An Investigation Of A Case Of Brooder Pneumonia In A Commercial Broiler Operation In Central Province, Sri Lanka

W.S Gothami, M.E.S.A.De S Ariyaratne, Amal.D Premarathna, M.D.N Jayaweera

Abstract: This study was carried out in a commercial broiler farm with 5000 birds. High mortality (27.3%) among 5 days old chicks was reported in a broiler farms at central province in Sri Lanka on March 2014. Among 5000 birds, 3000 were affected and more than 500 birds were dead at the time of complaint. Affected birds showed signs of respiratory difficulties, off food and drooping feather apart from the high mortality. Flock has been treated with Greseofulvin (50mg/20L) for a period of one week. But still high number of deaths was observed. Post mortem examination was carried out in birds and samples were isolated from lungs, heart and liver. Impression smear was prepared from the lung and stained with Leishman stain. Fungal hyphae were observed in the impression smear. Greenish grey colour colonies were observed on SDA. Moreover, bacterial analysis of liver and spleen showed E coli infection. After analysing history, clinical signs and culture results the disease was diagnosed as Aspergillus pneumonia with E coli infection. With the treatment, daily mortality was reduced from 12% to 1% over a period of one month. But expected average weight was not gained.

Index Terms: Aspergillosis, brooder pneumonia, E coli

1 Introduction

Brooder pneumonia is a disease that mainly affects respiratory system but sometime infection may spread to visceral organs and it is caused by ubiquitous opportunistic soil saprophytes of genus Aspergillus. Although A. fumigates is the most commonly isolated organism, other species like A. flavus, A. niger, A. glaucus and A. terreus can also produce the same invasive infection. These organisms grow on organic matter in warm (25˚C) humid environment including damaged eggs in hatcheries, ventilation system, poultry litter and feed. Almost all avian species should be considered as potential hosts susceptible to infection including brooder stages of chicks, quails, pheasants, turkeys, pigeons, parrots etc.(Arne et al., 2011). It is commonly seen in the chicks below 10 days of age and the chicks below 3 days of age are highly susceptible. But it may develop in birds up to the age of 10 weeks. Occasionally, adult birds may also get affected by the Aspergillosis.

- *Dr. W.S.Gothami (BVSc.)*
  Central Veterinary Investigation Center, Veterinary Research Institute, Gannoruwa, Sri Lanka.
  E mail – Sugandhikagothami@yahoo.com
- *M.E.S.A.De S Ariyaratne*
  Central Veterinary Investigation Center, Veterinary Research Institute, Gannoruwa, Sri Lanka..
  E mail – sanupama.ariyaratne@gmail.com
- *Amal D Premarathna*
  Department of Veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science University of Peradeniya, Sri Lanka.
  E mail – amaldharmapriya@gmail.com
- *M.D.N Jayaweera*
  Central Veterinary Investigation Center, Veterinary Research Institute, Gannoruwa,Sri Lanka.
  E mail – mdnjayaweera@yahoo.com

Pathogenesis

High moisture content and warmth provide suitable environment for the growth of fungi. Especially Aspergillus species sporulate abundantly with every conidial head producing thousands of conidia with 2-3 mm of diameter which is small enough to reach alveoli of the lungs (Beernaert,et al., 2010). Inhalation of excess amount of fungal conidia produces severe clinical disease. Immunosuppression is the major factor that increases the susceptibility of birds for the infection. Stress alone or other factors related to confinement, poor husbandry practice, malnutrition, pre-existing disease and the prolong use of antibiotics and steroids further increase susceptibility. Clinical signs are variable and it depends upon factors like pathogenesis, affected systems, location of the lesions and the immunity of the bird.

Acute Aspergillosis

This form is characterized by variable morbidity and high mortality. Young, newly captive birds are susceptible. Inhalation of large number of spores induce the infection which has rapid onset of clinical signs followed by death. Dyspnea gasping, dropping wings, anorexia and lethargy are common sings.

Chronic Aspergillosis

Older captive birds that are subjected to long term stresses like malnutrition heat stress might get this form of the disease. Early signs are nonspecific. Behavioral signs, reduced appetite and weight loss are common signs. Aspergillosis may be found in the entire respiratory tract, commonly occurs in the posterior thoracic and abdominal air sacs. Tail-bobbing, open mouth breathing, audible respiratory sounds indicate lower respiratory disease. With disseminated Aspergillosis other systems are also get affected. Especially in the GIT, kidneys and CNS might have nodular lesions. Although very rare, ocular lesions can also be observed with the signs of keratitis, blepharitis, photophobia and periorbital swelling.

