually occurs via airborne conidia and the respiratory tract remains the main target of infection. However, other routes have been described, such as infection by ingestion of contaminated feed in herbivores. Characteristics of both host and fungus explain the particular susceptibility of birds to *A. fumigatus*. In other animals, other *Aspergillus* species represent significant threats, such as *A. sydowii* in corals.

The diagnosis of aspergillosis in animals is usually not straightforward. A diagnosis based on culture alone is not appropriate because *Aspergillus* fungi are ubiquitous. Positive cultures should therefore be supported by

the microscopic demonstration of narrow, hyaline, septate, branching hyphae within lesions or by serologic tests. The agar-gel double-diffusion test for serum antibody is a reliable technique for diagnosis; improved sensitivity may be possible with techniques such as ELISA. Immunofluorescent procedures can be used to identify hyphae in tissue sections [302]. Notably, the role of the newly identified *Aspergillus* species in causing disease in animals remains unclear, considering the fact that clinical observations and evolution of infections caused by non-*fumigatus* *Aspergillus* species may differ significantly from those by *A. fumigatus*.

Surveillance networks that incorporate sequence-based identification of clinical isolates are needed to determine the species distribution corresponding to various clinical manifestations of *Aspergillus* diseases in animals. *In vitro* susceptibility surveillance should be included particularly in those areas where azole resistance is emerging.

Finally, given the lack of guidelines and standards, and the poor quality of epidemiological data, diagnosis and treatment in animal *Aspergillus* disease would benefit from in-depth studies in these areas. Guidelines and recommendations for diagnosis and treatment of animal *Aspergillus* infections, similar to those proposed for human medicine, are urgently needed.

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Declaration of interest

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References

- Pitt JI. The current role of *Aspergillus* and *Penicillium* in human and animal health. *J Med Vet Mycol* 1994; 32(S1): 17–32.
- Heitman J. Microbial pathogens in the fungal kingdom. *Fungal Biol Rev* 2011; 25(1): 48–60.
- Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* 1999; 12(2): 310–350.
- Segal BH. Aspergillosis. *N Engl J Med* 2009; 360(18): 1870–1884.
- Arne P, Thierry S, Wang D et al. *Aspergillus fumigatus* in poultry. *Int J Microbiol* 2011; 2011: 746356.
- Tell LA. Aspergillosis in mammals and birds: impact on veterinary medicine. *Med Mycol* 2005; 43(Suppl 1): S71–S73.
- Meersseman W, Vandecasteele SJ, Wilmer A et al. Invasive aspergillosis in critically ill patients without malignancy. *Am J Respir Crit Care Med*. 2004; 170(6):621–625.
- Denning DW. Invasive aspergillosis. *Clin Infect Dis* 1998; 26(4): 781–803; quiz 804–785.
- Segal BH, Romani LR. Invasive aspergillosis in chronic granulomatous disease. *Med Mycol* 2009; 47(Suppl 1): S282–S290.
- Patterson K, Strek ME. Allergic bronchopulmonary aspergillosis. *Proc Am Thorac Soc* 2010; 7(3): 237–244.
- DeLone DR, Goldstein RA, Petermann G et al. Disseminated aspergillosis involving the brain: distribution and imaging characteristics. *AJNR Am J Neuroradiol* 1999; 20(9): 1597–1604.
- Greub G, Bille J. *Aspergillus* species isolated from clinical specimens: suggested clinical and microbiological criteria to determine significance. *Clin Microbiol Infect* 1998; 4(12): 710–716.
- Clancy CJ, Nguyen MH. Acute community-acquired pneumonia due to *Aspergillus* in presumably immunocompetent hosts: clues for recognition of a rare but fatal disease. *Chest* 1998; 114(2): 629–634.
- Stevens DA, Melikian GL. Aspergillosis in the ‘nonimmunocompromised’ host. *Immunol Invest* 2011; 40(7–8): 751–766.
- Peterson SW, Varga J, Frisvad JC. Phylogeny and subgeneric taxonomy of *Aspergillus*. In: Varga J, Samson RA (eds.) *Wageningen: Wageningen Academic Publishers*; 2008, 33–56.
- Peterson SW. Phylogenetic analysis of *Aspergillus* species using DNA sequences from four loci. *Mycologia* 2008; 100(2): 205–226.
- Punttenney SB, Wang Y, Forsberg NE, eds. *Mycotic infections in livestock: Recent insights and studies on etiology, diagnostics and prevention of Hemorrhagic Bowel Syndrome*. Southwest Nutrition & Management Conference; 2003 Pheonix, Tuscon: University of Arizona, Department of Animal Science, p. 49–63.
- Cole RJ, Kirksey JW, Dorner JW et al. Mycotoxins produced by *Aspergillus fumigatus* species isolated from moldy silage. *Agric Food Chem* 1977; 25: 826–830.
- Muntz FH. Oxalate-producing pulmonary aspergillosis in an alpaca. *Vet Pathol* 1999; 36(6): 631–632.
- Pickett JP, Moore CP, Beehler BA et al. Bilateral chorioretinitis secondary to disseminated aspergillosis in an alpaca. *J Am Vet Med Assoc* 1985; 187(11): 1241–1243.
- Severo LC, Bohrer JC, Geyer GR et al. Invasive aspergillosis in an alpaca (*Lama pacos*). *J Med Vet Mycol* 1989; 27(3): 193–195.
- Sarfati J, Jensen HE, Latge JP. Route of infections in bovine aspergillosis. *J Med Vet Mycol* 1996; 34(6): 379–383.
- Jensen HE, Olsen SN, Aalback B. Gastrointestinal aspergillosis and zygomycosis of cattle. *Vet Pathol* 1994; 31(1): 28–36.
- Perez V, Corpa JM, Garcia Marin JF et al. Generalized aspergillosis in dairy sheep. *Zentralbl Veterinarmed B*. 1999; 46(9): 613–621.

