Genus Aspergillus

Aspergillus is derived from the Latin word **"Aspergere,"** which means "**to scatter**". In 1729, Micheli defined the genus *Aspergillus*. are saprophytic mold and are found in decaying organic matter. The fungi grow commonly as the molds on the substrate surface as the contamination in the bread and potatoes.

Aspergillus is a very large genus containing about 250 species, which are currently classified into seven subgenera that are in turn subdivided into several sections comprised of related species (Raper and Fennell 1965, Geiser *et al.* 2007).

A. flavus is the principle medically important pathogen of both humans and animals, some other species in the *A. flavus* complex notably *A. oryzae, A. avenaceus, A. tamari, A. alliaceus* and *A. nomius* may cause rare mostly superficial infections (Hedayati *et al.*, 2007; de Hoog *et al.*, 2020).

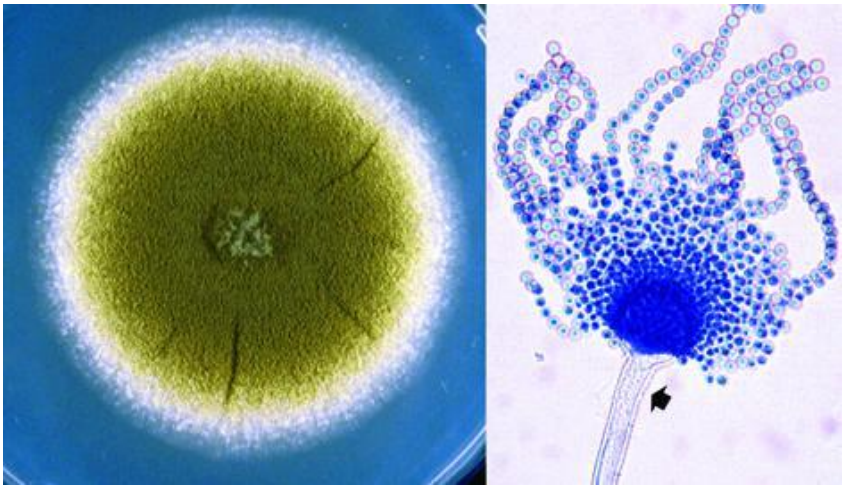
Classification

Kingdom: Fungi
Phylum: Ascomycota
Class: Eurotiomycetes
Order: Eurotiales
Family: Trichocomaceae
Genus: *Aspergillus*

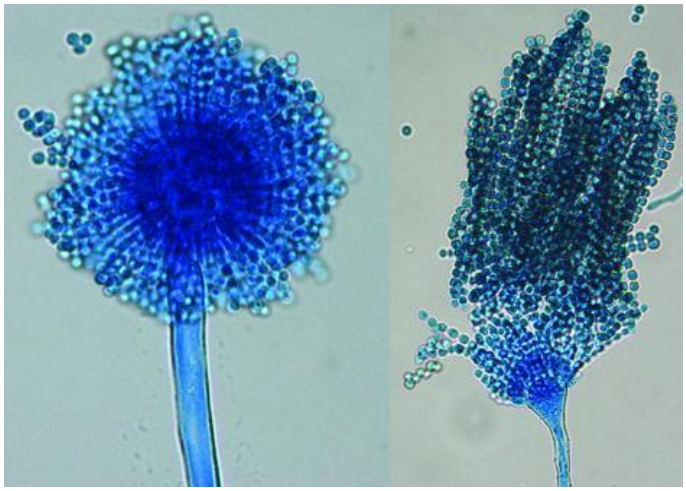
Aspergillus flavus

Morphology

On **Czapek Dox agar**, colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age. The reverse side is golden to red-brown. The reverse side is golden to red-brown. Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.



Culture and conidial head of *Aspergillus flavus*.



Conidial heads of *Aspergillus flavus*. Note: conidial heads with both uniseriate and biseriate arrangement of phialides may be present.

Phialides :In some species of Aspergillus the phialides are the first layer of cells on the surface of the vesicle. Phialides are sporogenous cells that produce conidia through a specialized apical budding process.

Aspergillus flavus has a worldwide distribution and normally occurs as a saprophyte in soil and on many kinds of decaying organic matter, however, it is also a recognized pathogen of humans and animals. It is a causative agent of **Aflatoxicosis**/**Aspergillosis in poultry**, **hepatotoxicity, and hepatocarcinogenic in poultry and in animals**.

