ASPERGILLOSIS IN FOWLS

By R. P. Asthana, M.Sc., D.I.C., Ph.D. (London)
(Mycologist to Government, C. P. and Berar, Nagpur)

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For the first time in this Province a serious and fatal outbreak of Aspergillosis in fowls has been encountered with during 1943. The disease was first brought to the notice of the author by Mr. G. D. Pani, Poultry Investigation Officer, and a systematic study of the disease has been taken up.

A. C. Mayer first observed the disease in 1815 in the bronchi and air-sacs of jay. Henry Grey has also mentioned that the disease is common in Great Britain. Thom and Church report that *Aspergillus fumigatus* caused death in birds in zoological gardens in different countries. Since then several other writers have mentioned cases of affection in different wild species of birds and domestic poultry. However, as far as known to the author this disease has not so far been reported from anywhere else in this country.

The outbreak of Aspergillosis was first detected in some fowls at Dhamtari during the end of May 1943. Severe and fatal outbreaks of the disease were later on reported from Government Poultry Farm, Telankheri, Nagpur, in chickens of 4 to 6 months old and practically all the young birds died during June, July and August. The disease was again reported in an epidemic form from Raipur, Khandwa, Chhindwara, Seoni and several other places in the Province. It was also observed that Rhode Island Red were more susceptable to the disease than Deshi or Black Minorca; White Leghorns being the most resistant of all the breeds. Mortality in chickens was very much higher than in adults.

During the course of investigation it was observed that in severe cases of infection the birds die overnight though apparently healthy the previous day. In less acute case the sick birds became dull and droopy with rise of body temperature, preferred darkness to light, lost appetite, and developed paralysis of the legs and wings. The birds exhibit frequent sneezing and coughing with rattle in the throat and gasp for breath. There is slimy discharge from nose and beak; the eyelids swell and have cheesy deposits. In later stages of the disease the bird develops diarrhœa and the feathers

at the vent are soiled. Such birds waste in condition and generally die within a week. In other less severe cases of infection the birds develop the above symptoms gradually and die within three to four weeks due to exhaustion.

The other common features which were observed in the diseased birds were paleness of body flesh, greyish necrotic growths and lumps of cheesy material in the lungs and air-sacs. In some cases bluish-green necrotic patches were found in the lungs while in others blood clots were noted when cut open, though appeared normal externally. Trachea and bronchi may show hæmorrhage along with cheesy deposits. The lumen of infected œsophagus was studded with greyish nodules resembling vitamin 'A' deffi-ciency lesions. The proventriculus may show hæmorrhage patches along with bluish-green growth at the junction of the proventriculus to the extension of the crop. The infected intestines were thickened. It was common to find both the cæcal pouches filled with cheesy puss with hæmor-rhage of mucus membrane. Cheesy deposits were prominent all over the abdominal cavity when peritonium was infected. Kidneys, ovaries and testes when infected were considerably enlarged and showed greyish or bluish-green deposits. Congestion of heart and hæmorrhages in bone-marrow and brain was of common occurrence.

Under sterile conditions lesions from all the infected organs were cultured on broth, rice-mesh agar and Sabouraud's media and practically in every case pure cultures of *Aspergillus sp.* were obtained. In several cases heart and cutaneous blood, when cultured on starch agar, gave a luxuriant growth of *Aspergillus sp.* within ten to twenty days. The fungus when cultured on slightly acidic medium is bluish-green in colour but it forms brown colonies on alkaline medium.

To prove the pathogenicity pure cultures of the isolated *Aspergillus sp.* were obtained and the disease was artificially transmitted to the healthy fowls in the following three ways: (1) spores were mixed with grain and the birds were fed on them; (2) spores were insuflated into the nostrils of healthy birds; (3) sub-cutaneous injections were carried out on the birds by a spore suspension in sterile distilled water. In the first two cases the experimental birds died on the 26th and 28th day respectively while by the third method death took place on the 11th day. Typical Aspergillosis symptoms were observed in all the experimental birds and on reisolation the same species of *Aspergillus* was obtained from the lesions.

A general study of the pathogen was taken up. The fungal colonies on rice-mesh agar are velvety to felted floccose, spreading, yellowish-green

to dark green to almost dark-brown in age. Reverse and substratum are colourless to yellow and occasionally reddish-brown to red in old cultures. The vegetative hyphæ are septate, hyaline and measure 2·8-3·2 μ in breadth (varying from 2·4-3·5 μ). Conidiophores are short, septate, densely crowded, light green in colour specially above, arise either from submerged or aerial hyphæ and enlarge towards the top into a flask-shaped vesicle. They measure 4·9–6·3 μ in breadth (varying from 4·2–6·6 μ) at their source, 9·1–9·8 μ (varying from 7–10·5 μ) in the middle and 11·2–12·6 μ (varying from 9·1–13·3 μ) at the base of the vesicle. The length of mature conidiophore is 329–350 μ (varying from 215·5–350 μ). The vesicle is smooth, green in colour, usually fertile only on the upper half and measure 18·9–21·7 μ in diameter (varying from 18·9–27·3 μ). All over the round part of the vesicle closely packed green coloured sterigmata are arranged in one series, run approximately parallel to the axis of conidiophores, bear conidia in chains and measure 6·3–7·7 $\mu \times$ 2·4–2·8 μ (varying from 5·6–8·4 $\mu \times$ 2·1–2·8 μ). Single conidium is faint green in colour but in mass dark-green. They are smooth, spherical and measure 2·8–3·2 μ in diameter (varying from 2·4–3·5 μ). The older conidia have thick and roughened wall (Fig. 1).

