ASPERGILLOSIS

ORIGINALLY PREPARED AS ASPERGILLOSIS - DACTYLARIOSIS IN 1978 BY:

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INTRODUCTION

Aspergillosis is the most common respiratory mycotic disease of poultry. Aspergillosis is defined as any disease condition caused by a member of the fungal genus *Aspergillus*. Avian aspergillosis most often occurs in a pulmonary form, and hence the synonyms brooder pneumonia and mycotic pneumonia often appear in the literature. *A. fumigatus* was first reported in the lungs of a bustard (*Otis taradaga*) in 1863. The species name was attributed to Fresenius, who applied the term "Aspergillosis" to this respiratory disease.

Aspergillosis occurs frequently in turkeys and chickens, all species of birds are probably susceptible. Aspergillosis occurs in both acute and chronic forms in poultry. Acute aspergillosis is usually characterized by outbreaks in young birds with high morbidity and high mortality. Chronic disease, which occurs in adult birds, is less common but economically important. Outbreaks occur when the organism is present in sufficient quantities to establish disease or when the bird's resistance is impaired by factors such as environmental stress, immunosuppression from concomitant diseases or inadequate nutrition.

ETIOLOGY

Aspergillus fumigatus is the most common etiologic agent of aspergillosis, but *A. flavus* and *A. niger* are also isolated in some cases. These organisms are ubiquitous, commonly occurring in decaying vegetative matter, soil, feed grain, and animal litter. The organism grows well on most common laboratory media, however Sabouraud's dextrose or potato dextrose agar are more selective. All *Aspergillus* species grow well at 25-37 C, however *A. fumigatus* also grows well at temperatures as high as 45 C.

EPIZOOTIOLOGY

Aspergillus spp. can penetrate egg shells and thus infect the embryo. Infected embryos may die or hatch with well developed lesions. If infected eggs break, large numbers of spores are released which contaminate the hatchery environment. Contamination of the hatcher or air system in hatcheries is usually responsible for outbreaks of aspergillosis in very young poults and chicks. Contaminated poultry liter is often the source of *Aspergillus spp.* in older birds. Respiratory infection usually follows inhalation of large numbers of spores from heavily contaminated feed, litter or environment. Conjunctival infections may occur from heavy exposure to airborne spores following traumatic injuries. Infections in the brain, posterior chamber of the eye or other visceral tissues result from systemic invasion from the respiratory tract. It is believed that healthy birds resist infection, but that resistance can be overwhelmed by massive exposure or impaired host defenses.

CLINICAL SIGNS AND LESIONS

Respiratory infections with *Aspergillus spp.* produce dyspnea, gasping ("gaping"), cyanosis and accelerated breathing. Gross lesions of the air sacs and lungs vary from small plaques to nodules from 1 mm to 7 mm in size that are white to yellow. Plaques may also be found at the level of the syrinx or adjacent to large bronchi in the lung. Occasionally, larger, thickened plaques with greenish fur-like growth of mold are found in the air sacs or on the coelom walls. Microscopically, infected lung tissues have lesions of focal pneumonia, multiple areas of necrosis and granulomas. Lesions in the air sac and on the inner surface of the thorax may show mycelia producing conidiophores with conidia.

Eye infections are usually unilateral and begin with lacrimation, followed by a conjunctivitis that may become chronic and are characterized by a yellow, cheesy plaque beneath the nictitating membrane. The vitreous humour and cornea may be involved.

Infection of the brain usually produces encephalitis with ataxia, incoordination, and/or torticollis. Gross brain lesions are usually circumscribed areas that range in color from white to yellow. In infected brain sections, the mycelia generally spread from individual foci into the surrounding tissue and show characteristic dichotomous branching. Mycelia are best demonstrated with fungal stains.