After *A. fumigatus*, *Aspergillus flavus* it is the second leading causative agent of aspergillosis. Since its **spore's size** is **big** (3-6 µm), it gets deposited in the upper respiratory tract causing fungal sinusitis. It also causes cutaneous infection and non-invasive fungal pneumonia. *A. flavus* produces **aflatoxin**, which can cause acute hepatitis, hepatocellular carcinoma, neutropenia, and aflatoxicosis in poultry birds.

Toxins produced by *Aspergillus flavus* and Aflatoxicosis

Aflatoxicosis represents one of the serious diseases of poultry, livestock and other animals. Aflatoxins (AF) are ubiquitous in corn-based animal feed and causes hepatotoxic and hepatocarcinogenic effects. The cause of this disease in poultry and other food-producing animals has been attributed to the ingestion of various feeds contaminated with A. flavus.

Aspergillus flavus, toxigenic fungus is known to produce a group of extremely toxic metabolites, structurally derivatives of difurocoumarin, aflatoxins are most commonly produced by strains of *Aspergillus flavus*, *A. parasiticus*, and *A. nominus*, although many other Aspergilli have aflatoxigenic capabilities. Named according to their blue or green fluorescence under UV light, there are four primary aflatoxins: aflatoxin B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2). Of these, AFB_1 is the most hepatotoxic, the most mutagenic, and the most prevalent worldwide of which aflatoxin B1 (AFB1) is most potent. Other aflatoxin includes types B_2, G_1, and G_2. Aflatoxin B_1 (AFB_1) is the most potent with the greatest toxigenic power, followed by G_1, B_2, and G_2.

Avian species especially chicks, goslings, ducklings and turkey poults are most susceptible to AFB1 toxicity. The toxic effects of AFB1 are mainly localized in liver as manifested by hepatic necrosis, bile duct proliferation, icterus and hemorrhage. Chronic toxicity in those birds is characterized by loss of weight, decline in feed efficiency, drop in egg production and increased susceptibility to infections. The incidence of hepatocellular tumors, particularly in ducklings, is considered to be one of the serious consequences of aflatoxicosis. Even though prevention and avoidance are the best way to control aflatoxicosis, natural contamination of crops with A. flavus is sometimes unavoidable. Such aflatoxin-contaminated feeds can

be decontaminated using various methods which mainly focus on physical removal or chemical inactivation of the toxins in the feeds. Moreover, dietary additives such as activated charcoal, phenobarbital, cysteine, glutathione, betacarotene, and selenium have also been reported to be effective in the reduction of aflatoxicosis in poultry.

What is the normal aflatoxin level in poultry feed?

The permissible level for poultry is **20 ppb**. The toxin production can take place in either preharvest or postharvest stage of the crop. The Food and Drug Administration and European Union have established 20 µg/kg and 10 µg/kg AFs as maximum level for poultry, respectively.[1 ppb equals 1 µg of substance per kg of solid (µg/kg)]

Aspergillus fumigatus

Morphology

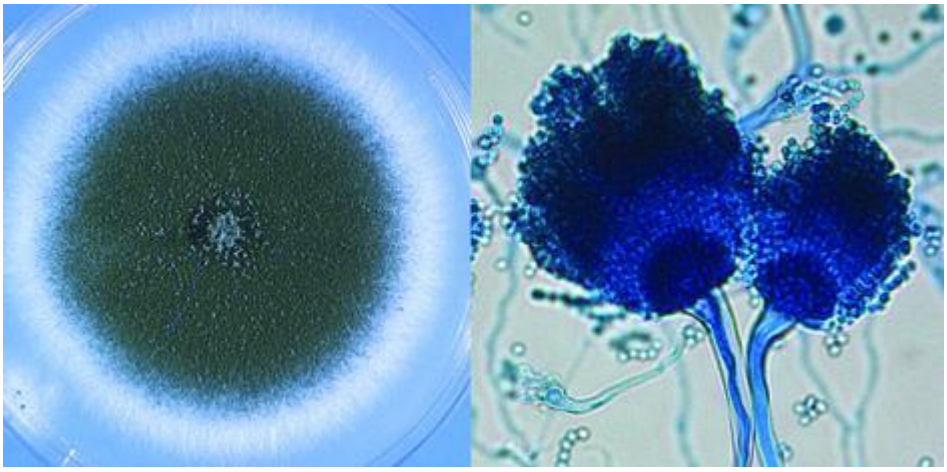
On **Czapek Dox agar**, Colonies of the *A. fumigatus* are velvety or powdery at first, turning to smoky green. The reverse side is white to tan. The conidiophore is smooth, phialides single (uniseriate), usually covering the upper half vesicle parallel to the axis of a stalk.