Diagnosis

Although ante-mortem diagnosis is challenging definitive diagnosis is made based on history, presence of characteristic lesions and demonstration of the organism by cytology or

histopathology with subsequent identification by culture. Microscopic lesions showing fungal elements within the granulomas can be suggestive but not useful in species identification because in vivo hyphae of filamentous fungi are very similar to each other. Thus, immunohistochemistry is applied to confirm disease and to differentiate it from many of the filamentous fungi (Beytut et al., 2004). Gross lesions are well developed in the chicks above 5 days of age. Lungs show almost uniform, raised pinhead size yellowish nodules. In the chronic form of the disease these granulomatous nodules can be found on the visceral organs as well. Air sacs are usually thickened with yellow colour plaques with cheesy consistency.

Prevention

TABLE 1: *COLOR OF THE COLONIES IN VARIOUS SPECIES OF FUNGI IN GENUS ASPEGILLUS (PATRON D.D., 2006)*

Species	Color of the colony
A.Flavus	Yellow – green color
A.fumigates	Blue green- gray
A.glaucus	Green with yellow areas
A.niger	Black
A.terres	Cinnamon to brown
A.clavutus	Blue-green

Prevention of Aspergillosis, stress factors and exposure to spores need to be minimized along with adopting strict hygiene and sanitation measures in brooder and hatchery. Dirty, broken and potentially contaminated eggs must be eliminated before setting in the incubator. An effective fungicide should be applied inside the setter soon after transfer of hatching eggs is complete. Feed with less moisture content should be given and the litter should be kept dry. Proper drainage is necessary to prevent water logging. It is necessary to maintain good ventilation, hygienic and stress-free environmental conditions inside the poultry farm. A good litter management practice needs to be followed and in between two flocks, treatment of new litter with antifungal agent is mandatory to prevent the disease. Feeders should be kept dry and clean to limit the fungal development. Affected and ill birds should be removed and culled. Both conventional and supportive treatments are required to control the infection. In mild form of disease, treatment is fruitful but when lesions are moderate to severe involving lungs and air sacs, therapy is often not successful even after combination of drugs are used. Various drugs like amphotericin-B, 5-fluorocytosine, and ketoconazole can be used to control the disease. Treating litter with Nystatin and Copper sulphate can reduce mold content. In outbreaks, drinking water with 1:2000 aqueous solution of Copper sulphate needs to be provided. Other drugs like enilconazole and fungicidin have also been tried on experimental basis.

2 MATERIALS AND METHODS

2.1 Sample collection

The farm was visited and relevant details were gathered. There are 4 poultry pens with 2000 birds in 3 pens and 1000 birds in the remaining pen. Two of the pens are being used to keep 5 days old chicks while other two pens have 11 days old birds. 16 chicks have been kept in each brooder and wood shavings have been used as litter. The wood shaving had been stored for 4 weeks before adding it as litter.

2.2 Sample for disease diagnosis

Five fresh carcasses selected haphazardly were brought to the Veterinary Investigation Center for disease diagnosis. Samples were collected aseptically from lungs, liver, yolk and heart for further investigations. Impression smears were obtained from the lesions present in the lungs and stained with Leishman and microscopic examination. A tissue piece taken from the lesions in the lungs was embedded and inoculated in the Sabaroad Dextrose Agar and incubated at 37˚C for three days. Samples from liver, heart and yolk cultured on blood agar and MacConkey agar to be incubated at 37˚C for 24 h. Furthermore, a portion of the colony was sub-cultured on EMB (Eosin Methylene Blue) agar and incubated at 37˚C for 24h.

3 RESULTS AND DISCUSSION

High mortality (27.3%) among 5 days old chicks was reported in the farm on March, 2014. Among 5000 birds, 3000 were affected and more than 500 birds were dead at the time of complaint. High mortality and morbidity, drooping feathers, off food together with respiratory difficulties have been observed. Flock has been treated with Greseofulvin (50mg / 20 l) for a period of one week. But still high number of deaths was observed. An investigation was carried out to diagnose and control the disease. Initially 15 birds were dead and the mortality was kept rising up to 820 birds. Affected birds have been showed signs of dyspnoea, anorexia and drooping feathers. Differential diagnosis; Brooder pneumonia,Gape worm (Syngamus trachea) infestation, New castle disease and Infectious Bronchitis. Post mortem examination of the carcasses revealed severe pulmonary pathology showing numerous whitish colors granulomatous foci uniformly distributed in both lungs, suggesting granulomatous inflammation in the lungs. Similarly visceral organs including intestines and peritoneal cavity were affected with the granulomatous lesions.

Figure: 01 *A bird showing gasping.*

Microscopic examination of the smear showed severe cellular infiltration with branched septate fungal hypae. A tissue piece taken from the lesions in the lungs was upon incubation greenish gray color colonies surrounded by white color margin were observed. Non hemolytic colonies were observed on Blood agar while pink color colonies were present on MacConkey agar. Gram stained smears were prepared and Gram negative rods were present. Metallic sheen was present on EMB agar indicating the presence of E.coli. Microscopic examination of the fungal colonies was carried out for further analysis. Sticky tape was used to take portion of the colony and it was stained with Lactophenol cotton blue. Then it was observed under microscope. Typical appearance of the sporangia specific to the Aspergillus species was observed. A branched septate hypae with numerous sporangia was present.

