

ASPERGILLUS SPECIES

Aspergillus species are saprophytic mould widely distributed in the environment. They are present in the soil and in decomposing organic matter. *Aspergillus fumigatus* can be found in overheated, poor quality hay and in compost heaps. Spores are dispersed in dust and in the air. The genus *Aspergillus* comprises of over 190 species but only a few of them are of clinical relevance causing opportunistic infections in humans and animals. *Aspergillus fumigatus* is the species most often implicated in tissue invasion. Other species commonly associated with aspergillosis are: *A. niger*, *A. flavus*, *A. terreus*, *A. deflexus*, *A. nidulans* and *A. flavipes*.

Most *Aspergillus spp* are grouped as fungi imperfecti while some are classified as ascomycetes. They possess septate hyphae which are hyaline in nature and about 8.0µm in diameter. Non-branching conidiophores can be seen to have emerged at right angle from a specialized hyphal foot cells. The tip of the conidiophores enlarged to form a vesicle bearing numerous flask-shaped phialides. The phialides produce chains of spherical pigmented conidia (phialoconidia) of about 5.0 µm in diameter and may be smooth or rough. In some species (*A. fumigatus*), the phialides are borne directly on the vesicle (uniseriate) while in others (*A. niger*), the phialides are borne on metulae attached to the vesicle.

Aspergillus species are aerobic and grow rapidly to form visible colonies after 2 to 3 days of incubation. The colour of the obverse side of the colonies varies with species and condition of culture. The different colours which may be may be blue-green, black, brown, yellow, or reddish can be used for presumptive identification. *Aspergillus fumigatus* can resist high temperature and grows at temperature ranging from 20 – 50 °C.

Clinical infections: respiratory infection develops following inhalation of *Aspergillus* spores. Occasionally, infection may occur following ingestion of spores in feed or following tissues invasion through trauma. Systemic infection is associated with immunosuppression. Mycotoxicosis occurs following expression of toxins by *Aspergillus spp* such as *A. flavus* growing in cereals and other foods.

Forms of aspergillosis:

- Allergic aspergillosis
- Colonizing aspergillosis: invasive aspergillosis, disseminated aspergillosis (other organs and the central nervous system), bronchopneumonia, naso-orbital aspergillosis,
- Aspergilloma: tumour in lungs and other body organ resulting from inhalation of *A. niger*

Poultry:

- i. Brooder pneumonia: Occurs in newly-hatched chickens in incubators
- ii. Pneumonia and air sacculitis: Most common in chickens and poults of up to 6 weeks of age. Older birds are occasionally affected.
- iii. Generalized aspergillosis: Dissemination of infection usually from the respiratory tract

Horses:

- i. Guttural pouch mycosis: confined to the guttural pouch and often unilateral
- ii. Nasal granulomas: Produces a nasal discharge and interferes with breathing. Fungi other than *Aspergillus spp* may initiate this condition
- iii. Keratitis: Localized infection following ocular trauma
- iv. Intestinal aspergillosis: enteric infection resulting in diarrhea in foals

Cattle:

- i. Mycotic abortion: occurs sporadically, produces thickened placenta and plaques on skin of aborted foetus
- ii. Mycotic pneumonia: uncommon condition of housed calves
- iii. Mycotic mastitis: may result from the use of contaminated intramammary antibiotic tubes
- iv. Intestinal aspergillosis: may cause acute or chronic diarrhea in calves

Dogs:

- i. Nasal aspergillosis: invasion of nasal mucosa and turbinate bones occurring sporadically
- ii. Otitis externa: *Aspergillus spp* may constitute part of a mixed infection
- iii. Dissemination aspergillosis: uncommon but may result in osteomyelitis or discospondylitis

Cats:

- i. Systemic aspergillosis: rarely encountered usually following immunosuppression

Diagnosis:

- i. Presumptive diagnosis is based on clinical signs.
- ii. Sample collection: biopsy samples, tissues from postmortem lesions.
- iii. Staining of tissue sections by methenamine silver impregnation or by PAS methods to reveal hyphal invasion.
- iv. For isolation, small tissue specimens are applied to the scarified surface of SDA and incubated aerobically at 37 °C for 2 to 5 days. Hyphae grow from specimen to form colonies. Colonies can be up to 5 cm in diameter after incubation for 5 days. The colour of the reverse side is pale

yellow to light tan. The colour of the obverse side is determined by the pigmentation of the conidia.

