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Phenotypic and Molecular characterization of *Aspergillus niger* from Otomycosis in Human and Domestic Cats

A Thesis

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By

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Abstract

aspergillus spp. Is a saprophytic opportunistic fungal pathogen that causes otomycosis in humans. Some fungal illnesses linked to zoonotic transmission are overlooked in public health efforts, necessitating the development of additional preventative techniques.

The study aims to Isolate Aspergillus niger from external ear of cats and their owners, Molecular characterization of A. niger by Conventional PCR, study the clinical manifestations for human and cats, detection of putative risk factors for infection with aspergillus in human and cats, detection of fumonisin and ochratoxin by PCR.

One hundred and six from the external ear swabs were taken from Domestic Cats attended to private veterinary clinics and cultured on Sabouraud dextrose agar. Fungal growth was identified as A. niger according to cultural characteristics. All A. niger positive samples were subjected to confirmatory step by conventional PCR using species-specific PCR primer pairs for A. niger (NIG1) and (Cmd A). Production of fumonisin and ochratoxin by A. niger confirmed by using specific primers.

A. niger was isolated from 11/106, (10.38%) ear swabs of Domestic Cats Breeders. All A. niger positive samples were positive in confirmatory step by conventional PCR using species-specific PCR primers for A. niger, (NIG1) and (Cmd A).

Swabs frequently were taken from ear of Domestic Cats Breeders at the age group (41-48) years, 24/106(22.64%) in which A. niger was isolated from 3/106, (2.83%). Significant correlation was reported between age group and isolation of *A. niger*. No significant correlation was recorded between sex, education levels, economic status and infection with A. niger.

Among cat breeders most cases were presented in autumn from ear of Domestic Cats Breeders, 60/106, (56.6%) compared with 46/106

(43.39%) in winter. *A. niger* was isolated frequently in winter from ear of Domestic Cats Breeders, 8/106, (7.54%) compared with 3/106 (2.83%) in autumn. Significant correlation (p value=0.038) was reported between season and isolation of *A. niger*.

A. niger was isolated frequently from ear of Domestic Cats Breeders, suffering from absence of earwax 7/106 (6.60%), compared with 4/106 4(3.77%), do not suffering from absence of earwax. Significant difference, (p value=0.000) and correlation (p value=0.024) were reported between absence of earwax and isolation of A. niger. A. niger was isolated from breeders whom practice swimming, 7/106, (6.60%), compared with 4/106, (3.77%) they do not practice swimming. No significant difference nor correlation were reported between swimming, and isolation of A. *niger*

A. niger was isolated mainly from breeders do not use of antibiotics eardrops, 7/106 (6.60%) compared with 4/106 (3.77%) from those using antibiotics eardrops. Significant difference (p value= 0.002) and correlation (p value= 0.013) were reported between using of Antibiotics eardrops and isolation of A. *niger*.

A. niger was isolated mainly from breeders do not expose to trauma of the external ear canal, 10/106 (9.43%) compared with 1/106 (0.94%) from those exposed to trauma. No significant difference (p value = 0.747) nor correlation (p value= 0.245) were reported between exposure to trauma and isolation of A. *niger*. No significant difference (p value = 0.095) no correlation (p value= 0.881) were reported between DM and isolation of *A. niger*.

A. niger was isolated equally from public workers, Free workers and housewives 3/106, (2.83%). No significant difference (p value= 0.526) nor correlation (p value = 0.882) were reported between occupation and isolation of *A. niger*. No significant correlation was reported between unilateral facial swelling; Nasal congestion; Sinus congestion and pain;

Cough; dyspnea; pain around the eyes and isolation of *A. niger* from Domestic Cats Breeders ear. Significant correlation was reported between chest pain; dizziness; tinnitus; ear itching and isolation of *A. niger* from ear of Domestic Cats Breeders.

A. niger was completely resistant to Ketoconazole and Fluconazole (11/11,100%). A total of 7/11 A. niger, (63.63%) were resistant to Itraconazole compared to 4/11, (36.36%) have intermediate zone of inhibition (14-18mm). Nystatin represents potent antifungal agent against *A. niger* with minimum zone of inhibition (17mm). A total of 9/11, (81.81%) of *A. niger* were susceptible to Nystatin and 2/9, (18.18%) have intermediate zone of inhibition (14-18mm). A total of 2/11, (18.18%) of A. niger were susceptible to Miconazole and 8/11, (72.72%) have Intermediate zone of inhibition (14-18mm) and only1/11, A. niger isolate, (9.09%) was resistant (<14 mm).

Fumonisin was detected in 7/11(63.64%) of *A. niger* isolated from ear of Domestic Cats Breeders. Ochratoxin was not produced by any isolates of A. niger, 0/11, (0%)

The total number of *A. niger* isolated from ear of Domestic Cats was 22/106, (20.75 %) All *A. niger* positive samples were subjected to confirmatory step by conventional PCR using species-specific PCR primer pairs for A. niger (NIG1) gene length 290 bp guaranteed to be *A. niger* and (Cmd A) gene length 245 bp guaranteed to be *A. niger*.

