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Aspergillus, Health Implication & Recommendations for Public Health Food Safety

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Recent outbreaks of Aspergillosis in chickens on farms throughout Trinidad have left the chicken consuming population shocked and frightened. At present there exists very little published information available to the population on Aspergillosis and its effect on health and food safety. The present paper examines some of the fundamental questions associated with the pathogenesis of *Aspergillus*, health implications and recommendation for public health food safety. It is hoped through education and access to information on *Aspergillus* will serve to alleviate fears about *Aspergillus* and to empower farmers and the chicken consuming population about methods of reducing, preventing and eliminating *Aspergillus*, thereby restoring confidence through the adoption of good agriculture practices, safe food handling practices, good sanitation practices and good hygienic practices when rearing, handling, processing, preparing, storing and transporting poultry.

Key words: *Aspergillus*, Aspergillosis, food safety, health implication

1. Introduction

Several recent outbreaks of Aspergillosis in Cumuto and Valencia farms in Trinidad, resulted in more than 6,000 chickens being culled, have left the chicken industry, processors and chicken consuming public fearful and dismayed. This may have resulted in decreased chicken sales and consumption at food service establishments throughout Trinidad and Tobago. Since poultry is one of the most frequently consumed meats in Trinidad the necessary steps and preventative mechanisms must be put in place to deal in a timely manner with any further and future outbreaks of Aspergillosis in Trinidad. The purpose of the present paper is to review past and current information on Aspergillosis and to provide recommendations on ways how to reduce, prevent or eliminate Aspergillosis and to restore confidence in chicken consuming public and the general public both locally, regionally and internationally.

2. What is Aspergillosis?

Aspergillosis is filamentous, increasingly common ubiquitous fungal infection of birds and occasionally other animals including man. It most frequently occurs when birds are exposed to large numbers of *Aspergillus* fungal elements through the respiratory tract. The genus *Aspergillus* includes over 185 species. Around 20 species have so far been reported as causative agents of opportunistic infections in man. Among these, *Aspergillus fumigatus* is the most commonly isolated species, followed by *Aspergillus flavus* and *Aspergillus niger*. *Aspergillus clavatus*, *Aspergillus glaucus* group, *Aspergillus nidulans*, *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus ustus* and *Aspergillus versicolor* are among the other species less commonly isolated as opportunistic pathogens. The fungus grows quite readily on ordinary laboratory culture media at room temperature, at 37 ^{o} C, and higher. Czapek's solution agar or Sabouraud's agar may be used. The colonies are green to bluish green at first and darken with age so as to appear almost black. The colonies vary from velvety to floccose.

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3. Where is *Aspergillus* found?

Aspergillus spores are commonly found in air, water, soil, plant debris, rotten vegetation, manure, sawdust litter, bagasse litter, animal feed, on animals and indoor air environment.

4. What is unique about *Aspergillus*?

Aspergillus may be considered unique among the fungi, since it can be readily identified using its gross macroscopic and microscopic characteristics. Some of *Aspergillus* noticeable macroscopic features include its growth rate, color of the colony and tolerance to temperature or thermotolerance.

5. Macroscopic features

Variations in growth rate can be used in the identification of *Aspergillus* species. *Aspergillus nidulans* and *Aspergillus glaucus*, grows the fastest among the molds. *Aspergillus nidulans* and *Aspergillus glaucus* grow slowly and may reach a colony size of 0.5-1 cm following incubation at 25°C for 7 days on Czapek-Dox agar. The remaining *Aspergillus* species grow at varying rates depending on the medium, reaching a diameter of 1-9 cm. *Aspergillus fumigatus* is unique among the species in that it can withstand temperatures 20 to 50 °C.

Aspergillus colonies have different colors which can be used in their identification. Some species of specific colors are given in Table 1.

Table 1. The color of the colony in various *Aspergillus* species

Species	Surface Color
A. clavatus	Blue-green
A. flavus	Yellow-green
A. fumigatus	Blue-green to gray
A. glaucus group	Green with yellow areas
A. nidulans	Green, buff to yellow
A. niger	Black
A. terreus	Cinnamon to brown
A. versicolor	White at the beginning turns to yellow, tan, pale green or pink

6. Microscopic features

The basic microscopic morphology is same for all species. However, some other microscopic structures are unique to certain species and constitute the key features for species identification together with the surface color of the colony (Table 2).

