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CASE REPORT

Aspergillosis concurrent with secondary bacterial infection in broiler chicks: a case report

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Abstract

Aspergillosis is an infectious
mycotic disease which mainly affects chicks at 1 to 3 weeks of age. This report describes an outbreak of aspergillosis in 13-day-old broiler chicks reared in an open-sided poultry house on deep litter system. The total number of birds kept in the affected house was 4000 with the mortality rate of 5%. The farmer noticed signs of weakness since the arrival of day-old chicks, and eventually, 200 chicks died within 13 days. Clinical signs observed were stunted growth, dyspnoea, torticollis, incoordination, swollen hock joint, and crooked toes. Postmortem findings revealed yellow to white caseous nodular lesion at the air sacs and lung with granular appearance upon cross section. The feed and organs samples were collected for microbiological and histopathological evaluation. *Aspergillus* spp. were isolated on Sabouraud dextrose agar (SDA) with whitish to grey-green appearance and stained using lactophenol cotton blue. Periodic acid-Schiff (PAS) staining in the brain tissue revealed the presence of fungal hyphae and vesicle. Histopathological findings revealed alveolar emphysema, atelectasis, thrombosis, and pneumonic lung with granulomatous tissue and granulomatous encephalitis. Culling of the affected birds and removal of the contaminated bedding and feed in the house are essential measures to control and prevent the disease occurrence. Treatment using fungicide such as copper sulphate is recommended to prevent further spread of the disease in the flock.

Keywords Aspergillosis · *Aspergillus* spp. · Clinical signs · Diagnostic work-ups · Pathogenesis · Broiler chicks

Introduction

Aspergillosis is an infectious mycotic disease which mainly affects chicks between 1 and 3 weeks old (Saad 2006). It is the most common opportunistic fungal infection of the respiratory tract in bird causing high morbidity and mortality leading to major economic loss in the poultry industry (Tell 2005). *Aspergillus* spp. are filamentous fungi which are ubiquitous in the environment, and they can be contaminant to the farm by contaminating hatching eggs in hatchery or breeder and also from poultry house. Predisposing factors causing infection of birds' respiratory system through air borne spores are poor sanitation of the house, feed contamination, and poor ventilation which promotes the fungal growth (Girma et al. 2016). The infection is acquired from the fungal spores travelling in the air which then enter the respiratory tract via the inhalation route. *Aspergillus* infection mainly affects lower respiratory tract with the most prominent lesions in the air sac and lung (Saif et al. 2008; Girma et al. 2016). It also may invade the brain tissue causing neurological deficit in the chicks. This case report describes the veterinary diagnosis of aspergillosis outbreak with concurrent secondary bacterial infection in a farm.

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Case details

History A total of 4000 broiler chicks were managed in an open-sided house on deep litter system in which the bedding is changed every 35 days. The chicks were vaccinated with Newcastle disease and infectious bronchitis (ND-IB) via drinking water at 5 days old. The farmer complained that the chicks were showing signs of weakness since arrival, and 200 chicks died within the first 13 days with a mortality rate of 5%; however, no treatment was administered. Upon clinical investigation, the most obvious clinical signs observed were stunted growth, raffled feathers, torticollis, incoordination, swollen hock joint, crooked toes, and dyspnoea.

Postmortem evaluation The postmortem findings of the culled chicks revealed the presence of yellow caseous nodule attached to the air sacs and lung (Figs. 1a and b). Besides, some chicks exhibited un-regressed Merkel diverticulum and unabsorbed yolk sac in the intestinal tract. White caseous nodules with firm structure were found in the brain (Fig. 1c). The differential diagnoses at that time were aspergillosis, Newcastle disease, and colisepticaemia. Feed and organ samples were collected for further diagnostic work-ups.