Colonia identification criteria:

- Temperature of growth (45-50°C) for identification of thermotolerant species (*A. fumigatus*).
- Presumptive identification can be made on the basis of colonial appearance and conidial arrangement on sporing heads (size, shape of vesicle, position of phialides; size, shape and colour of conidia).

Colonial appearance:

Only a small number of the species are responsible for the majority of infections in animals.

- *A. fumigatus* colonies rapidly become velvety or granular and bluish green with narrow white peripheries. Older colonies are slate-gray
- *A. niger* colonies are black and granular because of the large pigmentation of the sporing heads
- *A. flavus* colonies are yellowish green with a fluffy texture
- *A. terreus* colonies are cinnamon-brown with a granular texture

Because colonies of *Aspergillus fumigatus* are similar to those of *Penicillium spp* in appearance, microscopic differentiation of smear from colonies may be necessary. The conidiophores of *Penicillium spp* often possess secondary branches (metulae) bearing several phialides.

- v. Serological tests: procedure such as ELISA is reliable for identification in dogs
- vi. Molecular procedures such as PCR

vii. MUCORMYCOSIS

- viii. Mucormycosis refers to the mycosis caused by any of the fungal species belonging to the genera classified under the order 'Mucorales' in the class Zygomycetes. These genera include: *Rhizopus*, *Mucor*, *Rhizomucor* and *Absidia*. The term 'mucorales' originated from the Latin word '*mucere*' meaning 'to be mouldy or musty'. Members of the order 'Mucorales' include those Zygomycetes that reproduce asexually by formation of spores within sac-like sporangia and also sexually by forming zygospores.
- ix. Members of the order mucorales are commonly referred to as 'pin' mould because their dark sporangia resembles pin head, they are also called bread mould because they often grow on stale bread. They are widely distributed in nature as saprophytes in soil, dung, vegetation and other organic matters. Their spores are dispersed in air. They are transmitted through inhalation of spore to produce disease of the respiratory system or by ingestion leading to gastrointestinal disorder and associated lymph nodes. Disseminated infection may occur where the organisms invade the circulatory system and form foci of infection in different organs. Infection may be associated with immunosuppression.
- x. Identification of Mucorales**
- xi. *Rhizopus*, *Mucor*, *Rhizomucor* and *Absidia* grow well on SDA at 37 °C (*Rhizomucor* are thermophiles which will survive and grow at temperature up to 60 °C). Their growth is inhibited by cycloheximide. They grow rapidly and cover the entire surface of the agar plate. The colonies could be fluffy, woolly or cottony with gray-white, brown, brownish-gray colour. The fungi grow from border to border in the Petri dish without producing distinct colony margin. As colonies mature, gray-brown colour with black pepperlike stipples are produced indicating the production of sporangia. The genera are differentiated on the basis of microscopic morphologic features.
- xii. Generally, at microscopic they possess ribbon-like aseptate hyphae (there may be septa in older culture), sporangia that is borne on top of a sporangiophore (branched or unbranched) terminating in a bulb-like structure termed columella. Within the sporangia are numerous sporangiospores. The

columella may rest within another structure referred to as apophysis. They also have root-like structures called rhizoids for anchoring into the medium on which they are growing.

xiii. Differentiating morphological features of members of mucorales

Feature	<i>Rhizopus</i>	<i>Mucor</i>	<i>Rhizomucor</i>	<i>Absidia</i>
Sporangia	Spherical	Large spherical	Spherical	Pear-shaped
Sporangiophore	Simple but occasionally branched	Simple but tend to branch	Simple but may branch	Branch freely
Apophysis	Not prominent	Absent	Not prominent	Prominent
Rhizoids	Coarse, spiked and located underneath sporangiophores	Absent	Poorly developed found between sporangiophores	Delicate, located between sporangiophores

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