Table 2. Microscopic features of various *Aspergillus* species

Species	Characteristics of structures supporting asexual spores	Shape of vesicles
A. clavatus	Long, smooth	Clavate shape
A. flavus	Colurless, round	Round, radiate
A. fumigatus	Short, smooth, colourless, greenish	Round, columnar head
A. glaucus group	Variable length, smooth, colourless	Round, radiate to very loosely columnar head
A. nidulans	Short, smooth, brown	Round, columnar head
A. terreus	Short, smooth, colourless	Round, compactly, colourless head
A. versicolour	Long, smooth, colourless	Round, loosely, radiate head
A. niger	Long, smooth, colourless or brown	Round, radiate

7. What are the signs of *Aspergillus* infection?

Signs are physical manifestation of a disease condition. Common signs associated with Aspergillosis infection include difficulty with breathing in which forced or labored breathing may occur. There may be increased thirst, fever, diarrhea blindness and inflammation of the brain and membranes surrounding the brain may occur in the later stages resulting in increased morbidity and mortality.

8 Gross Lesions

Gross lesions may appear in the air passages and bronchi of chickens. Lesions vary in size from pinhead or miller seed up to the size of a pea and these nodules may appear in the thoracic and abdominal cavities of chickens, ducks, geese and pigeons. Nodules may also appear in the cells of the liver that may affect its cellular functions.

9. What are the health implications of *Aspergillus*?

Aspergillus spp. can cause disease in birds and man. Three different disease states are observed in man:

(i) Infections that can arise from the weakening effects of aspergillosis e.g. colonization of lung cavities due to tuberculosis, neoplasms or new growths in lungs and kidneys. Almost any organ or system in the human body may be involved. Onychomycosis, sinusitis, cerebral aspergillosis, meningitis, endocarditis, myocarditis, pulmonary aspergillosis, osteomyelitis, otomycosis, endophthalmitis, cutaneous aspergillosis, hepatosplenic aspergillosis, as well as *Aspergillus fungemia*, and disseminated aspergillosis may develop. Nosocomial occurrence of aspergillosis due to catheters and other devices is also likely. Construction in hospital environments constitutes a major risk for development of aspergillosis particularly in neutropenic patients.

(ii) Allergic reactions to *Aspergillus* spp. e.g. allergic bronchopulmonary aspergillosis.

(iii) Toxic reactions occur as a result of toxins produced by Aspergillus spp. e.g. mycotoxins such as aflatoxin which are carcinogenic and may induce hepatocellular carcinoma or liver cancer.

10. What current trends exist for the incidence and distribution of *Aspergillus*?

The incidence of Aspergillosis in USA is 1-2 per 100,000 per year. The incidence rate is approximately 1 in 100,000 or 2,720 people in USA. Incidence extrapolations for USA for Aspergillosis are 2,720 per year, 226 per month, 52 per week, 7 per day, 0 per hour, 0 per minute, 0 per second. In Trinidad the incidence of Aspergillosis is underreported and there exists very little documented information on its incidence and prevalence.

11. What drugs can be used to treat Aspergillosis?

The efficacy of treatment depends on the timeliness in diagnosing the disease condition, state of health of the individual, the potency of the drug used to prevent, reduce, eliminate or better manage Aspergillosis. In vitro testing showed that administering antibiotics; amphotericin B, itraconazole or voriconazole to patients are able to reduce the effects of *Aspergillus* spp. However, some *Aspergillus* spp. are resistant to a few of these antibiotics for example *Aspergillus fumigatus* is resistant to itraconzole.

The use of novel antifungal agents, such as the echinocandins has shown some promising results in vivo and vitro against *Aspergillus*. Some positive benefits have been derived by the use of antibiotic-antifungal

(amphotericin B-echinocandins) combinations against *Aspergillus* in animal models.

Another promising therapeutic intervention against *Aspergillus* is the use of novel azoles (e.g., voriconazole, posaconazole, or ravuconazole), glucan synthesis inhibitors (e.g., caspofungin, V-echinocandin, FK463) and liposomal nystatin.

12. How can aspergillosis be diagnosed?

Aspergillosis can be diagnosed by observing the symptoms and signs demonstrated by suspected birds and animals. Confirmation of diagnosis can be done by cultural methods in the laboratory. Further confirmation can be done at necropsy or examination of birds or animals at death and noting visible masses of fungus that are visible to the naked eye particularly in the lungs, air sacs, thoracic and abdominal cavities.

13. Who are susceptible to *Aspergillus* infection?

(i) Chicken handlers. Those people who are involved in cleaning pens, feeding, transporting and taking care of chickens on the farm.
(ii) People who live on farms or close to farms. Since the spores can be easily carried in the air and inhaled by potential victims.
(iii) People who handle chicken and who do not exercise proper hygienic practices can transfer spores on their person to other susceptible individuals.
(iv) People with weakened immune systems, aged, pregnant, infants and young children are more susceptible to infection by *Aspergillus*.
(v) Animals that share feeding and watering utensils.