In current study, cats with age (2-6) months were frequently infected with *A. niger* 8/106, (7.54%) followed by those with (12- 16) months 7/106, (6.60%). *A. niger* was not recover from old age, (17-21) months, (27-31) months. No significant difference nor correlation were reported between age of Domestic Cats and infection with *A. niger*.

Current study revealed that 66/106 of Domestic Cats were males (62.26%) while female represent 40/106 (37.73%). A total of 16/106

(15.09%) males were infected with *A. niger* isolated from ear versus 6/106(5.66%) for females. No significant correlation was reported between sex of Domestic Cats and infection with *A. niger*. Females appear to be at risk of getting *A. niger* infection at (1.813) time than males.

No significant correlation was reported between body weight; breed; hair length; ear cleaning interval; Bathing frequency of Domestic Cats; Season and infection with *A. niger*.

No significant correlation was recorded between head shaking; head tilting; rubbing against wall; ulcers around the nostrils; malodorous; unilateral ear drooping; lethargy; sneezing and isolation of *A. niger*. Significant correlation was recorded between Blackish waxy discharge of ear; Erythematous lesion of ear and isolation of *A. niger*, (p value=0.012).

A. niger was completely resistant to Ketoconazole and Fluconazole (22/22,100%) with minimum zone of inhibition, (0mm). A. niger was resistant to Itraconazole which is the second antifungal agent in the scale, with minimum zone of inhibition for *A. niger*, 10mm. A total of 20/22, (90.90%), *A. niger* were resistant to Itraconazole compared to 2/22, (9.09%) have intermediate zone of inhibition (14-18mm). Nystatin represents potent antifungal agent against *A. niger* with minimum zone of inhibition (15mm). A total of 15/22, (68.18%) of *A. niger* were susceptible to Nystatin and 7/22, (31.81%), have intermediate zone of inhibition (14-18mm).

Miconazole minimum zone of inhibition (10 mm). A total of 17/22, (77.27%) of *A. niger* have Intermediate zone of inhibition (14-18mm) and only5/22, (22.27%), *A. niger* isolate have resistant (<14 mm).

Fumonisin was detected in 7/17(41.17%) of *A. niger* isolated from ear of Domestic Cats. Ochratoxin was not produced by any isolates of *A. niger*, 0/17, (0%).

Chapter One

Introduction

1. Introduction

aspergillus spp. Are fungi that thrive on dead organic matter and can cause otomycosis in humans, as stated by (Goodale et al., 2016). Certain fungal infections, whether they are actual pathogens or opportunistic ones linked to the spread of zoonoses, are overlooked in public health initiatives, highlighting the need for additional prevention strategies (Seyedmousavi *et al.,* 2015). Fungi struggled to adapt to the human host environment due to the strong immune response it can trigger, posing a fatal risk to immunocompromised patients (Hernández-Chávez *et al.,* 2017). People in the United States who interact with pets like cats and dogs experience numerous infections annually, including both mild skin conditions and severe systemic diseases (Rahman *et al.,* 2020).

Skin fungal infections (such as skin dermatophytosis or other skin conditions) contracted from cats and dogs are likely the most frequent illnesses linked with pets (Łagowski *et al.,* 2019). Numerous zoonotic diseases have the potential to be transmitted between pets and humans (Chowdhury *et al.,* 2021). The problem's full scope is unclear due to numerous zoonotic diseases going unreported to health authorities or being underdiagnosed (Varela *et al.,* 2022). Owning pets has advantages in emotional, health, and social aspects, but domestic animals can also spread zoonotic infections (Ansari-Lari and Oroji, 2023). A significant survey discovered that roughly 75% of households have intimate interactions with animals (Stull *et al.,* 2013).

1.1 Otomycosis in Human

Due to the perfect combination of warmth and moisture for fungal growth, otomycosis, a surface fungal infection of the outer ear canal, is usually observed in tropical and warm regions throughout the summer around 60 various types of fungi contribute to otomycosis, although the

predominant ones, aspergillus and Candida, can lead to complications with bacteria (Mokhtar, 2022).

Even though Aspergillus niger is considered an opportunistic pathogen, a previous study mentioned its ability to induce otomycosis in healthy individuals without any pre-existing conditions (Opperman and Copelyn, 2020). In a study performed by adhavan (2020) it was noticed that A. niger was the main causative agent of otomycosis growing on ear wax and shed skin in the outer ear canal

1.2 Otomycosis in Cats

Otomycosis in cats is a condition of the external ear canal, resulting from a fungal infection. For the condition to develop, there typically has to be a shift in the external ear canal environment or harm to the skin in the canal. Due to their preference for warm, damp conditions, fungi find the excessive wax in the ear canal to be an ideal environment for growth. Persian cats and other long-haired breeds with furry ears are more prone to ear infections, especially in warm, moist conditions. While otomycosis is more common in dogs, it can also be frequently seen in cats. Excessive cleaning or scratching caused by an ear mite infestation is the primary reason for otomycosis in cats. A cat that is in good health and has no ear injuries is quite resilient against fungal infections. In certain instances, a surplus of a specific type of fungi in the environment can lead to the infection of a healthy ear. It is crucial to keep in mind that fungal infections in other areas of the body often signal an underlying disease process. Therefore, if the veterinarian cannot identify any underlying reason for the ear infection, further examinations may be necessary to identify potential overall health problems (Hartmann *et al.,* 2013).

