Toxic Mold and Mycotoxins

Instructor: Gregory Möller, Ph.D.
University of Idaho

Learning Objectives

• Understand the relationship between mold growth, their potential mycotoxins, and disease.
• Explore the environmental conditions for mold growth.
• Understand the major species of toxic molds and their disease endpoints.
• Review the route of exposure of mycotoxins, general pharmacologic effects and clinical disease.
• Discuss some recent mycotoxin outbreaks.

Fungi and Mycotoxins

• > 100,000 species of fungi
• Mycotoxins: Substances produced by fungi that are harmful to animals and humans
• > 300 mycotoxins isolated
  – ~ 30 well-characterized and considered harmful to animals and humans (more?)

Mold Growth → Mycotoxin Production

• Substrate (plant) specific
• Environmental: (field and storage)
  – Temperature
  – Humidity
  – Moisture
  – Oxygen
• Crop damage:
  – Parasites
  – Drought
  – Pesticides

Mycotoxin Observations

• Not all moldy feeds/foods contain mycotoxins
• Not all feeds/foods containing mycotoxins are ‘toxic’
• Feed/food does not have to look moldy to be contaminated
• May not be uniformly distributed

Toxic Mold Disease Endpoints

• Allergy
  – Sensitization to mold or mold products
• Mycosis
  – Direct infection by fungi
• Irritation
  – Mechanical effects of spores, mycelial debris, VOCs
• Mycotoxicosis
  – Response to toxin (mycotoxin)
### Toxic Molds

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Allergy</th>
<th>Mycosis</th>
<th>Irritation</th>
<th>Mycotoxicosis</th>
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</thead>
<tbody>
<tr>
<td>Stachybotrys</td>
<td>+</td>
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<tr>
<td>Coccidioides</td>
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<td>Claviceps</td>
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<tr>
<td>Fusarium</td>
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<td>Aspergillus</td>
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* + reported, ± possible, - not reported
* Fung et al., Clinical Tox. 36: 79-86 (1998)

### Mycotoxins

- Resting stage secondary metabolites of fungus/mold
  - Low MW, not required for growth
  - Polyketide, amino acid, or terpene precursor
- Why? Ecological biochemistry?
  - Storage products? Competitive advantage?
- Beneficial uses
  - Antibiotics, other drugs
- Adverse effects
  - Toxic, carcinogenic

### Stachybotrys

- *S. chartarum* (aka. *atra*)
- Hay and cellulose products common substrates
- Water-damaged buildings: 'toxic' house mold

### Stachybotryotoxicosis

- Animals: clinical progression
  - Irritation of mouth, throat, and nose...
  - Shock...
  - Dermal necrosis...
  - Leukopenia...
  - Pulmonary (alveolar, bronchiolar, interstitial) inflammation and hemorrhage...
  - Nervous disorder; death

### Straw contamination

Straw contaminated with *S. chartarum* (top); clean straw (bottom). Persons handling contaminated straw can develop stachybotryotoxicosis.

Mold growth on water-damaged interior wall
Stachybotryotoxicosis

- 19th Century Russia: numerous veterinary and human epidemics
  - ATA “Alimentary Toxic Aleukia”
- 1931 Ukraine
  - Inhalation of mold from hay and contaminated bedding
- Occupational cases
  - Cottonseed oil plants
  - Grain elevators; malting plants
  - Textile mills; twine factories

1996: Employees at a German horticultural facility developed painful, inflamed lesions on their fingertips followed by scaling off of the skin after handling decomposable paper pots infested with S. chartarum

Toxins from S. chartarum

Trichoverroid trichotecenes:
- Trichodermin: $R_1 = R_2 = Ac$
- Verrol: $R_1 = OH; R_2 = H$
- Roridin L-2:
- Satratoxin H

Phoma hypotrichina

Stachybotryotoxicosis

- Humans: inhalation, dermal exposure
- Clinical progression:
  - Dermatitis
  - Inflammation of mucous membrane
  - Upper respiratory symptom
  - Fever
  - Neutropenia
  - Headache, fatigue