. *A. fumigatus* is a fast grower; the colony size can reach 4 ± 1 cm within a week when grown on Czapek-Dox agar at 25°C. *A. fumigatus* is a thermophilic species, with growth occurring at temperatures as high as 55°C and survival maintained at temperatures up to 70°C.

No sexual stage is known for this species.

Aspergillus fumigatus is characterized by the formation of a conidiophore with columnar conidial heads consisting of flask-shaped vesicles, uniseriate phialides, and long chains of conidia. Conidia are bluish-green to pale green, generally hydrophobic, and have a size of 2.5–3.5 µm.

Iron and zinc are of prime importance for *A. fumigatus* growth.



Culture and conidial heads of *Aspergillus fumigatus.*

A. fumigatus, due to the small size of the conidia, spores reach the alveoli in the lungs. It can germinate to hyphae and cause endothelial damage. It produces **gliotoxin**, inhibiting phagocytosis, which can evade the immune defense mechanism. *Aspergillus fumigatus* can infect the skin, eye, and other organs, causing aspergilloma and **allergic bronchopulmonary aspergillosis**. In the immunocompromised condition, aspergillosis can be fatal, leading to death.

Avian Aspergillosis (Brooder's Pneumonia, Mycotic Pneumonia, Pneumomycosis)

Avian Aspergillosis is an infectious fungal disease of poutrly birds, world wide in distribution, characterized mainly by respiratory symptoms, appears to be sporadic, frequently affecting only individual birds aging **7–40 days old** , caused by *Aspergillus fumigatus*, filamentous fungus , ubiquitous, opportunistic pathogen that produces large amounts of small-sized conidia in the air. characterized by dyspnoea, gasping, and inappetence, resulting in high morbidity and mortality, thus inducing significant economic losses in poultry. The chronic form of aspergillosis is sporadic, which generally occurs in older birds, especially breeders in poultry, and causes lesser mortality

Aspergillus fumigatus is considered as a major respiratory pathogen in birds. This filamentous fungus was first found in the lungs of a bustard (*Otis tarda*) in 1863 by Fresenius.

A. fumigatus develops and sporulates easily in poor quality bedding or contaminated feedstuffs in indoor farm environments. **Inadequate ventilation and dusty conditions increase the risk of bird exposure to aerosolized spores**.

Etiology: *Aspergillus fumigatus* is a common cause of avian aspergillosis. However, several other mold species, such as other species like *A. flavus*, *A. niger*, *A. nidulans,* and *A. terreus* may also be isolated from avian cases of aspergillosis (sometimes in mixed infections) but much less frequently than *A. fumigatus*.

High mortality rates can be seen in chicks and poults that inhale large numbers of spores (Conidia in the air) during hatching or when placed on bedding contaminated with mold spores. In older birds, infection is caused primarily by inhalation of spore-laden dust from contaminated litter, feed, or dusty range areas.

Pathogenesis
Virulence represents the ability of a pathogen to invade the host, overcome its natural defences, and proliferate subsequently in the organism. When sensing a favourable environment, *A. fumigatus* conidia germinate and concurrently produce enzymes that degrade organic materials into nutrients for further assimilation. The fungus secretes various enzymes like proteases and toxic secondary metabolites.

Gliotoxin is a highly immunosuppressive mycotoxin produced by various isolates of *A. fumigatus*. Concentrations exceeding 20 μg/g and 70 μg/g have been detected in poultry feedstuffs and in tissues obtained from turkeys with airsacculitis, respectively. Turkey blood peripheral lymphocytes, when exposed to high levels of gliotoxin, either died or exhibited a lower lymphoblastogenic response.

Clinical Signs
The most **common clinical signs** of aspergillosis include:
Dyspnoea, labored breathing, fever, inappetence.

In chickens and turkeys, the lungs and airsacs are most frequently involved. **Pulmonary lesions are commonly characterized by white to yellow plaques and nodules** a few millimeters to several centimeters in diameter. In rare cases, birds may present with **diffuse pulmonary congestion** only. Occasionally, **mycelial masses may be seen within the air passages** on gross examination.

Acute aspergillosis may include a variety of nonspecific clinical signs: anorexia, lethargy, ruffled feathers, respiratory signs, polydipsia, polyuria, stunting, or sudden death.

In chicks, contaminated *in ovo* or during hatching, the disease, commonly known as **brooder pneumonia**, is highly fatal in the first ten days of life and results in a major respiratory distress . Also causes omphalitis where the primary cause was *A. fumigatus* have been investigated in young turkeys . In poultry farms, mortality rate may rise slightly or increases suddenly, peaks during a few days, and then returns to initial state.