Though several Aspergillus species account for Aspergillus Pneumonia, Colony morphology on SDA and microscopic appearance of the fungus were highly suggestive of an Aspergillus fumigatus infection. Considering the history, clinical signs, postmortem finding and laboratory tests this was diagnosed as Aspergillus pneumonia with secondary E coli infection. However, polymerase chain reaction should be performed for accurate identification of the species. Aspergillus species are ubiquitous organisms. Improperly stored wood shavings were suspected as the source of the infection. The fungus produces number of conidia under favorable environmental conditions. Moldy feed, wet litter may act as sources for the infection. In this farm they have used wood shavings during the brooding period. According to the farmer, those are improperly stored and used after 3-4 weeks. This might be the source of infection in this farm. The farmer was used CuSO4 to control the infection and a solution prepared by dissolving 10 g of CuSO4 in 500 L of water was given in drinking water.

A B C

Figure: 03 *(A) Septate hypae present in the impression smear, (B) Greenish gray color fungal colonies on SDA, (C) microscopic appearance of sporangium*

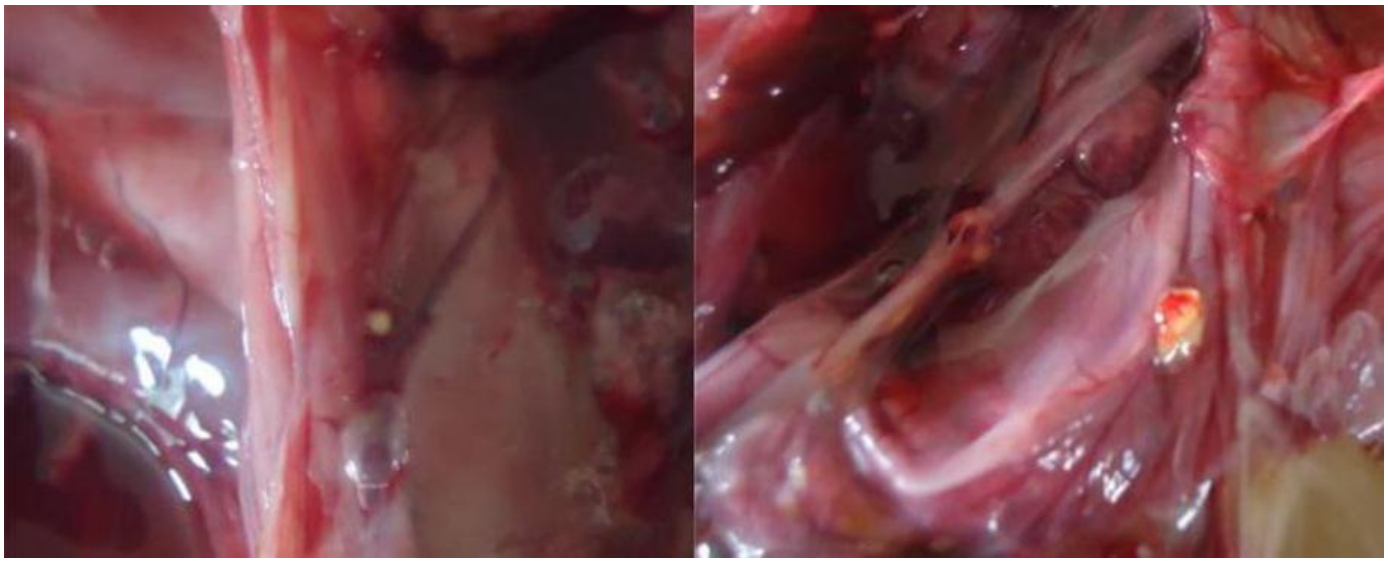

Figure: 02 *Yellowish color nodules present in the viscera*

4 Conclusion

Our study included limited these chicks were previously treated with greseofulvin (500mg/20L) but they had not responded well. Since this is an incurable disease, farmer was advised to remove the affected birds and disinfect the pens with a CuSO4 solution. Furthermore, farmer was encouraged to use fresh wood shaving and maintain proper storage conditions.

5 Abbreviation

CNS: Central nervous system
GIT: Gastrointestinal tract
SDA: Sabouraud Dextrose Agar
PCR: polymerase chain reaction

CuSO4: Copper sulfate

6 COMPETING INTERESTS

The author(s) declare that they have no competing interests.

7 END SECTIONS

7.1 Authors' contributions

W.S Gothami and M.D.N Jayaweera conceived the study, and conducted most of the laboratory experiments; analyzed and interpreted experimental results. A.D premarathna Conducted manuscript preparations; W.S Gothami and M.E.S.A.De S Ariyaratne carried out laboratory experiments. All authors read and approved the final manuscript.

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