DIFFERENTIAL DIAGNOSIS

Aspergillosis must be differentiated from other respiratory and mycotic diseases. *Dactylaria gallopava* is the second most common respiratory mycotic disease of poultry. It may cause a brooder pneumonia-like condition with formation of small cream colored nodules in the lungs during the first two weeks of life. Like *Aspergillus spp.* the organism often spreads to the brain causing encephalitis and to the eye causing ophthalmitis. Clinical signs and lesions of dactylariosis resemble those of encephalitis. Lesions in the brain range from diffuse infection involving an entire lobe to abscesses confined to specific areas. The color of these lesions vary from gray-brown to reddish-tan. *Aspergillus spp.* and *D. gallopava* must be differentiated by culture or histopathology. *D. gallopava* grows of Sabouraud's dextrose as a brownish, velvet-like colony that causes the surrounding media to turn red to brown. *Aspergillus fumigatus* grows as a green colony with white margins. Microscopically, the mycelia of *D. gallopava* are smaller in diameter than those of *Aspergillus spp.* and do not exhibit dichotomous branching. Numerous giant cells are characteristic of microscopic brain lesions caused by *D. gallopava*.

Phycomycosis, caused by species of *Rhizopus, Absidia* and *Mucor* are rare in the respiratory tract of birds but may cause nodules within the lungs which grossly resemble those caused by *A. fumigatus* or *D. gallopava*.

Penicillium cultures look very similar to *A. fumigatus* in gross examination. *A. fumigatus* and *Penicillium* may be differentiated based on microscopic examination of a wet mount or tape mount of the spore producing bodies.

PREVENTION, CONTROL AND TREATMENT

Aspergillosis in young chicks and poults can be controlled though breeder management and hatchery sanitation. Eggs which are cracked or of poor shell quality should not be set. Thoroughly clean, disinfect and fumigate egg storage areas, incubators and hatchers. Clean, disinfect and dry all egg flats, racks and baskets (trays) between each use. Monitor hatchery environment for mold contamination.

Use only dry, clean litter and mold-free feeds. Store feeds and litter properly to inhibit growth of mold. Keep humidity in mid-range, the fungus multiplies during the wet period producing abundant spores which then become aerosolized when they become dry. Mold inhibitors are available as feed additives, as well as for disinfection and fumigation. Good cleaning to remove organic debris prior to disinfection is the most important factor in preventing the disease.

Treatment is usually not worthwhile due to expense.

SLIDES

1. Eggs contaminated by *Aspergillus spp.* during incubation.

2. Young chicks showing clinical signs of aspergillosis (brooder pneumonia); gasping or "gaping".

3. Adult turkeys showing clinical signs of aspergillosis; respiratory difficulty and cyanosis.

4. Chickens with the clinical signs of dactylariosis. Lateral recumbency, circling and tremors are commonly observed. Similar signs are found in birds with aspergillosis when the brain is involved.

5. Neurogenic torticollis in a turkey poult caused by *Dactylaria*. Similar signs are found in birds with aspergillosis when the brain is involved.

6. Bird with yellow to white mycotic nodules in the lung.

7. Bird, fulminant *Aspergillus sp.* infection, nodules in lungs and air sacs.

8. Fruiting *Aspergillus sp.* conidia in the body cavity of a bird.

9. Turkey, chronic mycotic panophthalmitis. Globe is shrunken, lack of clarity to cornea, posterior chamber is filled with exudate and necrotic tissue. Iris may also be involved.

10. Turkey, mycotic encephalitis. Gross lesion, circumscribed area with white to yellow discoloration in cerebrum.

11. Turkey, dactylariosis. Gross lesion, circumscribed area with white to yellow discoloration in cerebellum.

12. (Hematoxylin-eosin stain, X 10) Sagital section of brain from slide #11.

13. (Hematoxylin-eosin stain, X 160) Same lesion as in slide #11. Granulomatous encephalitis. Focal accumulation of multinucleated giant cells and macrophages in random arrangement. Hyphae do not exhibit dichotomous branching and are much thinner than those of *Aspergillus sp.*

14. (Periodic acid-Schiff stain, X 100) Same lesion as in slide #11. Organisms are darker red than surrounding tissue, note organisms within multinucleated giant cells.

15. (Periodic acid-Schiff stain, X 80) Bird, phycomycosis. Granuloma marked by accumulation of multinucleated giant cells and macrophages in random arrangement. Hyphae are wider than *Dactylaria* and irregular in shape.