Over 180 species of aspergillus are found in the environment and can lead to illness in cats and dogs with weakened immune systems or excessive exposure to fungus (Elad and Segal, 2018). Typically, the fungal

infection that predominantly affects the sinus/orbital area in cats can escalate to involve the entire body in more serious situations. There are two types of aspergillosis that cats can get: Sinonasal aspergillosis and Sino-orbital aspergillosis is the most common and contagious kind. Sino-orbital aspergillosis (SOA) is currently present in half of the reported cases. Tiny aspergillus spores are inhaled and then grow in the sinuses, nasal cavity, and nose to cause infection. The spread of aspergillosis could be caused by a weakened immune system (Hartmann *et al.*, 2013). Therefore, the recent study aimed to:

1.3 Aims and objectives:

[1] Morphological characterization of Aspergillus niger from external ear of cats and their owners

[2] Molecular detection of A. niger by Conventional PCR

[3] Study the clinical manifestations for human and cats

[4] Detection of putative risk factors for infection with aspergillus in human and cats

[5] Determination antifungal susceptibility of isolated fungi in ear swab sample collected from cats and owners with otomycosis

Chapter two

Literature Review

2. 1Otomycosis

Otomycosis is a fungal infection that affects the outside of the ear. It is most common in warmer climates, especially during the summer months when the weather is warm and humid (Deshmukh *et al.,* 2014). Otomycosis can be caused by roughly 60 different species of fungi, the most common being aspergillus and Candida. Bacteria can occasionally worsen the situation (Bojanović *et al.,* 2023). According to Kiakojuri *et al.*, (2021), the most common fungi responsible for otomycosis are A. fumigatus, A. niger, A. flavus, and Candida albicans. Patients presenting with symptoms of otitis externa had a 7% frequency of otomycosis, a frequent condition found in general otolaryngology practices.

After a bacterial infection affects the local host immune system of the ear, it is uncertain whether the fungi are the real infectious agents or colonization species (Valand and Umakhanth, 2021). The risk factors include humidity, cerumen, ear instrumentation, and the increased use of topical antibiotics and steroids (Krishna, 2020). Fungal ear infection might present as a primary or secondary infection (Awungafac *et al.,* 2024).This occurs more frequently in humid places and during hot seasons, but it is less common during cold seasons. Bojanović *et al.,* (2023) suggest that fungal ear infections can be induced by swimming, foreign bodies in the external auditory canal (EAC), poor personal hygiene, hearing aids, diabetes, and tympanic membrane perforation. Patients with otomycosis may have itching, otalgia, mild discharge, hearing loss, and the sense of a foreign body in the external auditory canal (Vivas and Matthew, 2023). Additionally, epithelial and fungal debris, inflammation, pain, and suppuration are all possible effects. Grayish-white substance is usually seen when inspecting the ears. According to Jayachitra (2018), Aspergillus niger is the most common fungi in otomycosis. Geographical location can influence strain variation (Görür *et al.,* 2019).

2.2 Etiology of Otomycosis

A wide variety of fungi can cause otomycosis. Candida and aspergillus are the two most common fungi that cause otomycosis (Sarvestani *et al.,* 2022) According to (Ziaee *et al.,* 2018), aspergillus is a widespread fungus that grows both indoors and outdoors. Every day, they inhale harmless aspergillus spores. However, it can also result in health problems for some people, such as allergic responses and lung infections (Baxi *et al.*, 2014). The fungi that inhabit human bodies are diverse in sort. Candida is a form of yeast that typically resides in mouth, throat, and intestines in addition to being on their skin. Certain species can cause various forms of candidiasis, including oral thrush (Lin *et al.,* 2017).

In recent years, the number of *instances* of otomycosis has increased. According to experts, the increased use of broad-spectrum antibiotics, steroids, and antibiotic ear drops is the cause of this surge (Mofatteh *et al.,* 2021). According to (Zhao and Zhuxiang Chen, 2022), certain experts postulate that medications modify the pH levels in the ear canal and eliminate any viruses and bacteria, hence facilitating the growth of the fungus.

Impaired immunological responses play a significant role in otomycosis (Viswanatha, 2011). Otomycosis is more common in patients with compromised immune systems. They might experience additional difficulties and otomycosis recurrences. If the infection gets to the temporal bone in skull, that's one serious consequence. According to (Di Lullo *et al.,* 2021), this may be lethal if left untreated.