25. Perez V, Corpa JM, Garcia Marin JF et al. Mammary and systemic aspergillosis in dairy sheep. *Vet Pathol* 1998; **35**(4): 235–240.
26. Las Heras A, Dominguez L, Lopez I et al. Intramammary *Aspergillus fumigatus* infection in dairy ewes associated with antibiotic dry therapy. *Vet Rec* 2000; **147**(20): 578–580.
27. Knudtson WU, Kirkbride CA. Fungi associated with bovine abortion in the northern plains states (USA). *J Vet Diagn Invest* 1992; **4**(2): 181–185.
28. Dobesova O, Schwarz B, Velde K et al. Guttural pouch mycosis in horses: a retrospective study of 28 cases. *Vet Rec* 2012; **171**(22): 561.
29. Guillot J, Collobert C, Gueho E et al. *Emericella nidulans* as an agent of guttural pouch mycosis in a horse. *J Med Vet Mycol* 1997; **35**: 433–435.
30. Sweeney CR, Habecker PL. Pulmonary aspergillosis in horses: 29 cases (1974–1997). *J Am Vet Med Assoc* 1999; **214**(6): 808–811.
31. Pace LW, Wirth NR, Foss RR et al. Endocarditis and pulmonary aspergillosis in a horse. *J Vet Diagn Invest* 1994; **6**(4): 504–506.
32. Johnson PJ, Moore LA, Mrad DR et al. Sudden death of two horses associated with pulmonary aspergillosis. *Vet Rec* 1999; **145**(1): 16–20.
33. Cook WR. Observations on the aetiology of epistaxis and cranial nerve paralysis in the horse. *Vet Rec* 1966; **78**(12): 396–406.
34. Markus R, Deegen E, Drommer W et al. Guttural pouch mycosis. *J Equine Vet Sci* 2005; **25**(4): 150–156.
35. Hattel AL, Drake TR, Anderholm BJ et al. Pulmonary aspergillosis associated with acute enteritis in a horse. *J Am Vet Med Assoc* 1991; **199**(5): 589–590.
36. Kendall A, Brojer J, Karlstam E et al. Enilconazole treatment of horses with superficial *Aspergillus* spp. rhinitis. *J Vet Intern Med* 2008; **22**(5): 1239–1242.
37. Sharman MJ, Mansfield CS. Sinonasal aspergillosis in dogs: a review. *J Small Anim Pract* 2012; **53**(8): 434–444.
38. Peeters D, Clercx C. Update on canine sinonasal aspergillosis. *Vet Clin North Am Small Anim Pract* 2007; **37**(5): 901–916, vi.
39. Meler E, Dunn M, Lecuyer M. A retrospective study of canine persistent nasal disease: 80 cases (1998–2003). *Can Vet J* 2008; **49**(1): 71–76.
40. Sharman M, Paul A, Davies D et al. Multi-centre assessment of mycotic rhinosinusitis in dogs: a retrospective study of initial treatment success (1998 to 2008). *J Small Anim Pract* 2010; **51**(8): 423–427.
41. Peeters D, Day MJ, Clercx C. An immunohistochemical study of canine nasal aspergillosis. *J Comp Pathol* 2005; **132**(4): 283–288.
42. Adamama-Moraitou KK, Pardali D, Day MJ et al. *Aspergillus fumigatus* Bronchopneumonia in a Hellenic Shepherd Dog. *J Am Anim Hosp Assoc* 2011; **47**(2): e13–e18.
43. Kim SH, Yong HC, Yoon JH et al. *Aspergillus niger* pulmonary infection in a dog. *J Vet Med Sci* 2003; **65**(10): 1139–1140.
44. Southard C. Bronchopulmonary aspergillosis in a dog. *J Am Vet Med Assoc* 1987; **190**(7): 875–877.
45. Billen F, Clercx C, Le Garerres A et al. Effect of sampling method and incubation temperature on fungal culture in canine sinonasal aspergillosis. *J Small Anim Pract* 2009; **50**(2): 67–72.
46. Schultz RM, Johnson EG, Wisner ER et al. Clinicopathologic and diagnostic imaging characteristics of systemic aspergillosis in 30 dogs. *J Vet Intern Med* 2008; **22**(4): 851–859.
47. Zhang S, Corapi W, Quist E et al. *Aspergillus versicolor*, a new causative agent of canine disseminated aspergillosis. *J Clin Microbiol* 2012; **50**(1): 187–191.
48. Dagleish MP, Foster G, Howie FE et al. Fatal mycotic encephalitis caused by *Aspergillus fumigatus* in a northern bottlenose whale (*Hyperoodon ampullatus*). *Vet Rec* 2008; **163**(20): 602–604.
49. Barley J, Foster G, Reid B et al. Encephalitis in a northern bottlenose whale. *Vet Rec* 2007; **160**(13): 452.
50. Abdo W, Kawachi T, Sakai H et al. Disseminated mycosis in a killer whale (*Orcinus orca*). *J Vet Diagn Invest* 2012; **24**(1): 211–218.
51. Kwon-Chung KJ, Sugui JA. *Aspergillus fumigatus*—what makes the species a ubiquitous human fungal pathogen? *PLoS Pathog* 2013; **9**(12): e1003743.
52. Balajee SA. *Aspergillus terreus* complex. *Med Mycol* 2009; **47**(Suppl 1): S42–S46.
53. Fresenius GW. Beiträge zur mykologie. Frankfurt: Frankfurt a. M., Brönnner. 1863. p. 81–82.
54. Keller NP, Turner G, Bennett JW. Fungal secondary metabolism—from biochemistry to genomics. *Nat Rev Microbiol* 2005; **3**(12): 937–947.
55. Niyo KA, Richard JL, Niyo Y et al. Effects of T-2 mycotoxin ingestion on phagocytosis of *Aspergillus fumigatus* conidia by rabbit alveolar macrophages and on hematologic, serum biochemical, and pathologic changes in rabbits. *Am J Vet Res* 1988; **49**(10): 1766–1773.
56. Niyo KA, Richard JL, Niyo Y et al. Pathologic, hematologic, and serologic changes in rabbits given T-2 mycotoxin orally and exposed to aerosols of *Aspergillus fumigatus* conidia. *Am J Vet Res* 1988; **49**(12): 2151–2160.
57. Bennett JW, Klich M. Mycotoxins. *Clin Microbiol Rev* 2003; **16**(3): 497–516.
58. Lewis RE, Wiederhold NP, Chi J et al. Detection of gliotoxin in experimental and human aspergillosis. *Infect Immun* 2005; **73**(1): 635–637.
59. Mullbacher A, Eichner RD. Immunosuppression in vitro by a metabolite of a human pathogenic fungus. *Proc Natl Acad Sci U S A* 1984; **81**(12): 3835–3837.
60. Spikes S, Xu R, Nguyen CK et al. Gliotoxin production in *Aspergillus fumigatus* contributes to host-specific differences in virulence. *J Infect Dis* 2008; **197**(3): 479–486.
61. Askew DS. *Aspergillus fumigatus*: virulence genes in a street-smart mold. *Curr Opin Microbiol* 2008; **11**(4): 331–337.
62. Sugui JA, Pardo J, Chang YC et al. Gliotoxin is a virulence factor of *Aspergillus fumigatus*: gliP deletion attenuates virulence in mice immunosuppressed with hydrocortisone. *Eukaryot Cell* 2007; **6**(9): 1562–1569.
63. Reeves EP, Messina CG, Doyle S et al. Correlation between gliotoxin production and virulence of *Aspergillus fumigatus* in *Galleria mellonella*. *Mycopathologia* 2004; **158**(1): 73–79.