Microbiological and molecular biology evaluation The lung and feed samples were cultured on the Sabouraud dextrose agar (SDA), and within 48 h of post-incubation, there was whitish, grey to green centre with a puffy appearance on the SDA. Identification of the fungal was conducted using scotch tape method and stained with lactophenol cotton blue. *Aspergillus* spp. were identified based on the microscopic characteristics of conidiophore, vesicle, metulae, phialides, and conidia (Fig. 2) (McClenny 2005; Diba et al. 2007). The bacteriological works were done according to standard manual of bacterial culture, and the identification of bacteria was made through biochemical tests and culture on selective agars. Molecular test was not conducted due to limited funding. *Staphylococcus* spp. and *Micrococcus* spp. were isolated and identified from the brain, heart, air sac, and kidney. The gram staining revealed clusters of cocci and tetrad cocci, respectively, coagulase test negative and positive growth on mannitol salt agar which confirm the particular bacteria. For the yolk sac and hock joint samples, *Escherichia coli* were isolated and identified as the gram staining revealed short and long rod bacteria, fitted with all biochemistry tests as well as positive growth on the eosin methylene blue (EMB) agar with green metallic sheen colonies. To rule out Newcastle disease, reverse transcriptase polymerase chain reaction (RT-PCR) test was conducted by homogenising and filtering the samples of trachea, lung, spleen, and ceca tonsils. Ribonucleic acid (RNA) was later extracted from the filtered suspension, and PCR assay was performed following standard protocol. However, the results were demonstrated negative. Haemagglutination inhibition (HI) test was also done by detecting the antibodies against Newcastle disease virus. Serum samples from the sick birds were collected and run for HI test following standard laboratory protocol. Results of antibodies titreClick here to enter text. showed normal value.

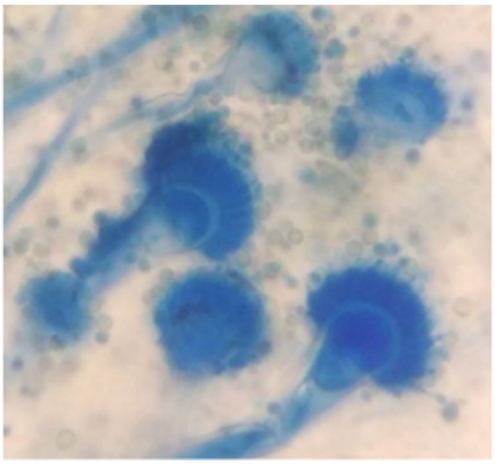

Fig. 2 Microscopic appearance of *Aspergillus* spp. (lactophenol cotton blue, ×100 magnification)

Histopathological examination Histopathological examination was carried out to investigate the histopathological lesion and changes caused by the infectious agents from the affected organs. The histopathological examination was conducted as previously described by Reduan et al. (2020). Haematoxylin and eosin (H&E) staining on the lung tissue showed

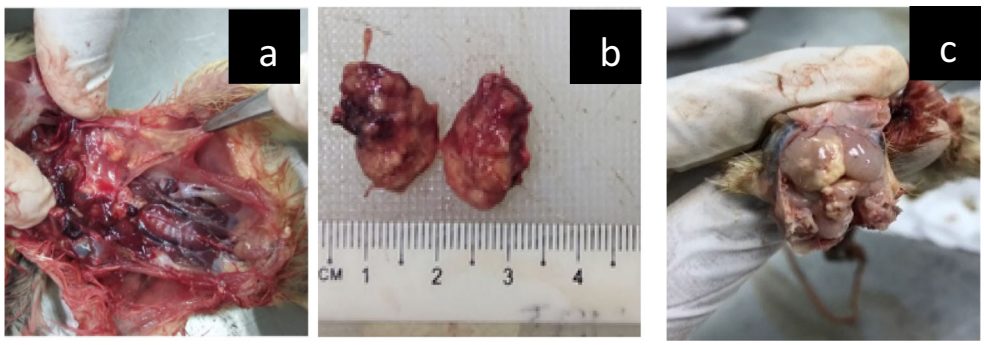

Fig. 1 Yellow to white caseous nodular plaque formation in the **a** air sac and **b** lung and **c** brain

granulomatous lesion characterised with rounded, thick encapsulation with fibroblast and infiltration of heterophils, monocytes, and macrophages with fistulous tract formation (Fig. 3a). In the other section of the lung, caseating granuloma structure is surrounded with thin encapsulation and a newly formed pyogranuloma structure (Fig. 3b). Other lesions that were observed in the lung tissue include alveolar emphysema, atelectasis, and thrombosis. In the brain section, granulomatous tissue formation was identified with a rounded, encapsulated area of fibrous tissue form on the wall, and there was disruption in the tissue architecture. In the centre of the granuloma, there was infiltration of heterophils, monocytes, and macrophages with fistulation (Fig. 3c). There was also a presence of rounded multinucleated giant cell structure in the brain with fused cytoplasm in the centre and fused macrophages nuclei (Fig. 3d). Histopathological examination using periodic acid-Schiff (PAS) staining visualised presence of fungal hyphae and vesicle in the brain tissue which stained magenta for glycoprotein and mucin substances (Fig. 3e).