2.3 Zoonotic Mycosis

Public health initiatives, whether caused by actual pathogens or opportunists connected to the spread of zoonoses, disregard some fungal

infections, necessitating the need for more preventive techniques (Guarro *et al.,* 1999). Because the infected fungi did not adapt adequately to the human host environment, immunocompromised patients may experience a severe immune response that is potentially lethal (Zhang *et al.*, 2015). Every year in the United States, people who come into contact with pets like cats and dogs get a variety of infections, from serious systemic diseases to superficial cutaneous ailments (Overgaauw *et al.*, 2020). Most likely, the most common diseases linked to pets are fungal skin infections (skin dermatophytosis or other skin disorders) contracted by cats and dogs. Humans and pets can contract a variety of zoonotic diseases. Health authorities either underdiagnose or fail to report many zoonotic infections, making it challenging to fully understand the extent of the problem (Peter *et al.*, 2007).

2.4. Aspergillus niger

According (Douglas *et al.*, 2021) the aspergillus fungus species can cause aspergillosis, which can manifest in various ways, from non-invasive localized infections to invasive focal or diffused infections. First-line treatments for this illness include antifungal azole medications. Mycological findings over the last 20 years have improved the understanding of pathogenic aspergillus species and the effectiveness of azole treatments as a first line of defense (Nicola et al., 2019). Technological developments in DNA identification and polyphasic taxonomy have made previously unidentified and unknown species more widely known (Hong *et al.*, 2005). aspergillus species are considered opportunistic pathogens. This progress has linked diseases in humans and other animal species to cryptic aspergillus species, previously misidentified as *aspergillus fumigatus*. (Samson *et al.*, 2014). Accurately identifying cryptic species is crucial since infections with these species are frequently linked to invasive diseases with high fatality rates that are resistant to

standard antifungal treatments (Kidd *et al.,* 2020). Human invasive illness is caused by these fungi (Balajee *et al.,* 2006). and, surprisingly, are the most frequently reported cause of invasive Sino-orbital aspergillosis in cats (Stachler *et al.,* 2012). The biggest study on this illness in cats found that 92% of cases result in death (Barrs *et al.*, 2012).

2.4.1 History of *Aspergillus niger*

Aspergillus niger is the most prevalent and extensively researched species within the aspergillus genus, with respect to its shape, physiology, advantages, and consequences (Paulussen *et al.,* 2016). This accounts for its well-known decreased pathogenicity towards humans and animals. • Food chemist James Currie discovered in 1917 that Aspergillus niger produces significant amounts of citric acid when grown in a medium containing sugar. Currie isolated the acid and thoroughly investigated its advantages as a food preservative (Mudyiwa and Nyati, 2013). Additional research indicates that it can produce glucoamylase, -galactosidase, and numerous other industrially important enzymes. According to (Bhatia *et al.,* 2020), scientists deduced that *Aspergillus niger* possesses distinct strains with varying properties based on their findings and those of earlier morphological research investigations.

A team of scientists looking into the distinctive ochratoid generation by Aspergillus niger found various species that resembled the fungus in 2004 (Frisvad *et al.,* 2011). Subgenus Circumdati, section Nigri, is one of these species; its 15 related black spores resemble Aspergillus niger's seeds fairly closely. According to (Abd Mallick, 2019), these are A. awamori, A. carbonarius, A. foetidus, and A. tubingensis.

2.4.2 Classification of Aspergillus niger

According Webster&Weber (2007)., the Aspergillus niger mold belongs to the Nigri section of the *aspergillus* genus. The aspergillus genus includes widespread molds that are present in soil and water. Figure (2-1) displays these threads.

Kingdom: *Fungi*
Phylum: *Ascomycota*
Class: *Plectomycetes*
Order: *Eurotiales*
Genus: *aspergillus, Eurotium*

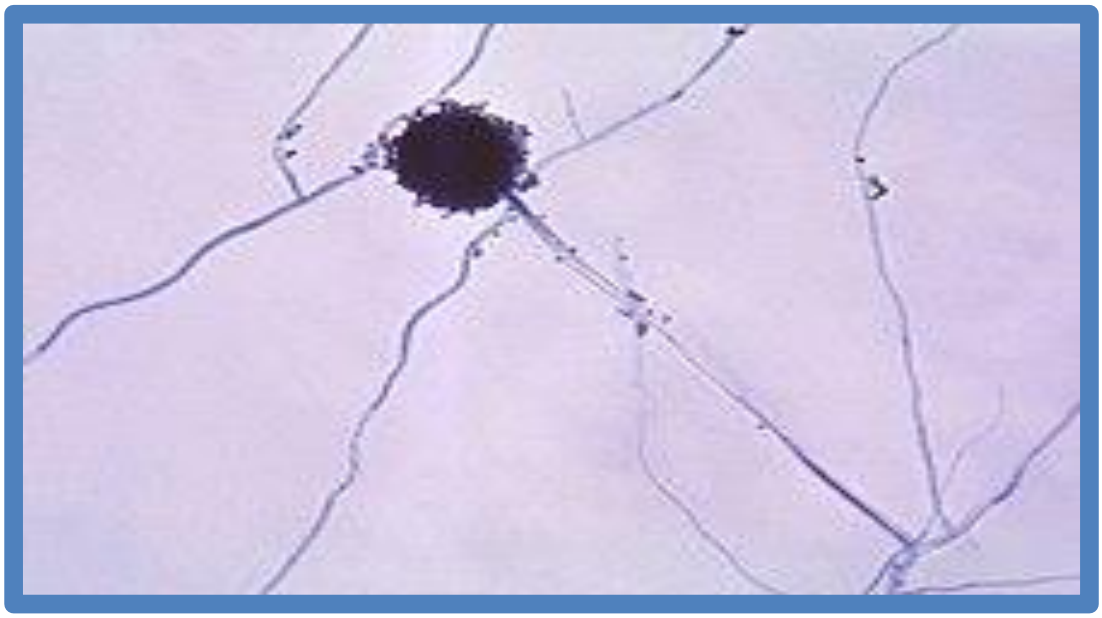

Figure (2-1) showing the microscopic appearance of *Aspergillus niger* conidial head (Varga *et al.*, 2011)