14. When Aspergillus is considered significant?

Aspergillus under suitable conditions produce spores that are ubiquitous in low numbers throughout the environment. The spores, similar to seeds of a plant, are very resilient to dryness and heat. Under ideal conditions, these spores can germinate into the actual plant form of the fungus and proliferate and produce more spores. In order for the fungus to grow, three key factors are needed: (1) substrate, otherwise known as food to support growth, (2) moisture and (3) favorable temperatures.

Fungi can grow on a variety of organic substances that favor their incidence and distribution. In the bird's environment, this can include bedding, manure, feed, on eggs, or even in eggs. Warm temperatures are ideal for mold growth, especially in the egg incubators or the brooder house. High humidity and wet conditions favor the proliferation of the plant forms. However, when dry conditions prevail, the spore forms predominate.

While *Aspergillus* can grow in the feed or litter, the real danger to the birds and even man is when the fungi begin to produce lots of spores. Dry, fine, dusty litter or feed will readily transport the spores directly into the upper and lower respiratory tract of birds, man and other animals. The conditions present in the nasal passages, eyes, trachea, lungs, and airsacs of the birds, man and animals are ideal for the fungus to grow. The result is a severe inflammation in these organs and death may result from suffocation.

15. What toxins are produced by *Aspergillus*?

Toxins are poisonous chemicals which are produced by *Aspegillus* spp. Several studies have revealed that toxins produced by *Aspergillus* can cause from minor skin irritations to severe destruction of body cells such as nerve and blood cells leading to convulsions, paralytic symptoms and finally death.

Toxins produced by *Aspergillus* are highly substituted coumarins and at least 18 closely related toxins are known. The toxicity of the six most potent aflatoxins in decreasing order are: B1>M1>G1>B2>M2>G2. These aflatoxins fluoresce under ultraviolet light and give the following characteristic colors:

(i) B1 & B2-blue
(ii) G1-green
(iii) G2-green-blue
(iv) M1-blue-violet
(v) M2-violet

The two main factors affecting aflatoxin formation are moisture and temperature. The optimum temperature for toxin production has been found to be between 24 to 28 ºC. The limiting moisture content for B1 and B2 on corn was 17.5 % at a temperature of 24 ºC. Overall, aflatoxin production has been observed over the water activity range of 0.93 to 0.98. The limiting water activity range has been found to be between 0.71 and 0.94.

16. What levels of aflatoxins are considered safe in foods?

Since *Aspergillus* is ubiquitous in low levels in the environment, the USFDA has allowed a maximum level of 20 ppb for aflatoxins in food, feed, nuts, peanuts, peanut products and pistachio nuts and 0.5 ppb for milk. The Codex Alimentarius has provided even more stringent guidelines for aflatoxins in food.

- 15 µg/kg aflatoxin in peanuts for further processing
- 0.05 µg/kg aflatoxin M1 in milk
- 50 µg/kg of patulin in apple juice
- 5 µg/kg of ochratoxin A in cereals and cereal products

17. How can *Aspergillus* be controlled or prevented?

As with most diseases, but especially with Aspergillosis, prevention is far more economically sound than attempting to manage the disease when it strikes. The following points are provided to help better manage Aspergillosis to prevent economic losses and human suffering.

a. In the hatchery

(i) Ensure proper cleaning and sanitation of incubators, hatchers and air duct systems.

(ii) Reject heavily soiled eggs.
(iii) Ensure proper temperature and humidity of egg storage rooms.
(iv) Elimination of cracked eggs.
(v) Special care need to be taken especially if eggs are injected with vaccines or antibiotics since these puncture holes can serve as easy access sites for *Aspergillus* to enter and infect eggs and chicks.
(vi) Develop and implement documented hatchery preventative programs.

b. On the farm
(i) Remove moldy litter.
(ii) Clean and sanitize pens and animal holding areas thoroughly.
(iii) Carefully select feed for animals ensuring that feed is not moldy. Do not overbuy feed or store feed under unsanitary conditions that would favor mold growth.
(iv) Carefully select litter as this may provide an ideal harborage for *Aspergillus*.
(v) Clean and sanitize utensils used in collecting milk from cows.
(vi) Clean and sanitize areas around feed hoppers and watering containers.
(vii) Design floors in pens and animal holding areas so they are sloped or graded with drain holes to remove any standing water.
(viii) Use copper sulphate solution diluted 1 part in 2,000 parts water and place in all drinking water. Hamycin at a concentration of 20mg per ml in the drinking water may also be used.
(ix) Remove flooded litter immediately as this would give off large amounts of spores when dried.
(x) Design pens and farms to ensure proper ventilation by removing warm moisture laden air to the outside.
(xi) Quarantine infected birds or animals. Remove uninfected birds and animals to a safe area to prevent further contamination.
(xii) Use a mold inhibitor in the feed for suspected outbreaks.
(xiii) Administer antibiotics as recommended by a competent authority or person.
(xiv) Cull infected birds or animals to prevent further contamination and possible public health hazards.

