

Mycotoxicosis

Aetiology Epidemiology Diagnosis Prevention and Control
Potential Impacts of Disease Agent Beyond Clinical Illness References

AETIOLOGY

Classification of the causative agent

Because the term “mycotoxicosis” encompasses a significant number of toxic agents, this technical card will focus on specific toxins most relevant to wildlife species.

Mycotoxins are compounds synthesised by moulds that cause toxic, non-infectious disease in the animals that ingest them. There are many mycotoxins, some of which are only synthesised by one organism and some of which are synthesised by many, and therefore have several potential clinical manifestations. It should be noted that not all isolates from a mould species known to produce toxins will do so. Synthesis of mycotoxins is induced by complex relationships between genetic composition and the environment; therefore, the presence of a specific organism does not confirm the presence of a toxin.

This technical card will focus on: aflatoxin, produced by *Aspergillus flavus* and *parasiticus*; ergot alkaloids, produced by *Claviceps purpurea*; and fusariotoxins such as fumonisin, the trichothecenes, and zearalenone, produced by *Fusarium* spp.

Resistance to physical and chemical action

Temperature: Incineration is recommended for disposal of contaminated crops and feed material; see *Occurrence* for more information

pH: *In vitro* models of rumen conditions suggest acidic states reduce clearance of mycotoxins

Chemicals/Disinfectants: Aluminosilicates and sodium bentonite effectively absorb aflatoxin; polymeric glucomannan and cholestyramine effectively adsorb multiple fusariotoxins; in the event of suspected mycotoxicosis, sodium hypochlorite can be used to rid surfaces, machines, and instruments of remaining mould

Survival: Microbes in the soil, namely *Bacillus* spp. and members of the phylum Actinobacteria, are critically important for aflatoxin degradation in the environment; protozoa are known to degrade multiple mycotoxins in the rumen

EPIDEMIOLOGY

Affected Species

Because mycotoxins are capable of affecting numerous species, the term “affected species” as used in this technical card will refer to species for which these compounds are currently an appreciable danger. It should be noted that there may be variability in species’ relevance due to inherent differences in species sensitivity, environmental differences, anthropogenic factors, route of exposure, et cetera.

Mycotoxins are of primary concern to humans and livestock because improper feed storage and preservation techniques encourage fungal growth and toxin production. Therefore, much of what is known about these toxins is from species relevant to agriculture and from experimental models. The toxicology of these substances is assumed to be fairly consistent across species and will be discussed as such.

- Aflatoxin
 - Birds are more susceptible than mammals and susceptibility among bird species is variable
 - Ruminants are more tolerant than monogastric species
 - Risk of exposure increases if the species of concern routinely consumes grains
 - Aquatic and marine species such as Nile tilapia (*Oreochromis niloticus*) and Pacific white shrimp/King prawn (*Litopenaeus vannamei*)
- Ergot alkaloids
 - Cattle (*Bos taurus*), equids (*Equidae*), sheep (*Ovis aries*), swine (*Sus scrofa*)
- Fusariotoxins
 - Fumonisin - equids (*Equidae*), hares and rabbits (*Leporidae*), swine (*Sus scrofa*)
 - Trichothecenes - sandhill cranes (*Grus canadensis*) and whooping cranes (*Grus americana*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*)
 - Zearalenone - cattle (*Bos taurus*), sheep (*Ovis aries*), rainbow trout (*Oncorhynchus mykiss*); swine (*Sus scrofa*) are exceedingly sensitive; poultry (*Galliformes*) are fairly resistant

Routes of Exposure

- Ingestion
- Some toxins are absorbed transdermally or cause damage to the skin if touched
- There is speculation that some moulds or mould fragments may be aerosolised and induce mycotoxicity if inhaled. However, this hypothesis is understudied and remains fairly controversial

Sources

- Mould-contaminated feed (e.g., grain, corn, peanuts) in the form of silage, bait, pelleted food, or crops in the field
 - Decomposing plant material can introduce mycotoxins into water sources (groundwater, runoff) and soil

Occurrence

Aflatoxins

Aflatoxins are highly toxic compounds. There are four main types of aflatoxin: B1, B2, G1, and G2, all of which form metabolites that are more toxic than the original compound. B1 is both the most common and the most toxic. Typically, aflatoxins are identified in contaminated grains but may be found in nuts, oilseeds, and corn that have been stored improperly. *Aspergillus* spp. require a substrate moisture content >14%, relative humidity >70%, and a temperature >21°C to proliferate in grains. In the field, drought stress, insect infestation, and other causes of plant damage can encourage fungal growth and toxin production.