64. Amitani R, Murayama T, Nawada R et al. *Aspergillus* culture filtrates and sputum sols from patients with pulmonary aspergillosis cause damage to human respiratory ciliated epithelium in vitro. *Eur Respir J* 1995; 8(10): 1681–1687.
65. Amitani R, Taylor G, Elezis EN et al. Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Infect Immun* 1995; 63(9): 3266–3271.
66. Tomee JF, Kauffman HF. Putative virulence factors of *Aspergillus fumigatus*. *Clin Exp Allergy* 2000; 30(4): 476–484.
67. Khoufache K, Puel O, Loiseau N et al. Verruculogen associated with *Aspergillus fumigatus* hyphae and conidia modifies the electrophysiological properties of human nasal epithelial cells. *BMC Microbiol* 2007; 7: 5.
68. Khoufache K, Cabaret O, Farrugia C et al. Primary in vitro culture of porcine tracheal epithelial cells in an air-liquid interface as a model to study airway epithelium and *Aspergillus fumigatus* interactions. *Med Mycol* 2010; 48(8): 1049–1055.
69. Brakhage AA, Liebmann B. *Aspergillus fumigatus* conidial pigment and cAMP signal transduction: significance for virulence. *Med Mycol* 2005; 43(Suppl 1): S75–S82.
70. Bhabhra R, Askew DS. Thermotolerance and virulence of *Aspergillus fumigatus*: role of the fungal nucleolus. *Med Mycol* 2005; 43(Suppl 1): S87–S93.
71. Tekai F, Latge JP. *Aspergillus fumigatus*: saprophyte or pathogen? *Curr Opin Microbiol* 2005; 8(4): 385–392.
72. Abad A, Fernandez-Molina JV, Bikandi J et al. What makes *Aspergillus fumigatus* a successful pathogen? Genes and molecules involved in invasive aspergillosis. *Rev Iberoam Micol* 2010; 27(4): 155–182.
73. Brakhage AA, Langfelder K. Menacing mold: the molecular biology of *Aspergillus fumigatus*. *Annu Rev Microbiol* 2002; 56: 433–455.
74. Wasylnka JA, Simmer MI, Moore MM. Differences in sialic acid density in pathogenic and non-pathogenic *Aspergillus* species. *Microbiology* 2001; 147(Pt 4): 869–877.
75. Gravelat FN, Beauvais A, Liu H et al. *Aspergillus* galactosaminogalactan mediates adherence to host constituents and conceals hyphal beta-glucan from the immune system. *PLoS Pathog* 2013; 9(8): e1003575.
76. Hohl TM, Feldmesser M. *Aspergillus fumigatus*: principles of pathogenesis and host defense. *Eukaryot Cell* 2007; 6(11): 1953–1963.
77. Chung D, Haas H, Cramer RA. Coordination of hypoxia adaptation and iron homeostasis in human pathogenic fungi. *Front Microbiol* 2012; 3: 381.
78. Dagenais TR, Keller NP. Pathogenesis of *Aspergillus fumigatus* in invasive aspergillosis. *Clin Microbiol Rev* 2009; 22(3): 447–465.
79. Cooney NM, Klein BS. Fungal adaptation to the mammalian host: it is a new world, after all. *Curr Opin Microbiol* 2008; 11(6): 511–516.
80. Feng X, Krishnan K, Richie DL et al. HacA-independent functions of the ER stress sensor IreA synergize with the canonical UPR to influence virulence traits in *Aspergillus fumigatus*. *PLoS Pathog* 2011; 7(10): e1002330.
81. Wezensky SJ, Cramer RA, Jr. Implications of hypoxic microenvironments during invasive aspergillosis. *Med Mycol* 2011; 49(Suppl 1): S120–S124.
82. Amich J, Vicente-franqueira R, Leal F et al. *Aspergillus fumigatus* survival in alkaline and extreme zinc-limiting environments relies on the induction of a zinc homeostasis system encoded by the *zrfC* and *asf2* genes. *Eukaryot Cell* 2010; 9(3): 424–437.
83. Steiman R, Guiraud P, Sage L et al. Mycoflora of Soil around the Dead-Sea .1. Ascomycetes (Including *Aspergillus* and *Penicillium*), Basidiomycetes, Zygomycetes. *Systematic and Applied Microbiology* 1995; 18(2): 310–317.
84. Oren A, Gunde-Cimerman N. Fungal life in the Dead Sea. *Prog Mol Subcell Biol* 2012; 53: 115–132.
85. Kis-Papo T, Kirzhner V, Wasser SP et al. Evolution of genomic diversity and sex at extreme environments: fungal life under hypersaline Dead Sea stress. *Proc Natl Acad Sci U S A* 2003; 100(25): 14970–14975.
86. Buchalo AS, Nevo E, Wasser SP et al. Fungal life in the extremely hypersaline water of the Dead Sea: first records. *Proc Biol Sci* 1998; 265(1404): 1461–1465.
87. Zmeili OS, Soubani AO. Pulmonary aspergillosis: a clinical update. *QJM* 2007; 100(6): 317–334.
88. Yamauchi H, Takai Y, Yamasaki H et al. Thoracic mass in a cynomolgus macaque (*Macaca fascicularis*). *Vet Pathol* 2011; 48(4): E1–E5.
89. Dominguez MC, Chavez G, Trigo FJ et al. Concurrent cholangiocarcinoma, peritonitis, paratuberculosis, and aspergillosis in a goat. *Can Vet J* 2001; 42(11): 884–885.
90. Jensen HE, Schonheyder H, Jorgensen JB. Intestinal and pulmonary mycotic lymphadenitis in cattle. *J Comp Pathol* 1990; 102(3): 345–355.
91. Jacobson ER, Cheatwood JL, Maxwell LK. Mycotic diseases of reptiles. *Semin Avian Exo Pet Med* 2000; 9(2): 94–101.
92. Fedde MR. Relationship of structure and function of the avian respiratory system to disease susceptibility. *Poult Sci* 1998; 77(8): 1130–1138.
93. Brown RE, Brain JD, Wang N. The avian respiratory system: a unique model for studies of respiratory toxicosis and for monitoring air quality. *Environ Health Perspect* 1997; 105(2): 188–200.
94. Hoerr FJ. Clinical aspects of immunosuppression in poultry. *Avian Dis* 2010; 54(1): 2–15.
95. Buehler DM, Tieleman BI, Piersma T. How do migratory species stay healthy over the annual cycle? A conceptual model for immune function and for resistance to disease. *Integr Comp Biol* 2010; 50(3): 346–357.
96. Sharp NJH HC, Sullivan M. Canine nasal aspergillosis/penicilliosis. *Compend Cont Educ Pract Vet* 1991; 13: 41–49.
97. Barachetti L, Mortellaro CM, Di Giancamillo M et al. Bilateral orbital and nasal aspergillosis in a cat. *Vet Ophthalmol* 2009; 12(3): 176–182.
98. Hamilton HL, Whitley RD, McLaughlin SA. Exophthalmos secondary to aspergillosis in a cat. *J Am Anim Hosp Assoc* 2000; 36(4): 343–347.
99. Kano R, Itamoto K, Okuda M et al. Isolation of *Aspergillus udagawae* from a fatal case of feline orbital aspergillosis. *Mycoses* 2008; 51(4): 360–361.
100. Smith LN, Hoffman SB. A case series of unilateral orbital aspergillosis in three cats and treatment with voriconazole. *Vet Ophthalmol* 2010; 13(3): 190–203.