2.4.3 Epidemiology

Patients with allergic respiratory disease are at increased risk for a more severe clinical course due to the prevalence of *aspergillus* sensitization, which ranges from 15.3% to 38.0% (Agarwal *et al.*, 2023).

Forty percent or more of people with severe asthma have a fungus allergy, compared to ten percent of people with milder asthma and five percent of the overall population (Denning *et al.,* 2006). An aggregated prevalence of sensitization to aspergillus spp. was 39.1% in Cystic Fibrosis (CF) patients, according to a meta-analysis. The skin prick test showed a higher prevalence than the particular IgE assay (Alyasin *et al.,* 2018).

2.4.4 Transmission

aspergillosis infections have been reported in birds, animals, and humans worldwide (Arné *et al.,* 2021). So far as we can tell, the fungus spreads by means of airborne conidia. Fungal infections have been reported to occur after direct skin contact with infected substances or injuries. Fungus infections can affect several organs, including the skin, bones, lungs, sinuses, brain, heart, kidneys, and eyes (Pal, 2017; Fang and Latgé, 2018). happens when the host breathes in conidia that are floating in the air (Oliveira *et al.,* 2023). Sporadic cases of hospital-acquired diseases or those linked to dust exposure during building renovations or constructions have been reported (Schahawi and, 2018). Biomedical equipment contamination has been linked to rare outbreaks of cutaneous illness.

2.5 Pathogenesis of otomycosis caused by *Aspergillus niger* in human

While the pathogenic process of *Aspergillus niger* otomycosis in cats is comparable to that in humans, there are notable variances owing to anatomical and immunological factors **(Garcia-Bustos *et al.,* 2024).**

- **Exposure and Entry**: *A. niger* spores are often found in the environment and can enter the ear canal via a variety of routes, including polluted water, earphones, or the hands.

- **Adherence and Colonization**: The spores attach to the skin of the outer ear canal and start to germinate. They subsequently develop hyphae that penetrate the ear canal's epithelial cells, establishing a fungal colony.

- **Invasion and Tissue Damage**: The fungus invades the surrounding tissues, causing damage to the ear canal's epithelial lining. This can result in inflammation, edema, and the accumulation of fungal debris.

- **Immune Response**: The immune system responds to the fungal infection, causing an inflammatory reaction. Symptoms may include pain, itching, and ear drainage.

- *A. niger*, the most common storage fungus, is thought to pose a significant danger of contamination to stored items in tropical and warm climates worldwide (Mohapatra *et al.,* 2017). This mycotoxin-producing *A. niger* is causing havoc in the food and herbal medicine industries.

According to research, under ideal conditions, less than 10% of *A. niger* strains tested positive for ochratoxin A and fumonisin. Humans and other living beings can consume these mycotoxins, which can cause a variety of harmful effects such as immunotoxicity, carcinogenicity, and hepatotoxicity (Malir *et al.,* 2016). Some of the animal consequences include modifications to the synthesis of cytokines, which are signaling proteins and peptides, a shrinkage of immunological organs, and a reduction in antibody responses (Pinti *et al.,* 2016). There would be serious consequences for the poultry business if A. niger were to contaminate chicken feed. May and Adams (1997) have well-documented the susceptibility of certain animals to ochratoxin.

People with compromised immune systems often perceive *A. niger* as a dangerous allergen capable of causing lung infections. When inhaled, mycosis can be deep or systemic due to the tiny conidia and conidiophores

(Jarowiecki, 1990; Table 2-1). Certain conditions, such as local lesions in the middle ear, external ear, or postoperative cavities, can lead to the development of otomycosis (Kaur *et al.*, 2000; Kurnatowski and Kilipiak, 2001). Other chemicals that A. niger can make are oxalic acids, kojic acids, and cyclic pentapeptides. These chemicals can be moderately to severely toxic when they are first discovered (Ueno and Ueno, 1978). Aspergillus niger can generate oxalic acid crystals that can respiratory failure. (Nakagawa *et al.*, 1999)

Table (2-1) Human diseases caused by *Aspergillus niger*

Name of disease	Target organ	References
Weak immune system	Any organ of the body	Louthrenco et al. (1990)
Systemic mycosis	External body part	Louthrenco et al. (1990)
Ear infection	External auditory system	Padhye (1982), Walth and Pizzo (1986)
Aspergillosis	Lung	Ueno and Ueno (1978), Bennett (1979), Richard et al. (2006)
Asthma ana allergic alveolitis	Respiratory tract	Edwards and AlZubaidy (1977)