- Recovery (?)
- Cause of Infantile Pulmonary Hemorrhage (IPH)?
- CDC: Data insufficient to support association between S. chartarum and IPH

Coccidioides

- Coccidioides immitis

Bilateral nodular infiltrates with progressive coccidioidomycosis

Coccidioidomycosis

- First described - 1894 (California)
- Inhalation of fungal hyphae
- Most cases asymptomatic, self resolving

Coccidioidomycosis: The Americas

- San Joaquin Valley Fever
  - “Valley Fever”
- Endemic in Southwestern US
- CDC: “emerging disease”
  - Changing demographics
  - Immune compromised at risk
Coccidioidomycosis - Symptoms

- **Primary coccidioidomycosis:**
  - Acute bronchitis: fever, cough, chills, sore throat
  - Pneumonia
  - Leukocytosis
- **Clinical progression:**
  - Low grade fever
  - Anorexia, weight loss
  - Skin ulcerations - face, abdomen
  - Abscesses
  - Progressive cyanosis
  - Renal, hepatic involvement

Claviceps

- **Claviceps purpurea, paspalli**
- Grows in wet and overwintered grains: rye, barley, wheat
- Sclerotia or “ergots”
  - Hard-packed mycelium
- **“Ergotism”**
  - Gangrene and/or convulsions and gastrointestinal symptoms
  - Livestock: decreased weight gains, milk production, reproductive efficiency

Ergotamine

- Ergotamine: analogue of lysergic acid dimethylamide (LSD)
- Vasoconstrictor
- Hallucinations, gangrene
- **St. Anthony’s fire**

How Ergot Exposure Occurs

- **Claviceps purpurea – soil**
- Spores released when grain flowers
- Land on stigma – germinate – hyphae extend into the ovary
- Replaces the ovary – hardens ergot body or sclerotium; recycle

A new spin on Salem witches

By Peter H. Gott, M.D.

DEAR DR. GOTT,

Could the witches of Salem have suffered ergotism?

DEAR READER:

Historical documents indicate that 24 of the 30 witches suffered from “stag’s” that they had hallucinations and convulsions. The symptoms were similar to ergot poisoning, which is known to have caused gangrene and convulsions.

The symptoms are very similar to ergot poisoning, which is known to have caused gangrene and convulsions.

There are many other historical examples of ergotism, such as the case of the farmers who ate ergot-contaminated bread and died of convulsions and gangrene.

This has led to the theory that the Salem witch trials were caused by ergot poisoning, leading to the execution of innocent people.
**Ergot Toxins**

- Ergot alkaloids - sclerotia
  - Ergonovine
  - Ergovaline
  - Ergosine
  - Ergocristine
  - Ergotamine
    - Medicinally for vascular migraine, postpartum uterine hemorrhage in abortions
- Types and concentrations of alkaloids vary

**Ergot Pharmacological Effects**

- Vasoconstriction
  - Gangrene
- Serotonin agonist
  - Neurological effects
- Dopaminergic agonist
  - Agalactia

**Ergot Clinical Diseases**

- Vasoconstriction → gangrene (toes, fingers, ears, worse) – ‘frostbite’
  - Epidemics of gangrenous limbs / 40,000 people in France died (944 AD)
  - Pain, swelling, numbness
  - Necrotic tissue
  - Death
  - Abortion

**Ergot Clinical Diseases**

- Neurological / tremorgenic / convulsive form
  - Tingling (ants crawling under the skin)
  - Itching
  - Numbness
  - Twitching
  - Spasms
  - Seizures

- Action levels set for ergot in grains

**Fusarium**

- *F. sporotrichioides* and *graminearum*
- Corn, wheat, barley
- Veterinary and public health concerns
- Major toxins:
  - Trichothecenes, zearalenone, fumonisin

**Fusarium Mycotoxins**

- Trichothecenes, Zearalenone, Fumonisin
**Trichothecenes**

- Sesquiterpenoid tetracyclic compounds

> Have seen use as a biological warfare agent: “Yellow Rain”

**T-2 Toxin: Animal Toxicity**

- Digestive disorders:
  - Feed refusal, vomiting, bloody diarrhea, intestinal inflammation
- Hemorrhage
  - Stomach, heart, intestines, lung, bladder, kidney
- Edema
- Oral lesions
- Blood disorders
- Immunotoxic
- The oral LD$_{50}$ of T-2 toxin in animals ranges from 3 to 5 mg/kg, and the DRC is very steep.