101. Little S, Bienzle D, Carioto L et al. Feline leukemia virus and feline immunodeficiency virus in Canada: recommendations for testing and management. *Can Vet J* 2011; **52**(8): 849–855.
102. Barrs VR, Ujvari B, Dhand NK et al. Detection of *Aspergillus*-specific antibodies by agar gel double immunodiffusion and IgG ELISA in feline upper respiratory tract aspergillosis. *Vet J* 2015; **203**(3): 285–289.
103. Carrasco L, Mendez A, Jensen HE. Chronic bronchopulmonary aspergillosis in a horse with Cushing's syndrome. *Mycoses* 1996; **39**(11–12): 443–447.
104. Lepage OM, Perron MF, Cadore JL. The mystery of fungal infection in the guttural pouches. *Vet J* 2004; **168**(1): 60–64.
105. Hausteine SV, Kolterman AJ, Sundblad JJ et al. Nonhuman primate infections after organ transplantation. *ILAR J* 2008; **49**(2): 209–219.
106. Starzl TE, Fung J, Tzakis A et al. Baboon-to-human liver transplantation. *Lancet* 1993; **341**(8837): 65–71.
107. Jurczynski K, Gruber-Dujardin E, Widmer D et al. Invasive aspergillosis in a putty-nosed monkey (*Cercopithecus nictitans*) with adrenocortical Cushing's syndrome. *J Med Primatol* 2012; **41**(3): 172–175.
108. Wilk J, Lewis A, Lukas V. Dermatitis in a rhesus macaque (*Macaca mulatta*) experimentally infected with simian immunodeficiency virus. *J Med Primatol* 2008; **37**(Suppl 1): 25–28.
109. Kim K, Harvell CD. The rise and fall of a six-year coral-fungal epizootic. *Am Nat* 2004; **164**(Suppl 5): S52–S63.
110. Altizer S, Ostfeld RS, Johnson PT et al. Climate change and infectious diseases: from evidence to a predictive framework. *Science* 2013; **341**(6145): 514–519.
111. Rypien KL, Andras JP, Harvell CD. Globally panmictic population structure in the opportunistic fungal pathogen *Aspergillus sydowii*. *Molecular Ecology* 2008; **17**(18): 4068–4078.
112. Nagelkerken I, Wiltjer GA, Debrot AO et al. Baseline study of submerged marine debris at beaches in Curacao, West Indies. *Mar Pollut Bull* 2001; **42**(9): 786–789.
113. Nagelkerken I, Grol MG, Mumby PJ. Effects of marine reserves versus nursery habitat availability on structure of reef fish communities. *PLoS One* 2012; **7**(6): e36906.
114. Harvell CD, Kim K, Burkholder JM et al. Emerging marine diseases—climate links and anthropogenic factors. *Science* 1999; **285**(5433): 1505–1510.
115. Harvell CD, Aronson R, Baron N et al. The rising tide of ocean diseases: unresolved problems and research priorities. *Frontiers in Ecology and the Environment* 2004; **2**: 375–382.
116. Kim K, Harvell CD. Aspergillosis of sea fan corals: disease dynamics in the Florida Keys, USA. In: Porter JW, Porter K, eds. *The Everglades, Florida Bay, and coral reefs of the Florida Keys: an ecosystem sourcebook*. Boca Raton, Fla: CRC; 2002. p. 813–824.
117. Kim K, Harvell CD, Kim PD et al. Fungal disease resistance of Caribbean sea fan corals (*Gorgonia* spp.). *Marine Biology* 2000; **136**: 259–267.
118. Jolles AE, Sullivan PS, Alker AP et al. Disease transmission of aspergillosis in sea fans: inferring process from spatial patterns. *Ecology* 2002; **83**: 2373–2378.
119. Paddock MJ, Reynolds JD, Aguilar C et al. Recent region-wide declines in Caribbean reef fish abundance. *Curr Biol* 2009; **19**(7): 590–595.
120. Smith G, Ives L, Nagelkerken I, Ritchie K. Caribbean sea-fan mortalities. *Nature* 1996; **383**: 487.
121. Geiser DM, Taylor JW, Ritchie KB et al. Cause of sea fan death in the West Indies. *Nature* 1998; **394**: 137–138.
122. Ein-Gil N, Ilan M, Carmeli S et al. Presence of *Aspergillus sydowii*, a pathogen of gorgonian sea fans in the marine sponge *Spongia obscura*. *ISME J* 2009; **3**(6): 752–755.
123. Foley K, Fazio G, Jensen AB et al. The distribution of *Aspergillus* spp. opportunistic parasites in hives and their pathogenicity to honey bees. *Vet Microbiol* 2014; **169**(3–4): 203–210.
124. Foley K, Fazio G, Jensen AB et al. Nutritional limitation and resistance to opportunistic *Aspergillus* parasites in honey bee larvae. *J Invertebr Pathol* 2012; **111**(1): 68–73.
125. Bailey L. Infectious Diseases in honey bee. London: Land Book; 1963.
126. Gilliam M, Vandenberg JD. Fungi. 3rd ed. In: Morse RA, Flotum K eds. *A.I. Root Company*; 1997, 79–112.
127. Jensen AB, Aronstein K, Flores JM et al. Standard methods for fungal brood disease research. *J Apic Res* 2013; **52**(1): 1–20.
128. Illiam M, Taber S, Richardson GV. Hygienic behaviour of honey bees in relation to chalkbrood disease. *Apidologie* 1983; **14**: 29–39.
129. Burnside CE. Fungous diseases of the honey bee. US Department of Agriculture Technical Bulletin. 1930: 149.
130. Hornitzky M, Stace P, Boulton JG. A case of stonebrood in Australian honey bees (*Apis mellifera*). *Aust Vet J* 1989; **66**(2): 64.
131. Vojvodic S, Jensen AB, James RR et al. Temperature dependent virulence of obligate and facultative fungal pathogens of honey bee brood. *Vet Microbiol* 2011; **149**(1–2): 200–205.
132. Lionakis MS, Kontoyiannis DP. *Drosophila melanogaster* as a model organism for invasive aspergillosis. *Methods Mol Biol* 2012; **845**: 455–468.
133. Arvanitis M, Glavis-Bloom J, Mylonakis E. Invertebrate models of fungal infection. *Biochim Biophys Acta* 2013; **1832**(9): 1378–1383.
134. Yanong RP. Fungal diseases of fish. *Vet Clin North Am Exot Anim Pract* 2003; **6**(2): 377–400.
135. Girling SJ, Fraser MA. Treatment of *Aspergillus* species infection in reptiles with itraconazole at metabolically scaled doses. *Vet Rec* 2009; **165**(2): 52–54.
136. Frye FL, Dutra FR. Mycotic granulomata involving the forefeet of a turtle. *Vet Med Small Anim Clin* 1974; **69**(12): 1554, 1556.
137. Buenviaje GN, Ladds PW, Melville L et al. Disease-husbandry associations in farmed crocodiles in Queensland and the Northern Territory. *Aust Vet J* 1994; **71**(6): 165–173.
138. Tappe JP, Chandler FW, Liu SK et al. Aspergillosis in two San Esteban chuckwallas. *J Am Vet Med Assoc* 1984; **185**(11): 1425–1428.
139. Myers DA, Isaza R, Ben-Shlomo G et al. Fungal keratitis in a gopher tortoise (*Gopherus polyphemus*). *J Zoo Wild Med* 2009; **40**(3): 579–582.
140. Miller DL, Radi ZA, Stiver SL et al. Cutaneous and pulmonary mycosis in green anacondas (*Eunectes murinus*). *J Zoo Wild Med* 2004; **35**(4): 557–561.