Mortality events in many avian species are commonly associated with migration or overwintering, as stopover sites frequently coincide with agricultural areas.

Ergot alkaloids

Ergot alkaloids have been implicated in human disease for centuries. Today, these compounds are most relevant to veterinary species, as modern agricultural techniques have significantly reduced their availability in the human food supply. *Claviceps* spp. are fungal parasites of grasses and are commonly found in the environment. This mould's resting body, called a sclerotium, replaces the grain or seed in a variety of forage and grass species. Sclerotia are black, elongated structures that contain hyphae as well as toxic alkaloids such as ergotamine and ergonovine. Ergotism is typically observed in the late summer when seed heads are maturing.

Fusariotoxins

Fusarium is a large and taxonomically diverse genus; it should be noted that reclassification and nomenclature changes are common so this fungus may have a different name in older literature. *Fusarium* spp. are capable of synthesizing more than 60 toxic compounds, including zearalenone (also known as F-2 toxin), fumonisin, and trichothecenes like vomitoxin (deoxynivalenol), T-2 toxin, diacetoxyscirpenol (DAS), neosolaniol, and isoneosolaniol. Fusariotoxins are unusual in that they tend to be produced during colder times of the year. Common substrates for toxin-producing *Fusarium* spp. include corn, grains, peanuts, and forages. Many fusariotoxins have been identified in grasses intended for pasture grazing, poultry feed, and other agricultural products.

These fungi are commonly found in the environment, typically as plant pathogens or feed contaminants. They grow well in wet field conditions during the summer and fall. Generally, 17°-25°C is preferable for fungal growth; the toxins themselves, however, are mostly produced at cooler temperatures ranging between 4°-24°C.

DIAGNOSIS

Aflatoxins

There is significant variation in species' susceptibility to aflatoxins due to differences in cytochrome P450 and glutathione transferase systems. Regardless of species, the liver is the target organ. Tissue residues are no longer detectable 1-3 weeks after exposure.

Ergot alkaloids

Concentrations of 200-600 parts per billion are believed to induce clinical signs, depending on the alkaloid composition and affected species. Clinical signs may not develop until 2-6 weeks after ingestion.

Fusariotoxins

There are multiple fumonisin toxins, but fumonisin B₁ is the most commonly discussed. Clinical signs can vary widely between species due to differences in sphingolipid metabolism and mechanisms of interference.

Clinical disease secondary to trichothecene intoxication is widely variable in appearance and timeframe. These compounds are able to inhibit protein synthesis via multiple molecular targets, thereby affecting many organ systems and stages of protein production. Although there are many trichothecene compounds, all contain an epoxide residue that is responsible for their toxicity.

Zearalenone and its metabolites are 17 β -oestradiol analogues and are often classified as a mycoestrogens or nonsteroidal oestrogens. It should be noted that while these compounds are exceedingly potent, they are not considered to be particularly toxic.

Clinical diagnosis

Aflatoxins

Intoxicated waterbirds are often found acutely dead. Antemortem, individuals may be lethargic, blind, flightless, and tremulous. Some birds may display wing-flapping, and many are less aware of their surroundings. Chronic toxicity in any species often manifests insidiously and vaguely, with signs such as inappetence, weight loss, and ill-thrift. In ruminants specifically, aflatoxins depress feed efficiency, reduce volatile fatty acid production, reduce milk production, and compromise ruminal function and motility.

The pathophysiology of aflatoxins is primarily characterised by severe liver damage, which causes icterus and haemorrhage due to reduced synthesis of platelets and clotting factors. Immunosuppression and concurrent infectious disease is common with chronic exposure. Aflatoxin B1 is mutagenic and among the most carcinogenic natural substances known; it is also believed to be teratogenic.

Ergot alkaloids

Toxicity is primarily characterised by vasoconstriction and central nervous system stimulation followed by depression. The severity and extent of disease in cattle is dose-dependent, but typically begins as lameness that initiates in the forelimbs and progresses to the hindlimbs. Pain and swelling of the distal limbs, namely the pastern and fetlock joints, is also present. As disease progresses, animals lose sensation in affected areas and signs of dry gangrene secondary to vasoconstriction become apparent. Necrosis and sloughing of skin and deeper tissues can occur in any affected area, including limbs, tail tips, and pinnae.