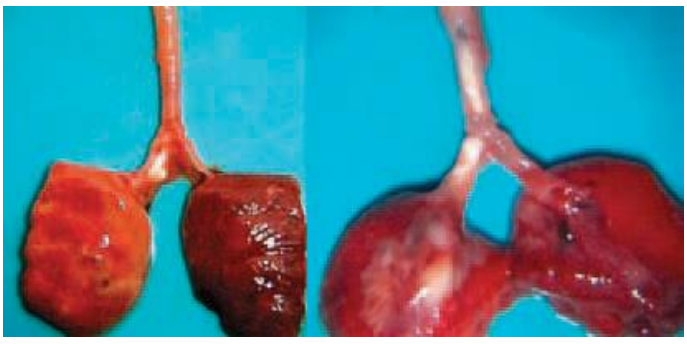
Respiratory signs include dyspnoea, gasping, hyperpnoea with panting, nonproductive coughing, wheezing, cyanosis, and sometimes nasal discharge.

In the chronic form, dyspnoea, depression, dehydration, and emaciation are described. Nervous system involvement causes ataxia, tremor, opisthotonos, lateral recumbency, torticollis, seizures, convulsions, lameness, and hind limb paresis. Occurrence of nervous and ophthalmic troubles one week after an acute episode of aspergillosis has been reported in a turkey flock. Cloudiness of the eye with severe conjunctivitis and turbid discharge were associated with paralysis in broiler breeders.

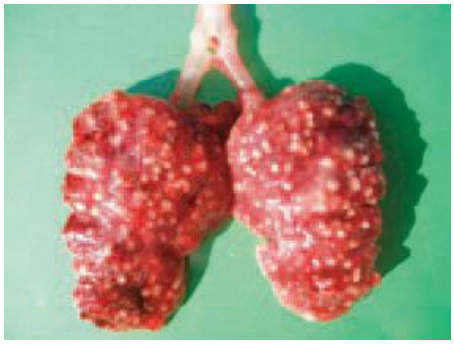
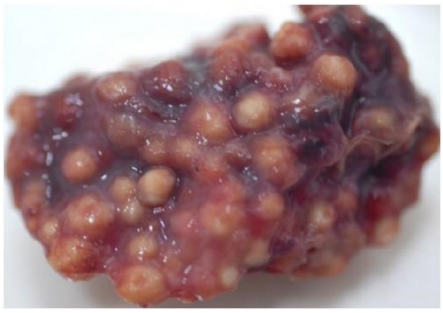
Lesions consist of white to yellowish granulomas ranging from miliary (<1 mm in diameter) to large roughly spherical granulomatous nodules (>2 cm) involving serosae and parenchyma of one or multiple organs. Single or multiple necrotic areas are visible on cut surfaces. The primary location of lesions is the air sacs and lungs although oesophagus, proventriculus, gizzard, small intestine, liver, kidney, spleen, skin, trachea, peritoneum, brain, eye, muscle, or heart may be involved.

An ocular form is seen in chickens and turkeys as mycotic keratitis, in which large plaques may be expressed from the medial canthus.

Aspergillosis is an acute or chronic respiratory disease. In rare instances, peritoneal, visceral or systemic lesions could be observed. It is caused by *Aspergillus fumigatus*. Dyspnoea, enhanced, tense and heavy breathing are observed. Sometimes, rales and cyanosis could occur.



In the acute form, a serous fibrinous pneumonia is observed. Within the trachea and the main bronchi, usually near the bifurcation, obturation masses of coagulated fibrinous exudate are detected.

In the nodular form, multiple grey whitish or yellowish dense nodes in the lungs are observed
Numerous nodules in the lung of a duck with acute aspergillosis (Arne, et al., 2011)

Rarely, as a systemic manifestation, Aspergillus granulomas in the brain could be detected, when spores are transported via the blood circulation.

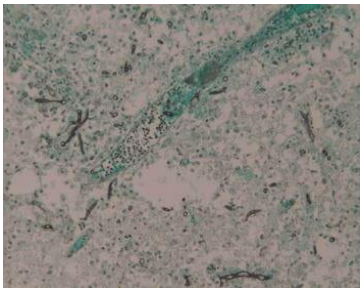
Diagnosis of Aspergillosis (Brooder's Pneumonia)

Diagnosis of aspergillosis is most frequently based on the clinical presentation and gross lesions. For confirmation, the presence of mold in the affected organs can be demonstrated by culture or by microscopic examination of fixed tissue.