141. Beernaert LA, Pasmans F, Van Waeyenberghe L et al. *Aspergillus* infections in birds: a review. *Avian Pathol* 2010; **39**(5): 325–331.
142. Maina JN. The Lung-Air Sac System of Birds: Development, Structure, and Function. Germany: Springer, Berlin Heidelberg New York; 2005, 159–174.
143. Martin MP, Bouck KP, Helm J et al. Disseminated *Aspergillus flavus* infection in broiler breeder pullets. *Avian Dis* 2007; **51**(2): 626–631.
144. Akan M, Haziroglu R, Ilhan Z et al. A case of aspergillosis in a broiler breeder flock. *Avian Dis* 2002; **46**(2): 497–501.
145. Wang DY, Hadj-Henni L, Thierry S et al. Simple and highly discriminatory VNTR-based multiplex PCR for tracing sources of *Aspergillus flavus* isolates. *PLoS One* 2012; **7**(9): e44204.
146. Jansson DS. Aspergillosis. In: Gavier-Widen D, Anna Meredith A, Paul Duff J, eds. Infectious Diseases of Wild Mammals and Birds in Europe 2012. p. 455–458.
147. Zinkl JG, Hyland JM, Hurt JJ. Aspergillosis in common crows in Nebraska. 1974. *J Wildl Dis* 1977; **13**(2): 191–193.
148. Rosen MN. Aspergillosis in wild and domestic fowl. *Avian Diseases* 1964; **8**: 1–6.
149. Neff JA. Outbreak of aspergillosis in mallards. *Journal of Wildlife Management* 1955; **19**: 415–416.
150. Mc Dougle HC, Vaught RW. An epizootic of aspergillosis in Canada geese. *Journal of Wildlife Management* 1968; **32**: 577–578.
151. Converse KA. Aspergillosis. In: Thomas NJ, Hunter DB, Atkinson CT, eds. Infectious Diseases of Wild Birds. Iowa, USA: Blackwell Publishing, 2008, 360–374.
152. Redig PT. Aspergillosis. In: Samour J, ed. Avian Medicine. Philadelphia: Mosby; 2000. p. 275–287.
153. Alvarez-Perez S, Mateos A, Dominguez L et al. Polyclonal *Aspergillus fumigatus* infection in captive penguins. *Vet Microbiol* 2010; **144**(3–4): 444–449.
154. Xavier MO, Soares MP, Meinerz ARM et al. Aspergillosis: a limiting factor during recovery of captive magellanic penguins. *Brazilian J Microbiol* 2007; **38**: 480–484.
155. Ainsworth GC, Rewell RE. The incidence of aspergillosis in captive wild birds. *J Comp Pathol* 1949; **59**: 213–224.
156. Bailey T, Silvanose CD, Naldo J et al. Health considerations of the rehabilitation of illegally traded houbara bustards (*Chlamydotis undulata macqueenii*) in the Middle East. *Oryx* 2000; **34**: 325–334.
157. Brand CJ, Windingstad RM, Siegfried LM et al. Avian morbidity and mortality from botulism, aspergillosis, and salmonellosis at Jamaica Bay Wildlife Refuge, New York, USA. *Colonial Waterbirds* 1988; **11**: 284–292.
158. Burr EW. Enzootic aspergillosis in wild red vented cockatoos in the Philippines. *Mycopathologia* 1981; **73**(1): 21–28.
159. Cork SC, Alley MR, Johnstone AC et al. Aspergillosis and other causes of mortality in the stitchbird in New Zealand. *J Wildl Dis* 1999; **35**(3): 481–486.
160. Nardoni S, Ceccherelli R, Rossi G et al. Aspergillosis in *Larus cachimans micaellis*: survey of eight cases. *Mycopathologia* 2006; **161**(5): 317–321.
161. Tarello W. Etiologic agents and diseases found associated with clinical aspergillosis in falcons. *Int J Microbiol* 2011; **2011**: 176963.
162. Perez J, Garcia PM, Mendez A et al. Outbreak of aspergillosis in a flock of adult ostriches (*Struthio camelus*). *Vet Rec* 2003; **153**(4): 124–125.
163. Simpson V. Spinal aspergillosis in pheasants. *Vet Rec* 2011; **169**(21): 562.
164. Barnett J, Booth P, Arrow M et al. Spinal aspergillosis in pheasants. *Vet Rec* 2011; **169**(17): 449–450.
165. Graczyk TK, Cockrem JF. *Aspergillus* spp. seropositivity in New Zealand penguins. *Mycopathologia* 1995; **131**(3): 179–184.
166. Bunting EM, Abou-Madi N, Cox S et al. Evaluation of oral itraconazole administration in captive Humboldt penguins (*Spheniscus humboldti*). *J Zoo Wildl Med* 2009; **40**(3): 508–518.
167. Kunkle RA, Sacco RE. Susceptibility of convalescent turkeys to pulmonary aspergillosis. *Avian Dis* 1998; **42**(4): 787–790.
168. ThroneSteinlage SJ, Sander JE, Brown TP et al. Disseminated mycosis in layer cockerels and pullets. *Avian Dis* 2003; **47**(1): 229–233.
169. Zafra R, Perez J, Perez-Ecija RA et al. Concurrent aspergillosis and ascites with high mortality in a farm of growing broiler chickens. *Avian Dis* 2008; **52**(4): 711–713.
170. d’Arc Moretti L, Dias RA, Telles EO et al. Time series evaluation of traumatic lesions and airsacculitis at one poultry abattoir in the state of Sao Paulo, Brazil (1996–2005). *Prev Vet Med* 2010; **94**(3–4): 231–239.
171. Lupo C, Le Bouquin S, Allain V et al. Risk and indicators of condemnation of male turkey broilers in western France, February–July 2006. *Prev Vet Med* 2010; **94**(3–4): 240–250.
172. Sato Y, Itagaki T. Fungal airsacculitis associated with multiple helminth infestations in a black-eared kite (*Milvus migrans*). *Avian Dis* 2010; **54**(2): 965–968.
173. Vestappen FALM, Dorrestein GM. Aspergillosis in Amazon parrots after corticosteroid therapy for smoke-inhalation injury. *J Avian MedSurg* 2005; **19**: 138–141.
174. Olias P, Gruber AD, Hafez HM et al. Molecular epidemiology and virulence assessment of *Aspergillus fumigatus* isolates from white stork chicks and their environment. *Vet Microbiol* 2011; **148**(2–4): 348–355.
175. Burco JD, Etienne KA, Massey JG et al. Molecular sub-typing suggests that the environment of rehabilitation centers may be a potential source of *Aspergillus fumigatus* infecting rehabilitating seabirds. *Med Mycol* 2012; **50**(1): 91–98.
176. Lair-Fullerenger S, Guillot J, Desterke C et al. Differentiation between isolates of *Aspergillus fumigatus* from breeding turkeys and their environment by genotyping with microsatellite markers. *J Clin Microbiol* 2003; **41**(4): 1798–1800.
177. Beytut E, Ozcan K, Erginsoy S. Immunohistochemical detection of fungal elements in the tissues of goslings with pulmonary and systemic aspergillosis. *Acta Vet Hung* 2004; **52**(1): 71–84.
178. Perelman B, Kuttin ES. Aspergillosis in ostriches. *Avian Pathol* 1992; **21**(1): 159–163.
179. Hamet N, Seigle-Murandi F, Steiman R. Contribution to the prophylaxis of chicks aspergillosis: study of the contamination of a hatchery by *Aspergillus fumigatus*. *Zentralbl Veterinarmed B* 1991; **38**(7): 529–537.
180. Vanderheyden N. Aspergillosis in psittacine chicks. In: Jackson G, ed. USA: Nashville, TN, 1993, 207.