Cattle suffering from toxicosis will become hyperthermic and develop signs of heat stress. Weight loss, tachypnoea, tachycardia, and hypersalivation are also apparent. There may be abnormal embryonic development in pregnant females. Neurologic signs such as ataxia, tremors, or hyperexcitability may occur. Sheep develop similar signs, as well as ulceration of the mouth and inflammation of the intestines. There is also a convulsive syndrome associated with ergotism in sheep. Swine develop ear and tail-tip necrosis. Pregnant sows may fail to properly develop udders, suffer from agalactia at parturition, and deliver undersized piglets. If they are not hand-fed, piglets will die from starvation. Birds may display a decrease in egg production.

Fusariotoxins

Leukoencephalomalacia, pulmonary oedema and hydrothorax, and hepatotoxicity have all been associated with fumonisin ingestion in domestic species. Laboratory studies suggest potential teratogenesis secondary to reduced folate uptake, manifesting primarily as neural tube defects. There is an epidemiologic association in humans with consumption of this toxin and oesophageal cancer.

Generally, trichothecenes induce anorexia, vomiting, hypotension, and gastrointestinal haemorrhage in many species. Many birds present with neurologic abnormalities including ataxia, poor balance, and paresis or flaccid paralysis of the neck and wings which manifests as drooping. Some trichothecenes have cytotoxic or immunosuppressive and bone marrow suppressive properties, as well. Feed refusal due to taste aversion is common, and toxicity is often self-limiting for that reason.

A report on captive whooping and sandhill cranes suggests that signs of trichothecene toxicity are vague but can include marked weight loss, diarrhoea, weakness, tongue and beak tip necrosis, recumbency, and rapid progression to death. Within 17 days, 240 of 300 animals were affected and 15 died.

Ingestion of zearalenone-contaminated feed can induce hyperoestrogenism, abortion, reduced conception rates, and reduced ovulation rates. Synergy between zearalenone and deoxynivalenol is known to induce T-cell apoptosis in a laboratory setting.

Lesions

- Many mycotoxins are immunosuppressive, therefore lesions associated with a secondary infection may be present
- Aflatoxins
 - Acute toxicity
 - Pale, swollen, and enlarged liver; haemorrhage is often present but the distribution is variable
 - Centrilobular hepatocellular necrosis and periportal proliferative inflammation
 - Bile ductule hyperplasia or fibrosis
 - Haemorrhagic inflammation of the gastrointestinal mucosa with glandular atrophy
 - Enlarged kidneys

- Multiple organs (including the subcutis, skeletal muscle, and heart) may be grossly haemorrhagic
 - Icterus
 - Chronic toxicity
 - Grossly small liver with hepatic fibrosis and multifocal regenerative nodules
- Ergot alkaloids
 - Indented lines of demarcation between healthy and gangrenous tissue on the limbs
 - Affected tissue may be cyanotic and hardened
 - Subcutaneous haemorrhage and oedema may be present on the proximal aspect of these lines
 - Tissue sloughing and ulceration
 - Pallor of exposed skin
 - Birds: disfigurement of the face, comb, and wattles
- Fusariotoxins
 - Ulceration of the skin, oral cavity, and upper gastrointestinal tract
 - Subcutaneous oedema
 - Pale and haemorrhagic skeletal muscle
 - Fumonisin
 - Interstitial pulmonary oedema and hydrothorax
 - Cyanosis
 - Liquefactive necrosis of cerebral white matter, often unilateral or asymmetrical
 - Hepatic necrosis
 - Icterus
 - Trichothecenes
 - Haemorrhage of the gastrointestinal tract
 - Abomasal ulceration
 - Papilla sloughing within the rumen
 - Contact dermatitis
 - Lymphoid tissue necrosis
 - Cranes: elevated serum uric acid with diffuse visceral and articular gout; haemorrhagic enteritis; necrosis of tongue and beak tips
 - Zearalenone
 - Enlarged mammary glands
 - Vulvar swelling
 - Vaginal or rectal prolapse
 - Oedematous uterus
 - Ovarian cysts
 - Germinal epithelial degeneration with altered sperm formation

Differential diagnoses

- Aflatoxins
 - Anticoagulant rodenticides (mild)
 - Pyrrolizidine alkaloid toxicosis
 - Other causes of acute liver injury (amatoxin ingestion, hepatitis, et cetera)
- Ergot alkaloids
 - Fescue foot
 - Summer syndrome/epidemic hyperthermia
 - Necrotising fasciitis
 - Trauma
 - Birds: avian influenza, infectious coryza
- Fusariotoxins
 - Fumonisin
 - Cardiogenic pulmonary oedema
 - Listeriosis
 - Other causes of acute liver injury (amatoxin ingestion, hepatitis, et cetera)
 - Trichothecenes
 - Avian botulism