18. How is *Aspergillus* transmitted to man and animal?

Aspergillus may be transmitted to animals in the following ways:
(i) Air can transport *Aspergillus* spores to considerable distances where they may be inhaled by susceptible birds and animals resulting in infection of these animals.
(ii) Water can also transport spores and can infect animals that share water containers.
(iii) Soil may contain high levels of *Aspergillus* spores that may contaminate birds and animals. Plant matter such as baggasse can contain high levels of *Aspergillus* spores which can serve to infect birds and animals.
(iv) Other animals. Wild birds and other animals may infect domesticated birds and animals with *Apergillus* spp when they come into contact with them.

19. *Aspergillus* may be transmitted to man in the following ways:
(i) Handling infected birds and animals.
(ii) Inhaling spores from infected feed, litter, birds, animals and surrounding environs.
(iii) Poor sanitation and hygienic conditions.
(iv) Eating undercooked contaminated poultry or animals.
(v) Using contaminated surface water to prepare food.
(vi) No human-to-human transmission has been reported.

20. How does *Aspergillus* affect public health food safety?

Can the mycotoxins produced by *Aspergillus* pose a public health hazard, if food is not cooked thoroughly?

Most mycotoxins are not broken down or destroyed by cooking temperatures and there is no safe way to salvage grain or food that has molded. Grains or food that has been contaminated with mycotoxins should be discarded safety and not fed to animals and pets.

21. What conditions may have caused the outbreak of Aspergillosis in Trinidad?
(i) Unsanitary conditions at farms.
(ii) Dirty litter, drinking and feeding utensils.
(iii) Unsanitary conditions at hatchery.
(iv) Poor hygienic practices by handlers at farms.
(v) Failure to detect early warning signs.
(vi) Failure to report unexpected death of chicks at farms to relevant authorities in a timely manner.
(vii) Delays in confirmation of Aspergillosis by laboratories.
(viii) Delays in culling affected chicks.

Conclusions and recommendation
(i) Adopt and implement sanitation standard operating procedures on farms and areas where animals are reared for human consumption.
(ii) Farmed animals including poultry should be placed in a controlled environment where no contact with outside or wild animals should be allowed so as to minimize the spread of *Aspergillus*.
(iii) Adopt good agricultural practices in the rearing, handling, processing, transport of animals and their products for human consumption.
(iv) Adopt and develop regular surveillance of animals on farms to detect early outbreaks of Aspergillosis by relevant authorities.
(v) Develop necessary laboratory services for the early detection of *Aspergillus*.

(vi) Poultry and other farm animals should be healthy at the time of slaughter and this should be reflected in the pre- and post mortem certificates of health.
(vii) Diseased animals should not be allowed to enter the food chain and reach the consumer.
(viii) All outbreaks of Aspergillosis should be monitored carefully and affected farms quarantined to prevent the spread of the disease.
(ix) Animals in an advanced disease states should be culled and disposed of safely to minimize the spread of *Aspergillus*.
(x) All incoming feed, litter and water should be inspected thoroughly to ensure that they are free from *Aspergillus*. Any signs of mold activity or presence in feed, litter or water should be refused and discarded appropriately.
(xi) Litter must be carefully selected and changed on a regular basis.
(xii) Animals should be slaughtered and stored under sanitary conditions at the appropriate temperatures.
(xiii) Poultry and other meats should be cooked thoroughly at an internal temperature of: Poultry-83 ºC, Beef-77 ºC, Pork-77 ºC, and Lamb-75 ºC.
(xiv) Poultry and meats should be bought only from reputable sources.
(xv) Animals infected with Aspergillosis should be either treated with the necessary antibiotics, antifungal agents, or chemicals under the supervision of a veterinarian.
(xvi) Water sources given to animals should be chlorinated.
(xvii) Watering and feeding utensils should be cleaned and sanitized regularly.
(xviii) It should be noted that Aspergillosis is difficult to treat and prevention is far better than cure and the necessary proactive steps should be adopted as described herein to prevent, reduce and/or eliminate Aspergillosis.

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