181. Dyar PM, Fletcher OJ, Page RK. Aspergillosis in turkeys associated with use of contaminated litter. *Avian Dis* 1984; **28**(1): 250–255.
182. Beckman BJ, Howe CW, Trampel DW et al. *Aspergillus fumigatus* keratitis with intraocular invasion in 15-day-old chicks. *Avian Dis* 1994; **38**(3): 660–665.
183. Dalton JR, Ainsworth H. Mycotic keratoconjunctivitis in 12-day-old red-legged partridges (*Alectoris rufa*). *Vet Rec* 2011; **168**(22): 590.
184. Mohammed MA, Sokkar SM, Batrawi A et al. Natural and experimental nodular dermatomycosis in chickens. *Avian Pathol* 1980; **9**(2): 185–192.
185. Suedmeyer WK, Bermudez AJ, Fales WH. Treatment of epidermal cysts associated with *Aspergillus fumigatus* and *Alternaria* species in a silky bantam chicken. *J Avi Med Surg* 2002; **16**: 133–137.
186. Chute HL, Witter JF, Rountree JL et al. The pathology of a fungous infection associated with a caponizing injury. *J Am Vet Med Assoc* 1955; **127**(942): 207–209.
187. Tsai SS, Park JH, Hirai K et al. Aspergillosis and candidiasis in psittacine and passeriforme birds with particular reference to nasal lesions. *Avian Pathol* 1992; **21**(4): 699–709.
188. van Veen L, Dwars RM, Fabri THF. Mycotic spondylitis in broilers caused by *Aspergillus fumigatus* resulting in partial anterior and posterior paralysis. *Avian Pathol* 1999; **28**: 487–490.
189. Olias P, Hauck R, Windhaus H et al. Articular aspergillosis of hip joints in turkeys. *Avian Dis* 2010; **54**(3): 1098–1101.
190. Cortes PL, Shivaprasad HL, Kiupel M et al. Omphalitis associated with *Aspergillus fumigatus* in poults. *Avian Dis* 2005; **49**(2): 304–308.
191. Julian RJ, Goryo M. Pulmonary aspergillosis causing right ventricular failure and ascites in meat-type chickens. *Avian Pathol* 1990; **19**(4): 643–654.
192. Richard JL, Dvorak TJ, Ross PF. Natural occurrence of gliotoxin in turkeys infected with *Aspergillus fumigatus*, *Fresenius*. *Mycopathologia* 1996; **134**(3): 167–170.
193. Richard JL, DeBey MC. Production of gliotoxin during the pathogenic state in turkey poults by *Aspergillus fumigatus* *Fresenius*. *Mycopathologia* 1995; **129**(2): 111–115.
194. Richard JL, Peden WM, Williams PP. Gliotoxin inhibits transformation and its cytotoxic to turkey peripheral blood lymphocytes. *Mycopathologia* 1994; **126**(2): 109–114.
195. Creekmore LH. Mycotoxins in in Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds. US Geological Survey 1999. p. 267–270.
196. Blout WP. Turkey “X” disease. *Turkeys* 1961; **9**(52): 55–58.
197. Kunkle RA. Aspergillosis. In: Saif YM, Barnes HJ, Glisson JR, eds. Diseases of Poultry. Ames, Iowa, USA: Iowa State University Press; 2008. p. 883–895.
198. Hoerr FJ. Mycotoxicoses. In: Saif YM, Barnes HJ, Glisson JR, eds. Diseases of Poultry. 12th ed. Iowa, USA: Iowa State University Press; 2008. p. 883–895.
199. Tasker S, Knottenbelt CM, Munro EA et al. Aetiology and diagnosis of persistent nasal disease in the dog: a retrospective study of 42 cases. *J Small Anim Pract* 1999; **40**(10): 473–478.
200. Talbot JJ, Johnson LR, Martin P et al. What causes canine sino-nasal aspergillosis? A molecular approach to species identification. *Vet J* 2014; **200**(1): 17–21.
201. Peeters D, Day MJ, Farnir F et al. Distribution of leucocyte subsets in the canine respiratory tract. *J Comp Pathol* 2005; **132**(4): 261–272.
202. Peeters D, Peters IR, Clercx C et al. Quantification of mRNA encoding cytokines and chemokines in nasal biopsies from dogs with sino-nasal aspergillosis. *Vet Microbiol* 2006; **114**(3–4): 318–326.
203. Day MJ. Canine sino-nasal aspergillosis: parallels with human disease. *Med Mycol* 2009; **47**(Suppl 1): S315–S323.
204. Vanherberghen M, Bureau F, Peters IR et al. Analysis of gene expression in canine sino-nasal aspergillosis and idiopathic lymphoplasmacytic rhinitis: a transcriptomic analysis. *Vet Microbiol* 2012; **157**(1–2): 143–151.
205. Mercier E, Peters IR, Farnir F et al. Assessment of Toll-like receptor 2, 4 and 9 SNP genotypes in canine sino-nasal aspergillosis. *BMC Vet Res* 2014; **10**: 187.
206. Coyner K. Otomycosis due to *Aspergillus* spp. in a dog: case report and literature review. *Vet Dermatol* 2010; **21**(6): 613–618.
207. Barrs VR, Halliday C, Martin P et al. Sinonasal and sino-orbital aspergillosis in 23 cats: aetiology, clinicopathological features and treatment outcomes. *Vet J* 2012; **191**(1): 58–64.
208. Barrs VR, Talbot JJ. Feline aspergillosis. *Vet Clin North Am Small Anim Pract* 2014; **44**(1): 51–73.
209. Barrs VR, Beatty JA, Lingard AE et al. Feline sino-orbital aspergillosis: an emerging clinical syndrome. *Aust Vet J* 2007; **85**(3): N23.
210. Barrs VR, van Doorn TM, Houbraken J et al. *Aspergillus felis* sp. nov., an emerging agent of invasive aspergillosis in humans, cats, and dogs. *Plos One* 2013; **8**(6): e64871.
211. Kano R, Shibahashi A, Fujino Y et al. Two cases of feline orbital aspergillosis due to *Aspergillus udagawae* and *A. viridinutans*. *J Vet Med Sci* 2013; **75**(1): 7–10.
212. Whitney BL, Broussard J, Stefanacci JD. Four cats with fungal rhinitis. *J Feline Med Surg* 2005; **7**(1): 53–58.
213. Labelle AL, Hamor RE, Barger AM et al. *Aspergillus flavus* keratomycosis in a cat treated with topical 1% voriconazole solution. *Vet Ophthalmol* 2009; **12**(1): 48–52.
214. Lee SH, Barnes WG, Schaetzel WP. Pulmonary aspergillosis and the importance of oxalate crystal recognition in cytology specimens. *Arch Pathol Lab Med* 1986; **110**(12): 1176–1179.
215. Kimmerling EA, Fedrick JA, Tenholder MF. Invasive *Aspergillus niger* with fatal pulmonary oxalosis in chronic obstructive pulmonary disease. *Chest* 1992; **101**(3): 870–872.
216. Park SJ, Chung CR, Rhee YK et al. Chronic pulmonary aspergillosis due to *Aspergillus niger*. *Am J Respir Crit Care Med* 2012; **186**(10): e16–e17.
217. Jensen HE, Halbaek B, Lind P et al. Development of murine monoclonal antibodies for the immunohistochemical diagnosis of systemic bovine aspergillosis. *J Vet Diagn Invest* 1996; **8**(1): 68–75.
218. Hill MW, Whiteman CE, Benjamin MM et al. Pathogenesis of experimental bovine mycotic placentitis produced by *Aspergillus fumigatus*. *Vet Pathol* 1971; **8**(2): 175–192.