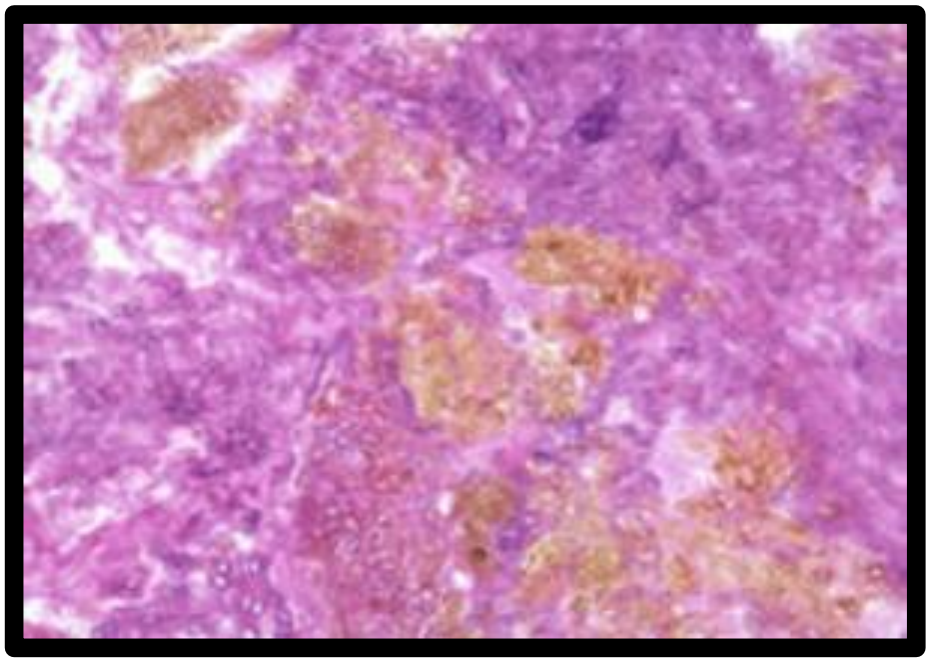

Fig (2.2): Pulmonary oxalosis due to fungus ball of *Aspergillus niger*. The band of necrosis is due to the diffusion of oxalate crystals (Richard *et al.*, 2008)

2.6 Pathophysiology of Aspergillus niger-induced otomycosis in Cat

Aspergillus niger is one of several fungi that can cause otomycosis, an infection of the outer ear. There are usually multiple stages to the pathogenesis of *Aspergillus niger* otomycosis in cats (Tutun *et al.*, 2021).

- **Exposure to the fungus:** Cats can eat *Aspergillus niger* spores found in soil, decomposing organic materials, and tainted food.

***Aspergillus niger* spores colonize**: the outer ear canal of cats by adhering to the epithelial cells that line it. After that, they set up shop in the epithelial cells after germinating and producing hyphae.

- **The fungus invades neighboring tissues:** as it multiplies, harming the ear canal's epithelial lining in the process. as a result, the cat may have pain, edema, and inflammation.

- **The cat's immune system reacts:** by causing an inflammatory reaction to the fungal infection. When this occurs, the immune system may release cytokines and other mediators, exacerbating the otomycosis-related discomfort and itching.

• The fungi that cause the sickness include *A. niger* and *Candida albicans*. The second can cause a dark mat to form in the ear canal, however this is not always the case (Adhavan, 2020). The gold standard for treating otomycosis is to clean the ear canals and use topical antifungal medicine. aspergillosis, an environmental fungal ailment, can affect cats and dogs with weakened immune systems or those exposed to high concentrations of the fungus (Seyedmousavi *et al.,* 2015). Although a fungal infection in cats typically just affects the sinuses orbital region, it can spread throughout the body in extreme circumstances.

aspergillosis can manifest in two ways in cats: first sinonasal aspergillosis SNA characterization by the present local signs of nasal when the infection is chronic and second Sino-orbital aspergillosis SOA is the most common, invasive kind. SOA some time may be represents a stretch of SNA to orbital and subcutaneous When tiny aspergillus spores inhaled through the air settle into the nasal passages, sinuses, and nasal cavity, they cause an infection. A compromised immune system could be the cause of the spread of aspergillosis (Hartmann Katrin *et al.,* 2013).

2.7. Detection and diagnostic strategies of *Aspergillus niger*

2.7.1. Pulmonary Computed tomography (CT)

The use of pulmonary Coronary angiography (CT) The use of pulmonary angiography in the early diagnosis of invasive aspergillosis (IA) can be beneficial when conventional imaging or computed tomography yields likely negative results, particularly in cases of blood vessel occlusion caused by lesions-induced suspicious fungus (Stanzani *et al.,* 2015).

However, using magnetic resonance imaging (MRI) of the lungs as an alternate recommended way of diagnosis in some chosen patients with hemoptysis (AII) could substitute the CT scan (Yan *et al.,* 2015; Sodhi *et al.,* 2016).