1. History of / detection of growth of mould in the litter, feed specifically in rainy season is of prime importance.

2. Confirmation by **Isolation and identification of *Aspergillus fumigatus*** from the lesions in the lungs. A piece of affected tissue may be collected, the lesion(s) may be sampled using a swab, or one of the plaques can be teased apart. Most commonly, the sample would then be placed on Sabouraud-Dextrose agar or some other medium specific for the growth of mold.

3. Most commonly based on clinical presentation and gross lesions
   Nodular form, multiple grey whitish or yellowish dense nodes in the lungs.
   Aspergillosis should be strongly suspected when debilitated birds with respiratory distress do not respond to antibiotic treatment and when careful history reveals the presence of underlying environmental or immunosuppressive factors.
4. Confirmation is by histopathology
   Haematoxylin-eosin stain is often augmented with periodic acid-Schiff, Grocott, and Gomori's methenamine silver stains in order to display fungal elements in embedded tissue sections. The fluorescent optical brightener blankophor proves to be a valuable tool for demonstrating *Aspergillus* sp. Hyphae. Histopathologic examination using a special fungal stain reveals granulomas containing mycelia.



Grocott, and Gomori's methenamine silver (GMS) stained cerebellar histomicrograph
Photo by Dr. S.M. Williams

5. Immunohistochemistry, with monoclonal or polyclonal antibodies, is a powerful and accurate tool to identify *A. fumigatus* in lesions.

Prevention and Control/Intervention Strategies

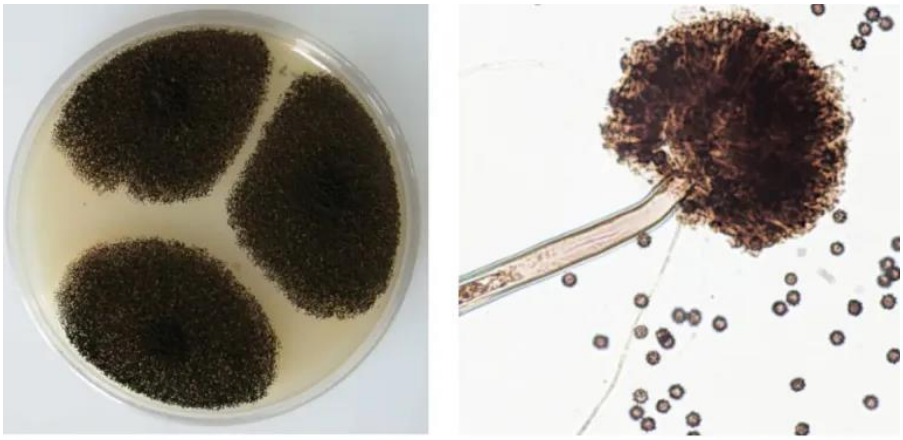
- Although numerous antifungal protocols have been proposed to cure birds with aspergillosis, treatment of the disease in poultry farms is virtually impossible.
- No vaccine is available.
- Specific biosecurity measures against *Aspergillus* contamination rely primarily on prevention.
- Dust and mouldy litter or feed should be avoided.
- A good litter management combined with daily assessment of its quality throughout the lifetime of the flock is the key to prevention of the disease.
- Bed, like feeders, should be kept dry, nondusty, and clean in order to limit fungal development.
- Control of relative humidity through appropriate ventilation should be verified to prevent wet litter.
- Sporadic or repeated antifungal treatment may be useful in order to control environmental contamination. Spraying of fungistatic agents like thiabendazole , nystatin, or copper sulphate contributed to decreased fungal contamination of beddings. Enilconazole may be sprayed, fogged, or nebulised to decontaminate surfaces or indoor volume.
- Finally, effects of stressors like beak trimming and high stocking densities should be minimized.

Aspergillus niger

Colonies of the *A. niger* are woolly at first, white to yellow, then dark brown to black. The reverse side is white to yellow. The conidiophore is of variable length; phialides are biseriate, i.e., arranged in two rows, covering the entire vesicle, forming a radiate head.

A. niger is also known as the black mold and causes infection in food and vegetables. In immunocompromised patients, it can cause invasive pulmonary aspergillosis. People exposed to horticulture are more likely to inhale its spores. *A. niger* can also cause otomycosis (infection of the ear). Some of the strains of *Aspergillus niger* also produce the mycotoxins called **ochratoxin A**.

Aspergillus niger, a fungus known for its high radiation resistance, is widely used in biotechnology and a candidate for pyomelanin production, a molecule with UV-C radiation shielding activity.



Aspergillus niger

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