**T-2 Toxin: Human Toxicity**

- Dermal exposure: local cutaneous necrosis and inflammation
- Oral exposure: lesions to the upper gastrointestinal tract (ATA)
  - Because of the lipophilic nature of trichothecenes, they are rapidly and completely absorbed from the GIT and quickly distributed to all major organs.
  - The mechanism by which T-2 toxin causes cell death is ribosomal binding and inhibition of protein synthesis.
- Ocular exposure: corneal injury.

**Metabolism of T-2 Toxin**

- Metabolites are less toxic than T-2

**T-2 Dermal Toxicity**

- Skin lesions on the back of a hairless guinea pig at 1 day after application of (bottom to top) 25, 50, 100, or 200 ng of T-2 toxin in 2 µL of methanol.

When human skin is exposed in vivo to small amounts of trichothecene mycotoxins, severe cutaneous irritations develop and can last 1 to 2 weeks after acute exposure.

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Wannemacher and Weiner, 1997
Alimentary Toxic Aleukia Toxicosis (ATA)

- **First stage:** Immediately or several days after consumption of grain products contaminated with trichothecene mycotoxins.
  - Inflammation of the gastric and intestinal mucosa causes vomiting, diarrhea, and abdominal pain. In most cases, excessive salivation, headache, dizziness, weakness, fatigue, and tachycardia accompany this stage, and fever and sweating may also be present.

- **Second stage:** the leukopenic or latent stage—which is characterized by leukopenia, granulopenia, and progressive lymphocytosis.
  - When the ingestion of the toxic-contaminated food is not interrupted or if large doses are consumed, the next stage develops.

- **Third stage:** Characterized by the appearance of a bright red, or dark cherry-red, petechial rash on the skin of the chest and other areas of the body.
  - At first, the petechiae are localized in small areas, but they then spread and become more numerous. In the most severe cases, extensive ulceration and gangrenous processes develop in the larynx, leading to aphonia and death by strangulation. At the same time, affected individuals have severe hemorrhagic diathesis of the nasal, oral, gastric, and intestinal mucosa.

- **Fourth stage:** The necrotic lesions heal and the body temperature falls; the recovery stage begins.
  - During this period, exposed patients are susceptible to various secondary infections, including pneumonia. Convalescence is prolonged and can last for several weeks. Usually, 2 months or more are required for the blood-forming capacity of the bone marrow to return to normal.

Zearalenone (ZEN)

- **F. graminearium and F. sporotrichiodes**
  - Corn, wheat, barley, oats, sorghum, hay
  - High humidity, low temperature
  - Autumn harvest in upper Midwest US
  - Often coincident with T-2

- **Swine (>0.1 ppm):** estrogenic effects
  - Vulvovaginitis, swollen mammae
- **Swine (50-100 ppm):** decreased reproduction
  - Cycling, conception, ovulation, implantation
- **Boars (>0.1 ppm):** feminization
  - Testicular atrophy, enlarged nipples
- **Cows:** decreased conception rates

Fumonisin

- **F. moniliforme** (universal in corn)
  - Corn, wheat, barley, oats, sorghum, hay
  - High humidity, low temperature
  - Autumn harvest in upper Midwest US
  - Often coincident with T-2
  - Horses, pigs most susceptible
  - FB1 most toxic