219. Jensen HE, Schonheyder H. Experimental murine mycotic placentitis and abortion. A potent animal model. *J Exp Anim Sci* 1993; 35(4): 155–160.
220. Karaman I, Karaman A, Boduroglu EC et al. Invasive *Aspergillus* infection localized to the gastric wall: report of a case. *Surg Today* 2013; 43(6): 682–684.
221. Eggimann P, Chevolet JC, Starobinski M et al. Primary invasive aspergillosis of the digestive tract: report of two cases and review of the literature. *Infection* 2006; 34(6): 333–338.
222. Tresallet C, Seman M, Hoang C et al. Gastric perforation from potential primary digestive aspergillosis. *Surgery* 2010; 148(1): 158–159.
223. Garcia ME, Duran C, Cruzado M et al. Evaluation of molecular and immunological techniques for the diagnosis of mammary aspergillosis in ewes. *Vet Microbiol* 2004; 98(1): 17–21.
224. Morgavi DP, Boudra H, Jouany JP et al. Effect and stability of gliotoxin, an *Aspergillus fumigatus* toxin, on *in vitro* rumen fermentation. *Food Addit Contam* 2004; 21(9): 871–878.
225. Finnie JW, Windsor PA, Kessell AE. Neurological diseases of ruminant livestock in Australia. II: toxic disorders and nutritional deficiencies (vol 89, pg 247, 2011). *Australian Veterinary Journal*. 2011; 89(8): 311–311.
226. Lorette AP, Colodel EM, Driemeier D et al. Neurological disorder in dairy cattle associated with consumption of beer residues contaminated with *Aspergillus clavatus*. *J Vet Diagn Invest* 2003; 15(2): 123–132.
227. Blomme E, Del Piero F, La Perle KMD et al. Aspergillosis in horses: a review. *Equine Vet Educ* 1998; 10: 86–93.
228. Cook W. The clinical features of guttural pouch mycosis in the horse. *Vet Rec* 1968; 83: 336–345.
229. Lane JG. The management of guttural pouch mycosis. *Equine Vet J* 1989; 21(5): 321–324.
230. Ludwig A, Gatineau S, Reynaud MC et al. Fungal isolation and identification in 21 cases of guttural pouch mycosis in horses (1998–2002). *Vet J* 2005; 169(3): 457–461.
231. DesLions JA, Guillot J, Legrand E et al. Aspergillosis involving the frontal sinus in a horse. *Equine Vet Educ* 2000; 12(5): 248–250.
232. Sansom J, Featherstone H, Barnett KC. Keratomycosis in six horses in the United Kingdom. *Vet Rec* 2005; 156(1): 13–17.
233. Wada S, Hobo S, Ode H et al. Equine keratomycosis in Japan. *Vet Ophthalmol* 2013; 16(1): 1–9.
234. Scotty N. Equine keratomycosis. *Clin Tech Equine Pract* 2005; 4: 29–46.
235. Gaarder JE, Rebhun WC, Ball MA et al. Clinical appearances, healing patterns, risk factors, and outcomes of horses with fungal keratitis: 53 cases (1978–1996). *J Am Vet Med Assoc* 1998; 213(1): 105–112.
236. Kalkanci A, Ozdek S. Ocular fungal infections. *Curr Eye Res* 2011; 36(3): 179–189.
237. Thomas PA. Current perspectives on ophthalmic mycoses. *Clin Microbiol Rev* 2003; 16(4): 730–797.
238. Reidarson TH, McBain JF, Dalton LM. Diagnosis and treatment of fungal infections in marine mammals. 4th ed. In: Fowler ME, Miller RE, eds. London: WB Saunders; 1999, 478–485.
239. Migaki G, Jones SR. Mycotic diseases in marine mammals. In: Howard EB, ed. Boca Raton, FL: CRC Press; 1983.
240. Dagleish MP, Patterson IA, Foster G et al. Intracranial granuloma caused by asporogenic *Aspergillus fumigatus* in a harbour porpoise (*Phocoena phocoena*). *Vet Rec* 2006; 159(14): 458–460.
241. Prah S, Jepson PD, Sanchez-Hanke M et al. Aspergillosis in the middle ear of a harbour porpoise (*Phocoena phocoena*): a case report. *Mycoses* 2011; 54(4): e260–e264.
242. Rubin RH. Overview: pathogenesis of fungal infections in the organ transplant recipient. *Transpl Infect Dis* 2002; 4(Suppl 3): 12–17.
243. Marty FM, Rubin RH. The prevention of infection post-transplant: the role of prophylaxis, preemptive and empiric therapy. *Transpl Int* 2006; 19(1): 2–11.
244. Silveira FP, Husain S. Fungal infections in solid organ transplantation. *Med Mycol* 2007; 45(4): 305–320.
245. Scott HH. Tuberculosis in Man and Lower Animals: A Study in Comparative Pathology: Medical Research Council; 1930, 270.
246. Barnes PD, Marr KA. Aspergillosis: spectrum of disease, diagnosis, and treatment. *Infect Dis Clin North Am* 2006; 20(3): 545–561, vi.
247. Cacciuto E, Rossi G, Nardoni S et al. Anatomopathological aspects of avian aspergillosis. *Vet Res Commun*. 2009; 33(6): 521–527.
248. Cafarchia C, Romina Paradies R, Figueredo LA et al. A case of equine aspergillosis: a novel sampling procedure for diagnosis. *Journal of Equine Veterinary Science* 2012; 32: 634–637.
249. Arca-Ruibal B, Wernery U, Zachariah R et al. Assessment of a commercial sandwich ELISA in the diagnosis of aspergillosis in falcons. *Vet Rec* 2006; 158(13): 442–444.
250. Burco JD, Ziccardi MH, Clemons KV et al. Evaluation of plasma (1→3) beta-D-glucan concentrations in birds naturally and experimentally infected with *Aspergillus fumigatus*. *Avian Dis* 2012; 56(1): 183–191.
251. Cray C, Watson T, Rodriguez M et al. Application of galactomannan analysis and protein electrophoresis in the diagnosis of aspergillosis in avian species. *J Zoo Wildl Med* 2009; 40(1): 64–70.
252. Cray C, Watson T, Arheart KL. Serosurvey and diagnostic application of antibody titers to *Aspergillus* in avian species. *Avian Dis* 2009; 53(4): 491–494.
253. Cray C, Reavill D, Romagnano A et al. Galactomannan assay and plasma protein electrophoresis findings in psittacine birds with aspergillosis. *J Avian Med Surg* 2009; 23(2): 125–135.
254. Ivey ES. Serologic and plasma protein electrophoretic findings in 7 psittacine birds with aspergillosis. *Journal of Avian Medicine and Surgery* 2000; 14: 103–106.
255. Deem SL. Fungal diseases of birds of prey. *Vet Clin North Am Exot Anim Pract* 2003; 6(2): 363–376.
256. Franca M, Cray C, Shivaprasad HL. Serologic testing for aspergillosis in commercial broiler chickens and turkeys. *Avian Dis* 2012; 56(1): 160–164.
257. Maertens J, Verhaegen J, Demuyneck H et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. *J Clin Microbiol* 1999; 37(10): 3223–3228.