- Gastroenteritis
 - Ruminal acidosis
 - Stachybotryotoxicosis
 - Caustic material exposure
 - Bone marrow suppression
 - Other causes of immunosuppression (stress, infection, malnutrition, et cetera)
 - Birds: other causes of uraemia (e.g., renal failure)
- Zearalenone
 - Reproductive tract infection
 - Other plant oestrogens
 - Iatrogenic oestrogen administration

Laboratory diagnosis

Samples

For isolation of toxin

- For all mycotoxins discussed, feed or gastrointestinal contents may be frozen or dried for storage and shipment to prevent fungal growth and toxin production post-collection
- Presence of toxin or sclerotia can vary significantly throughout a field or source of feed; be sure to obtain multiple representative samples for accurate assessment
- Aflatoxins
 - Liver
 - Kidney
 - Urine
 - Milk

Serological tests

- Serology is not used to detect mycotoxins

Procedures

Identification of toxin

- Aflatoxin
 - Gross and microscopic assessment of lesions
 - Measurement of aflatoxin concentrations in feed and/or gastrointestinal content via chromatography or mass spectrometry; aflatoxin M1, a metabolite of aflatoxin B1, can be identified in liver, kidney, urine, and milk in cases of significant exposure
- Fusariotoxins
 - Gross and microscopic assessment of lesions
 - Measurement of toxin concentrations in feed and/or gastrointestinal content via chromatography or mass spectrometry
 - Polymerase chain reaction (PCR) can be used to identify organisms and toxin genes
 - Due to their unique hydrophilic nature, fumonisins can be extracted with aqueous methanol or aqueous acetonitrile and quantitated via high-performance liquid chromatography (HPLC) with fluorescent detection
 - Detection of the *fum1* gene (encodes for a fumonisin precursor) via loop-mediated isothermal amplification (LAMP) assay
- Ergot alkaloids
 - Identification of sclerotia in feed

Serological tests

- Serology is not used to detect mycotoxins

PREVENTION AND CONTROL

Sanitary prophylaxis

- Routinely clean and refill bird feeders with fresh grains and seed to prevent the growth of mould.
 - Feed provided to birds should not exceed aflatoxin concentrations of 20-100 parts per billion, depending on the species and ages present.
- The World Health Organisation (WHO), in conjunction with the Food and Agriculture Organisation of the United Nations (FAO), routinely performs risk assessments for mycotoxins and have published recommendations for minimising exposure:
 - Inspect harvested foods for evidence of mould and discard products that are discoloured, shrivelled, broken, or obviously contaminated with mould.
 - Avoid damaging plants and harvested foods both before and during the drying and storage process. Monitor crop conditions for drought, insect infestation, and other signs of damage.
 - Purchase and consume foods as fresh as possible and ensure they are properly stored. Additionally, maintaining a diverse diet aids in reducing exposure potential.
- Harvesting crops early and drying them rapidly significantly reduces the risk of mould growth.
- Competitive exclusion utilising non-toxigenic isolates is an active area of research that holds promise for reducing toxigenic mould burdens in the field.
- If an agricultural field is observed to have high levels of mould or toxin in any given year, deep plowing and wildlife deterrence is recommended to reduce animal exposure.

Medical prophylaxis

- Do not consume meat from animals that have died from mycotoxicosis.
- If grain or forage is suspected to be contaminated with *Fusarium* spp., wear gloves before collecting samples to prevent contact with skin. Wear masks to prevent inhalation of fungal spores.
- Increased dietary antioxidants and free radical scavengers are believed to attenuate toxic metabolite formation and aid in metabolic clearance.

POTENTIAL IMPACTS OF DISEASE AGENT BEYOND CLINICAL ILLNESS

Risks to public health

- Aflatoxins pose a significant threat to people living in areas suffering from food scarcity and other agricultural challenges. Regulations and resources in technologically advanced countries often reduce or eliminate aflatoxin concentrations in the food supply, whereas these mechanisms are not present in countries with fewer technological resources. This can result in more frequent ingestion of contaminated food. There are data to suggest liver cancers are significantly more common in populations from these regions.
- Some mycotoxins, such as the trichothecenes, are considered potential agents of bioterrorism.

Risks to agriculture

- Crops contaminated with mycotoxins are typically destroyed or diverted into animal feed. Exposed animals may suffer from reduced growth rates, ill-thrift, reduced immune responses, and death. Additionally, animal products such as meat, eggs, and milk can contain residues of the metabolite aflatoxin M1, rendering them unsafe for human consumption and potentially creating a significant economic burden.
- Zearalenone is a common contaminant of many feeds used in aquaculture and has been shown to induce abnormal gonadal development and sex differentiation in male rainbow trout. There are data to suggest the offspring of fish exposed to zearalenone have an increased mortality risk.