- **Neurotoxicity: Equine leukoencephalomalacia (ELEM)**
  - “Moldy corn toxicosis”
  - Rapid onset (few hours)
  - Feed and water refusal, lameness, ataxia, paralysis
  - Severe cerebral edema, focal malacia (softening), liquefaction of white matter

- **Pulmonary Edema: Porcine pulmonary edema syndrome (PPE)**
  - Hydrothorax and lung edema
  - Usually fatal

- **Liver cancer and liver toxicity**
Fumonisin B1: Animal Toxicity

- Horses (1-126 ppm):
  - Fatal ELEM
  - Liver toxicity at higher doses
- Swine (<1 – 5 ppm):
  - Low dose: hepatic toxicity
  - High dose: acute pulmonary edema, hepatic toxicity
- Sheep:
  - Nephritis

FDA-CVM: established action levels in animal feeds

Fumonisin Concern for Human Health

- Milk residues?
- Meat residues?

- 1996: 89% of corn grown in 3 areas of Costa Rica were contaminated with fumonisin

Fumonisin Carcinogenic Potential

- Carcinogen / promoter
- Esophageal cancer
  - South Africa, Italy, China, South Carolina
- Corn: staple, home brewed beer, moonshine, polenta

Aspergillus

- Dietary carcinogen

Aflatoxin B1

- From Aspergillus flavus
  - Universal food contaminant
  - Corn, peanuts, wheat, rice, etc.
- Animal carcinogen - 5 ppb
- FDA action level - 20 ppb

- Human liver carcinogen
  - Binds to N7 Gua; DNA-adduct
  - G → T in p53 codon 249
- Lung cancer risk
  - Respirable grain dusts > 4000 ppb AFB1
- Problem in food industry and grain handling
  - Harvesting
  - Transport
  - Storage
  - Processing
- Requires bioactivation
Cytochrome P450 (CYP) Reaction Sequence

\[
\begin{align*}
S + \text{O}_2 &\rightarrow S-OH \\
\text{Fe}^{2+} &\rightarrow \text{Fe}^{3+} \\
\text{P-450} &\rightarrow \text{flavoprotein-linked cytochrome P-450 reductase} \\
\text{FAD} &\rightarrow \text{FADH}_2 \\
\text{NADPH} &\rightarrow \text{NADP} \\
\text{G-6-PO}_4 &\rightarrow \text{Gluconate} \\
\text{G-6-PO}_4 &\rightarrow \text{G-6-PO}_4 \\
\end{align*}
\]

- Bioactivation

AFB1 Activation: Enzymology

\[
\begin{align*}
\text{AFB1} &\rightarrow \text{AFB1-8,9-epoxide} \\
\text{AFB1-8,9-epoxide} &\rightarrow \text{AFB1-8,9-dihydrodiol} \\
\text{AFB1-8,9-dihydrodiol} &\rightarrow \text{AFB1} \\
\text{AFB1} &\rightarrow \text{AFB1-N7-guanine} \\
\text{AFB1-N7-guanine} &\rightarrow \text{AFB1-GSH} \\
\text{AFB1-GSH} &\rightarrow \text{AFB1-M1} \\
\text{AFB1-M1} &\rightarrow \text{AFB1-Aflatoxicol} \\
\text{AFB1-Aflatoxicol} &\rightarrow \text{AFB1-Aflatoxin B1} \\
\end{align*}
\]

Aflatoxin: Etiological Role in Disease

- Indian childhood cirrhosis
- Hepatotoxicity
- Immunosuppression
- Kwashiorkor
- Carcinogenesis

Recent Aflatoxin Outbreaks

- Kenya: January to July, 2004
  - Outbreak of jaundice, liver failure
    - High fatality rate
  - 317 reported cases and 125 reported deaths
  - Consumption of maize – visibly discolored or moldy

Recent Aflatoxin Outbreaks

- Kenya: January to July, 2004
  - Range: 20 to 8,000 ppb
  - Widespread
  - Maize harvested when wet
  - Food shortage
  - Education needs
    - Harvesting, drying, storing