258. Garcia RS, Wheat LJ, Cook AK et al. Sensitivity and specificity of a blood and urine galactomannan antigen assay for diagnosis of systemic aspergillosis in dogs. *J Vet Intern Med* 2012; **26**(4): 911–919.
259. Billen F, Peeters D, Peters IR et al. Comparison of the value of measurement of serum galactomannan and *Aspergillus*-specific antibodies in the diagnosis of canine sino-nasal aspergillosis. *Vet Microbiol* 2009; **133**(4): 358–365.
260. Whitney J, Beatty JA, Martin P et al. Evaluation of serum galactomannan detection for diagnosis of feline upper respiratory tract aspergillosis. *Vet Microbiol* 2013; **162**(1): 180–185.
261. Kitasato Y, Tao Y, Hoshino T et al. Comparison of *Aspergillus galactomannan* antigen testing with a new cut-off index and *Aspergillus* precipitating antibody testing for the diagnosis of chronic pulmonary aspergillosis. *Respirology* 2009; **14**(5): 701–708.
262. Guillot J, Sarfati J, de Barros M et al. Comparative study of serological tests for the diagnosis of equine aspergillosis. *Vet Rec* 1999; **145**(12): 348–349.
263. Geiser DM, Klich MA, Frisvad JC et al. The current status of species recognition and identification in *Aspergillus*. *Stud Mycol* 2007; **59**: 1–10.
264. Balajee SA, Houburken J, Verweij PE et al. *Aspergillus* species identification in the clinical setting. *Stud Mycol* 2007; **59**: 39–46.
265. Sykes JE. Aspergillosis. In: Sykes JE, ed. Canine and feline infectious diseases. St Louis, Mo: Elsevier; 2010. p. 633–648.
266. Wood GL, Hirsh DC, Selcer RR et al. Disseminated aspergillosis in a dog. *J Am Vet Med Assoc* 1978; **172**(6): 704–707.
267. Uri N, Cohen-Kerem R, Elmalah I et al. Classification of fungal sinusitis in immunocompetent patients. *Otolaryngol Head Neck Surg* 2003; **129**(4): 372–378.
268. Lepage OM, Piccot-Crezollet C. Transarterial coil embolisation in 31 horses (1999–2002) with guttural pouch mycosis: a 2-year follow-up. *Equine Vet J* 2005; **37**(5): 430–434.
269. Church S, Wyn-Jones G, Parks AH et al. Treatment of guttural pouch mycosis. *Equine Vet J* 1986; **18**(5): 362–365.
270. Leveille R, Hardy J, Robertson JT et al. Transarterial coil embolization of the internal and external carotid and maxillary arteries for prevention of hemorrhage from guttural pouch mycosis in horses. *Vet Surg* 2000; **29**(5): 389–397.
271. Delfs KC, Hawkins JF, Hogan DF. Treatment of acute epistaxis secondary to guttural pouch mycosis with transarterial nitinol vascular occlusion plugs in three equids. *J Am Vet Med Assoc* 2009; **235**(2): 189–193.
272. Paré JA, Sigler L, Rosenthal KL. Microbiology: fungal and bacterial diseases of reptiles. In: Mader DR et al., ed. Reptile Medicine and Surgery. 2. St Louis: Saunders Elsevier; 2006 p. 217–239.
273. Schumacher J. Fungal diseases of reptiles. *Vet Clin North Am Exot Anim Pract* 2003; **6**(2): 327–335, vi.
274. Williams CJ, Brake J. Evaluation of application methods for control of *Aspergillus fumigatus* proliferation on the air cell membrane of in ovo injected broiler eggs. *Poult Sci* 2000; **79**(11): 1531–1535.
275. Qiang Z, Truong M, Meynen K et al. Efficacy of a mycotoxin binder against dietary fumonisin, deoxynivalenol, and zearalenone in rats. *J Agric Food Chem* 2011; **59**(13): 7527–7533.
276. Bursian SJ, Mitchell RR, Yamini B et al. Efficacy of a commercial mycotoxin binder in alleviating effects of ochratoxin A, fumonisin B1, moniliformin and zearalenone in adult mink. *Vet Hum Toxicol* 2004; **46**(3): 122–129.
277. Diaz DE, Hagler WM, Jr., Blackwelder JT et al. Aflatoxin binders II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia* 2004; **157**(2): 233–241.
278. Verweij PE, Howard SJ, Melchers WJ et al. Azole-resistance in *Aspergillus*: proposed nomenclature and breakpoints. *Drug Resist Updat* 2009; **12**(6): 141–147.
279. Snelders E, van der Lee HA, Kuijpers J et al. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 2008; **5**(11): e219.
280. Howard SJ, Webster I, Moore CB et al. Multi-azole resistance in *Aspergillus fumigatus*. *Int J Antimicrob Agents* 2006; **28**(5): 450–453.
281. Seyedmousavi S, Meletiadis J, Melchers WJ et al. In vitro interaction of voriconazole and anidulafungin against triazole-resistant *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2013; **57**(2): 796–803.
282. Seyedmousavi S, Melchers WJ, Mouton JW et al. Pharmacodynamics and dose-response relationships of liposomal amphotericin B against different azole-resistant *Aspergillus fumigatus* isolates in a murine model of disseminated aspergillosis. *Antimicrob Agents Chemother* 2013; **57**(4): 1866–1871.
283. Seyedmousavi S, Bruggemann RJ, Melchers WJ et al. Pharmacodynamics of anidulafungin against clinical *Aspergillus fumigatus* isolates in a nonneutropenic murine model of disseminated aspergillosis. *Antimicrob Agents Chemother* 2013; **57**(1): 303–308.
284. Seyedmousavi S, Bruggemann RJ, Melchers WJ et al. Efficacy and pharmacodynamics of voriconazole combined with anidulafungin in azole-resistant invasive aspergillosis. *J Antimicrob Chemother* 2013; **68**(2): 385–393.
285. Verweij PE, Snelders E, Kema GH et al. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009; **9**(12): 789–795.
286. ECDC. Risk assessment on the impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus* species. Stockholm: 2013.
287. Beernaert LA, Pasmans F, Van Waeyenberghe L et al. Avian *Aspergillus fumigatus* strains resistant to both itraconazole and voriconazole. *Antimicrob Agents Chemother* 2009; **53**(5): 2199–2201.
288. Wang DY, Gricourt M, Thierry S et al. Mutations in the cyp51A gene and susceptibility to itraconazole in *Aspergillus fumigatus* isolated from avian farms in France and China. *Poult Sci* 2014; **93**(1): 12–15.
289. Van Der Linden JW, Warris A, Verweij PE. *Aspergillus* species intrinsically resistant to antifungal agents. *Med Mycol* 2011; **49**(Suppl 1): S82–S89.
290. Verweij PE, Mellado E, Melchers WJ. Multiple-triazole-resistant aspergillosis. *N Engl J Med* 2007; **356**(14): 1481–1483.
291. Seyedmousavi S, Mouton JW, Melchers W et al. The role of azoles in the management of azole-resistant aspergillosis: from the bench to the bedside. *Drug Resist Updat* 2014; **17**(3): 37–50.

292. Gomez-Lopez A, Garcia-Effron G, Mellado E et al. In vitro activities of three licensed antifungal agents against spanish clinical isolates of *Aspergillus* spp. *Antimicrob Agents Chemother* 2003; 47(10): 3085–3088.
293. CLSI. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved standard-Second Edition. CLSI Document. M38-A2. Wane, PA: Clinical and laboratory Standards Institute; 2008.
294. Kontoyiannis DP, Lewis RE, May GS et al. *Aspergillus nidulans* is frequently resistant to amphotericin B. *Mycoses* 2001; 45: 406–407.
295. Pelaez T, Alvarez-Perez S, Mellado E et al. Invasive aspergillosis caused by cryptic *Aspergillus* species: a report of two consecutive episodes in a patient with leukaemia. *J Med Microbiol* 2013; 62(3): 474–478.
296. Coelho D, Silva S, Vale-Silva L et al. *Aspergillus viridinutans*: an agent of adult chronic invasive aspergillosis. *Med Mycol* 2011; 49(7): 755–759.
297. Varga J, Houbraken J, Van Der Lee HA et al. *Aspergillus calidoustus* sp. nov., causative agent of human infections previously assigned to *Aspergillus ustus*. *Eukaryot Cell* 2008; 7(4): 630–638.
298. Lass-Flörl C, Alastruey-Izquierdo A, Cuenca-Estrella M et al. In vitro activities of various antifungal drugs against *Aspergillus terreus*: Global assessment using the methodology of the European committee on antimicrobial susceptibility testing. *Antimicrob Agents Chemother* 2009; 53(2): 794–795.
299. Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A et al. Species identification and antifungal susceptibility patterns of species belonging to *Aspergillus* section Nigri. *Antimicrob Agents Chemother* 2009; 53(10): 4514–4517.
300. Cavallo M, Andreoni S, Martinotti MG et al. Monitoring environmental *Aspergillus* spp. contamination and meteorological factors in a haematological unit. *Mycopathologia* 2013; 176(5–6): 387–394.
301. Cafarchia C, Camarda A, Iatta R et al. Environmental contamination by *Aspergillus* spp. in laying hen farms and associated health risks for farm workers. *J Med Microbiol* 2014; 63(Pt 3): 464–470.
302. Thirion-Delalande C, Guillot J, Jensen HE et al. Disseminated acute concomitant aspergillosis and mucormycosis in a pony. *J Vet Med A Physiol Pathol Clin Med* 2005; 52(3): 121–124.