REFERENCES AND OTHER INFORMATION

- Adegbeye, M. J., Reddy, P. R., Chilaka, C. A., Balogun, O. B., Elghandour, M. M. M. Y., et al. (2020). Mycotoxin toxicity and residue in animal products: Prevalence, consumer exposure, and reduction strategies - A review. *Toxicon*, 177, 96-108.
- Bennett, J. W. & Kilch, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, 16(3), 497-516.
- Cai, G., Sun, K., Xia, S., Feng, Z., Zou, H., et al. (2020). Decrease in immune function and the role of mitogen-activated protein kinase (MAPK) overactivation in apoptosis during T lymphocyte activation induced by zearalenone, deoxynivalenol, and their combinations. *Chemosphere*, 255, 126999.
- Debevere, S., Cools, A., De Baere, S., Haesaert, G., Rychlik, M., Croubels, S., & Fievez, V. (2020). *In vitro* rumen simulations show a reduced disappearance of deoxynivalenol, nivalenol and enniatin b at conditions of rumen acidosis and lower microbial activity. *Toxins*, 12, 101.
- Deng, Y., Deng, Q., Wang, Y., Sun, L., Wang, R., et al. (2020). Tolerance and bio-accumulation of aflatoxin B1 in invertebrate *Litopenaeus vannamei* and vertebrate *Oreochromis niloticus*. *Aquaculture*, 524, 735055.
- Desjardins, A. E. & Proctor, R. H. (2007). Molecular biology of *Fusarium* mycotoxins. *International Journal of Food Microbiology*, 199(1-2), 47-50.
- Fouché, T., Claassens, S., & Maboeta, M. (2020). Aflatoxins in the soil ecosystem: an overview of its occurrence, fate, effects and future perspectives. *Mycotoxin Research*, 36, 303–309.
- Friend, M., & Franson, J. C. (1999). Chapter 37: Mycotoxins. In *Field Manual of Wildlife Diseases: General Field Procedures and Diseases of Birds* (pp. 284-353). Washington, D.C.: U.S. Dept. of the Interior, U.S. Geological Survey.
- Hoerr, F. J. (2019). Ergotism in poultry. Accessed 2020: <https://www.merckvetmanual.com/poultry/mycotoxicoses/ergotism-in-poultry>
- Oberhau, D. G. & Dabbert, C. B. (2001). Aflatoxin production in supplemental feeders provided for northern bobwhite in Texas and Oklahoma. *Journal of Wildlife Diseases*, 37(3), 475-480.
- Olsen, G. H., Carpenter, J. W., Gee, G. F., Thomas, N. J., & Dein, F. J. (1995). Mycotoxin-Induced disease in captive whooping cranes (*Grus americana*) and sandhill cranes (*Grus canadensis*). *Journal of Zoo and Wildlife Medicine*, 26(4), 569-576.
- Osweiler, G. D. (2014). Overview of mycotoxicoses. *Merck Veterinary Manual*. Accessed 2020: <https://www.merckvetmanual.com/toxicology/mycotoxicoses/overview-of-mycotoxicoses#v4718289>
- Osweiler, G. D. (2014). Ergotism. *Merck Veterinary Manual*. Accessed 2020: <https://www.merckvetmanual.com/toxicology/mycotoxicoses/ergotism>
- Wigmann, E. F., Meyer, K., Cendoya, E., Maul, R., Vogel, R. F., & Niessen, L. (2020). A loop-mediated isothermal amplification (LAMP) based assay for the rapid and sensitive group-specific detection of fumonisin producing *Fusarium* spp.. *International Journal of Food Microbiology*, 325, 108627.
- World Health Organisation (2018). Mycotoxins. *WHO*. Accessed 2020: <https://www.who.int/news-room/fact-sheets/detail/mycotoxins>
- Woźny, M., Dobosz, S., Hiwa, P., Gomulka, P., Król, J., et al. (2020). Feed-borne exposure to zearalenone impairs reproduction of rainbow trout. *Aquaculture*, 528, 735522.
- Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15, 129-144.

*

* *

The OIE will periodically update the OIE Technical Disease Cards. Please send relevant new references and proposed modifications to the OIE Science Department (scientific.dept@oie.int). Last updated 2020. Written by Samantha Gieger and Erin Furmaga with assistance from the USGS National Wildlife Health Center.