2.7.2 Microscopy and Culture

Culture is seeing the gold criterion however it might not always be obtainable or productive. Minimal turnaround time and careful diagnosis ease prompt and best treatment in fungal otitis media so preventing reverse result (Punia *et al.,* 2019). Fungal infection detection has historically relied on tissue culture as the primary diagnostic method. Furthermore, susceptibility testing is made possible by culture (Kozel and Wickes, 2014). Potato dextrose agar, or the slightly modified potato flakes agar, Sabouraud dextrose agar (SDA), SDA with antimicrobial agents, and BHI agar enhanced with blood and antimicrobial agents are a few examples of fungal culture(Sharma *et al.,* 2021)**.** Typically, fungal media are supplemented with antimicrobials such as gentamycin or chloramphenicol and cycloheximide (Acharya and Hare, 2022). The first two stop the growth of bacteria, whereas cycloheximide stops the growth of many of the fungi found in the environment that are usually regarded as pollutants. Large test tubes or Petri dishes can be used for culture. Although they have a greater surface area, the former are more likely to become dehydrated. The latter are less prone to drying out and safer to handle(Mahon and Lehman, 2022). Since many fungal infections develop at temperatures below 37°C, cultures are incubated between 25°–30°C and 37°C. To examine the morphological details, slide mounts should be created using lactophenol cotton blue dye after noting the colony characteristics, such as color and texture of growth.

2.7.3. Detection of *aspergillus* by Molecular Technique (PCR assay)

Molecular biological methods were developed for the diagnosis and detection of *Aspergillus niger* is the cause of otomycosis or other types causes infection, because they are very sensitive when compared to routine serological test rather than fungal culture. One of these tests is PCR technique is based on the amplification of aimed and removed DNA. all the process by the use of specific primers or probes designed for each targeted DNA, utilize of the molecular methods may alterations our knowing touching the microbial epidemiology of otomycosis (Aboutalebian *et al.,* 2019). Molecular methods such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and reverse transcription-PCR (RT-PCR) can be used to detect the genetic material of fungi in clinical samples. PCR is essential for multiplex testing because to its requirement for a small volume of fluid. as indicated by (Wallander *et al.,* 2012). aspergillus DNA identification in a patient's blood circulation is more clinically meaningful and less open to interpretation than detecting aspergillus DNA in other specimens using the same PCR technology (Pasqualotto, 2010; Barnes and White, 2016). Due to the rarity of *aspergillus fungemia*, the detection of a DNA signal in a blood sample using polymerase chain reaction sparked a protracted discussion over the origin and nature of this signal (Barton, 2013). A few phagocytic cells may carry nonviable *aspergillus*. *aspergillus* hyphae can attach to platelets, causing a decrease in fungal vitality. This can result in the detection of aspergillus DNA in the blood using PCR (Perkhofer *et al.,* 2008).

Various studies have demonstrated that aspergillus DNA can be identified in the bloodstream of patients with invasive aspergillosis (IA)

using polymerase chain reaction (PCR) on serum samples. Therefore, the presence or absence of this DNA is not significant (Barton, 2013).

2.7.4 Histopathology and Further Investigations

Though they aren't always feasible, histopathological analyses of tissue samples are invaluable. There is no information about the positive rate of these tests in the literature (Guarnerand Mary, 2011). Histopathological identification of fungal organisms in otomycosis provides a fast and somewhat credible diagnosis (Punia *et al.,* 2019). Imaging techniques like magnetic resonance (MR) and high-resolution computed tomography (HRCT) can also be highly beneficial.

2.7.5. Detection of Antigens-Antibodies by enzyme immunoassay (EIA)

The Enzyme Immuno assay (EIA) was employed to identify the presence of antibodies against aspergillus or to detect certain circulating antigens associated with it. Galactomannan is a polysaccharide that is a component of the cell wall of aspergillus. It is released during the growth of aspergillus in an infected host and is heat stable (Barton, 2013). The FDA has approved the use of the Platelia© sandwich enzyme linked immunoassay to identify the galactomannan antigen of aspergillus. This was supported by research undertaken by Bala and Higgins (2011); Fisher (2013); and Lamoth (2016). The concentration of Galactomannan (GM) in a patient's bloodstream is directly proportional to the amount of aspergillus present. When the aspergillus load is high, the GM levels rise. In contrast, the absence of GM indicates a low fungal load or a good prognosis for the infection (Hadrich *et al.,* 2012).

B-glucan, a component of the cell wall of many disease-causing fungi, can be used as a diagnostic marker for invasive fungi utilizing the

same enzyme immunoassay (EIA). It is regarded as a universal method for detecting fungi in general. Individuals with compromised immune systems or blood disorders are more vulnerable to the assay's antigens, especially those related to GM (Debets-Ossenkopp *et al.*, 2015; Warris and Lehrnbecher, 2017). The sensitivity of these antigen assays is also reduced as a result of fungal infection treatment.

as a result, additional approaches are required to establish precise diagnosis of IA in varied hosts (Marr *et al.*, 2005; Fisher, 2013).