This species of *Aspergillus* is very much similar to *Aspergillus fumigatus var. alpha.* Sion and Alexandresen in its cultural and pathogenic behavior. Dodge[1] and Thom and Church[2] have mentioned that this species of *Aspergillus* apparently cause severe epizootics in birds, less fatal in man, not reaching in epidemic proportions and pathogenic to laboratory animals. During the course of study it was observed that this species of *Aspergillus* is pathogenic to human beings, goats and rats as well though not to the same severity as in fowls. Mr. G. D. Pani while conducting certain feeding experiments with this pathogen accidentally got infected on his left cheek where probably there was a small cut due to shaving. An abscess developed that place with severe pain in back, feverishness, pain in the left cheek, at nausea and sleeplessness. The puss from his abscess was examined by the author and it was found full of spores which on culturing and examination proved to be that of *Aspergillus fumigatus.* Mr. Pani improved by tincture-iodine injections but subsequently had to proceed to the Tropical School of Medicine, Calcutta, for proper treatment.

The fungus thrives best during warm humid climates of this Province. Long periods of atomospheric humidity causes the fungus to thrive well and mostly during such periods epidemic of Aspergillosis is exhibited in fowls. Samples of poultry feed, *viz.*, grain and mash, from Government Poultry Farm, Telankheri, were examined for fungoid growth. On culturing

and examination the specimens were found infected with *Aspergillus fumigatus*. The spores of the pathogen are abundantly present in dry grass, damp soil, grain and mash during wet weather and gain access to the body by inhalation.

When heart and cutaneous blood from diseased fowls gave positive results for *Aspergillus fumigatus*, it was thought desirable to examine and culture the different parts of the eggs from infected pens. Eggs from infected and healthy pens were obtained from the Government Poultry Farm, Telankheri, Nagpur, and hundreds of cultures were taken from the washings of the eggs, shells, shell membrane, albumin, chalaza and yolk. *Aspergillus fumigatus* was obtained in most of the cases from the eggs of the infected pens from shell membrane, albumin and chalaza. In spite of several attempts the pathogen could not be isolated from yolk and shell. The cultures were taken either on rice-mesh agar or broth medium. Spring has isolated from eggs *Aspergillus glaucoides* and *Aspergillus heterocephalus* of which the former has been proved to be indistinguishable from *Aspergillus fumigatus* Fres. The *Aspergillus sp.* isolated from the inner contents of the eggs was slightly different from the species isolated from the diseased fowls. The hyphæ of such isolates is thread like, hyaline and measure 3·5–6·3 μ in breadth; the spores are roughened in outer wall, mostly sphærical, pale yellow in centre and dark brown at the periphery and measure 3·5–6·3 μ in diameter. Repeated sub-culturing however has later shown that it is indistinguishable from the species isolated from the lesions of diseased fowls. The difference exhibited at the early stage of isolation is most probably due to the different nature of the host. Transmission of the disease to healthy birds by the egg isolate could not be tried so far.

Sixteen fertile eggs from infected pen were kept for incubation but none of them hatched out. In a few cases dead embryos were seen on breaking the egg shells while in others opaque, non-motile, dense spot in the enlarged air-chamber were observed which on culturing gave colonies of *Aspergillus sp.* These experiments have shown that the disease is transmissible to the eggs when laid by sick birds. Further work on the disease is still in progress.

I am grateful to Mr. H. B. Shahi, Director of Veterinary Services, Government, C. P. and Berar, for according necessary facilities in this work. My sincerest thanks are due to Mr. G. D. Pani for bringing the disease to my notice and for his valuable help during the investigation. I am also thankful to Mr. R. A. Narke and Mr. S. L. Zargar for the help rendered by them.

FIG. 1. *Aspergillus fumigatus* ×900

Summary

1. A severe and fatal outbreak of Aspergillosis was reported from several places in the Central Provinces.

2. Young birds are most liable to an acute infection, but the older individuals are more resistant.

3. Symptoms of the disease are given.

4. *Aspergillus fumigatus* has been isolated from the heart and cutaneous blood, lungs and other organs of sick birds, eggs and poultry feed.

5. A detailed microscopic study of the organism is given.

6. Besides fowls the fungus has been found pathogenic to human beings, goats and rats.

7. Eggs from sick birds hardly ever hatched out and *Aspergillus fumigatus* has been isolated from most of them.

REFFRENCES

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2. Thom, C., and Church, M. B. .. *The Aspergilli*, 1926 Edition.
3. Gaiger, H. S., and G. D. Davies .. *Veterinary Pathology and Bacteriology*.