16. (Hematoxylin-eosin stain, X 70) Chronic lung lesion of aspergillosis, nodules are characterized by centers of eosinophilic cellular debris surrounded by a zone of multinucleated giant cells and epitheloid macrophages which in turn are surrounded by fibrous tissue and lymphocytes. *Aspergillus sp.* mycelia may be visible in necrotic centers in hematoxylin and eosin stained sections either as unstained or faintly basophilic staining mycelia.

SLIDES (CONTINUED)

17. (Gomori methenamine silver stain, X 70) Chronic lung lesion of aspergillosis, same lesion as in slide 16. Mycelia are better demonstrated with fungal stains as shown here. Mycelia are uniform in width (3-4 μm), septate, and branch dichotomously.

18. (Methenamine silver stain, X 500) Brain section from a two-week-old turkey poult with natural infection of aspergillosis showing the dichotomous branching and uniform width of mycelia.

19. (Methenamine silver stain, X 500) Brain section from a two-week-old chicken with natural infection caused by *D. gallopava*. Mycelial elements randomly present throughout the cortical tissue.

20. *A. fumigatus* on Sabouraud's dextrose media, three to five days of incubation. Typical colonies of *A. fumigatus* are blue green with a white edge.

21. *A. fumigatus* on Sabouraud's dextrose media, after five to ten days of incubation. The center is blue-green with a white edge. Upper left shows a mature colony, bottom right shows the colorless underside.

22. *A. flavus* on Sabouraud's dextrose media, colonies are yellow-green to brown.

23. *Aspergillus sp.* on Sabouraud's dextrose media, the colony shown here is typical of the dark colonies of *A. niger*, however all *Aspergillus sp.* colonies become flat and darken with age.

24. *D. gallopava* on Sabouraud's dextrose media. Brownish, velvet-like colony that causes the surrounding media to turn a red to brown color.

25. Other blue-green molds, such as *Penicillium* shown here, look very similar to *Aspergillus sp.* and must be differentiated microscopically.

26. (Methylene blue stain, X 274) *Aspergillus sp.*, small one-celled spores (conidia) irradiating out from swollen base (vesicle, see arrows). Mycelia are hyaline (transparent).

27. (Methylene blue stain, X 300) *Aspergillus sp.* characteristic conidiophore with flask shaped vesicle. Variation in form within *Aspergillus spp.* occur, therefore a mycologist should be consulted for identification of species.

28. (Methylene blue stain, X 500) *D. gallopava*, mycelium and characteristic spores.

29. (Methylene blue stain, X 349) *Penicillium*, small round spores borne in brush-like formations.

REFERENCES FOR FURTHER READING

Chute, H.L. and J.L. Richard. 1991. Fungal Infections. In Diseases of Poultry, 9th ed., edited by B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Yoder, Jr. pp. 326-339.

Dyar, P.M., O.J. Fletcher, and R.K. Page. 1984. Aspergillosis in turkeys associated with use of contaminated Litter. Avian Disease 28:250-255.

Eggert, M.J., and J.V. Barnhart. 1953. A case of egg-borne aspergillosis. Journal of the American Veterinary Medical Association 122:225.

Gauger, H.C. 1941. *Aspergillus fumigatus* infection in adult chickens. Poultry Science 20: 445-446.

Harry, E.G., and D.M. Cooper. 1970. The treatment of hatching eggs for the control of egg transmitted aspergillosis. British Poultry Science 11:269-272.

Peden, W.M., and K.R. Rhoades. 1992. Pathogenicity differences of multiple isolates of *Aspergillus fumigatus* in turkeys. Avian Diseases 36:537-542.

Ranck, F.M., Jr., L.K. George and D. H. Wallace. 1974. Dactylariosis: A newly recognized fungus disease of chickens. Avian Diseases 18:4-20.

Richard, J.L. and E.S. Beneke. 1989. Mycoses and Mycotoxicoses. In A Laboratory Manual for The Identification and Isolation of Avian Pathogens. 3rd ed. edited by H.G. Purchase, L.H. Arp, C.H. Domermuth and J.E. Pearson. pp. 70-76.

Richard, J.L., J.R. Thurston, W.M. Peden, and C. Pinello. 1984. Recent studies on aspergillosis in turkey poults. Mycopathologia 87:3-11.

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