2.7.6 Lateral Flow Cytometer

Using a lateral flow device, monoclonal antibodies (Mab) were used to combat unidentified exogenous antigens released during aspergillus infection. This result is seen as a significant step forward in the diagnosis of invasive aspergillosis in sensitive hosts (Heldt and Hoenigl, 2017).

2.8 Treatment of *Aspergillus niger*

2.8.1 Antifungal Therapy

- Several antifungal medications were used as the primary treatment for fungal infections. Fluconazole, also known as Diflucan, is a medicine used to treat fungal infections that affect various parts of the body. It works against both superficial and systemic fungal diseases. The FDA awarded first clearance in 1990. This drug is an azole antifungal, like ketoconazole and itraconazole. Fluconazole has various advantages over other antifungal medications, including the ability to be taken orally. The side effect profile of this medicine is mild. A study conducted by Badeeb in 2008 found that a single dose of this medicine is successful in curing vaginal yeast infections.
- Itraconazole is used to treat severe fungal or yeast infections. Itraconazole oral solution is used to treat oropharyngeal or

esophageal candidiasis, also known as thrush or oral thrush (Divya, 2014).

- Itraconazole capsules are used to treat fungal infections such as aspergillosis, blastomycosis, and histoplasmosis (commonly known as Darling's disease).
- Ketoconazole should be reserved for cases where alternative antifungal drugs are not feasible (Payne *et al.*, 2010). Ketoconazole has the potential to induce severe liver damage (Posch *et al.*, 2018).
- Miconazole is a topical antifungal drug available in the form of a skin cream. To treat fungal or yeast infections, follow the instructions and apply this cream directly to the affected area of your skin. Mode of Operation The action of this substance involves the inhibition of sterols in the fungal cell membrane, which leads to a modification in the permeability of the cell wall and subsequently results in the leakage of cellular contents (Ghobadi and Saeed Emami, 2022)
- Nystatin is prescribed for the treatment of oral and gastrointestinal fungal infections. Nystatin belongs to a category of antifungal drugs known as polyenes. The mechanism of action involves inhibiting the proliferation of pathogenic fungi responsible for causing infections (Vaishali and Wamik- Azmi, 2021).

2.8.2 Combined Antifungal Treatments

We deliberated on the medicinal and/or surgical approaches for managing aspergillus endophthalmitis. However, we should also consider the potential of combining antifungal medications, as suggested by Aydin et al., in 2007. The effectiveness of a combination antifungal medication in treating severe systemic fungal infections has been demonstrated (Drew *et al.*, 2013). Positive visual results have been documented following the use

of a combination of antifungal medications injected into the eye. In vitro studies have examined many forms of pharmacological interactions, including the study by Mith *et al.,* (2015) investigates the potential synergistic, indifferent, or antagonistic effects between these drugs. Amphotericin B, a type of polyene antifungal medication, is mostly used to treat systemic mycosis and endophthalmitis caused by filamentous fungi. (Klepser, 2011). It operates by binding to the sterols present on the outer surface of the fungi's cell membrane, resulting in the formation of pores and alterations in its permeability to substances (Kristanc *et al.,* 2019).

Voriconazole, an azole chemical, primarily acts by decreasing the levels of ergosterol, which is the main regulator of membrane integrity. The reference for the paper is Carrillo-Munoz *et al.,* (2019); Steinbach *et al.,* have reported that azoles have the capability to inactivate amphotericin B, which is assimilated into the outer layer of fungal cells.

The breakdown of the fungal cell membrane prevents the binding of amphotericin B (Blum *et al.,* 2013). Conversely, the amalgamation of amphotericin B with lipophilic triazoles, such as itraconazole, was found to be detrimental or inefficacious. Nevertheless, the combination of amphotericin B and voriconazole was found to be beneficial in certain experimental studies, albeit not all (Giguère, 2013).

2.9 Control of Aspergillus niger

Experimental use of antifungals should be depressed, prevent close contact with water, no maceration, trying not to get exposed to the trauma in the external ear canal and improve hygiene conditions (Ali *et al.,* 2018).

Antifungal ear drops, such as fluconazole and clotrimazole, have the ability to prevent several types of fungal ear infections (Kiakojuriet *et al.,* 2019). Your healthcare professional may also suggest using aluminum acetate or acetic acid ear drops to alleviate irritation in your ear canal. preventing the consumption of feed that has been contaminated with mold.

Prevention of moldy litter. Sanitizing and sterilizing feed and water utensils. Regularly moving feeders and water dispensers to prevent the accumulation of mold (Mithal *et al.*, 2015).

Regularly sanitize and sterilize the hatchery Enilconazole has been effectively utilized in commercial preparations to disinfect hatcheries and poultry farms, as demonstrated by **(**Seyedmousavi *et al.*, in 2015**)**.

2.10 Vaccination:

So far, there is no vaccine that protects or prevents infection (Jabber, 2023).

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التوصيف المظهري والجزيئي للرشاشيات السوداء من التهاب الأذن الفطري في الانسان والقطط المنزلية

رسالة مقدمة الى

مجلس كلية الطب البيطري - جامعة ديالى

وهي جزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري (الأمراض المشتركة)

من قبل

رسل مؤيد هادي

بإشراف

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