Treatment The final diagnosis of this farm was aspergillosis concurrent with secondary bacterial infection. Therefore, the severely infected chicks were culled. The flock was treated with 60 mL of copper sulphate ($CuSO_4$) in 16 L, via drinking water for 5 days. Streptomycin was also administered to the chicks at 300g/100 gallons of drinking water for 5 days to treat the secondary bacterial infection. The farmer was advised to change bedding regularly and disinfect the house with glutaraldehyde 30% w/v.

Discussion

Aspergillosis is a fungal disease which often causes severe financial loss due to the carcass condemnation and its public health significance and zoonotic concern. As aspergillosis has high prevalence in the environment of tropical countries (Rudramurthy et al. 2019), this disease is commonly observed and diagnosed in Malaysia's poultry farms (Chung et al. 2020). Chicks are usually infected by inhaling a large number of spores in the contaminated hatchery environment, or the infection may also be a result of the contaminated floor eggs in the breeder farm (Arné et al. 2011). Favourable conditions of wet and moist litter may have also contributed to the severity of the condition.

The main route of infection was through the inhalation of the fungal spores through the nares, trachea, and to the primary bronchi, then delivered to the thoracic and abdominal air

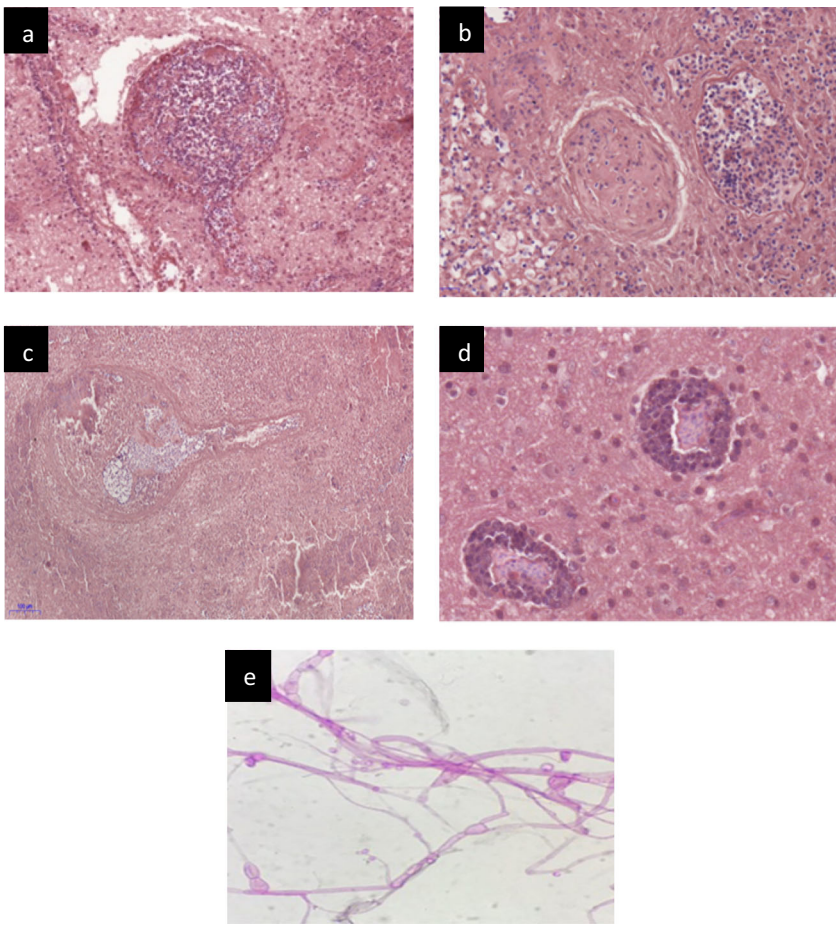

Fig. 3 **a** Granulomatous pneumonia (×10, 100μm); **b** caseating granulomatous pneumonia (×20, 50μm); **c** granulomatous encephalitis (×20, 50μm); **d** granulomatous encephalitis (×40, 20μm); **e** fungal hyphae and vesicle in the brain (PAS staining, ×100)

sacs before contacting the epithelial surfaces in the lungs (Nardoni et al. 2006). The fungal spores then released gliotoxin, an immunosuppressive mycotoxin of *Aspergillus* spp., and have proapoptotic effect (Kamei and Watanabe 2005; Sugui et al. 2007). It caused an impairment of the immune response towards removal of the spores from the lung. As the response developed, macrophages might fuse to form multinucleated giant cells, and collagen was produced by the stimulated fibroblast to form granuloma (McGavin and Zachary 2006; Leishangthem et al. 2015). Angio-invasive property of the fungus which has elastase enzyme can degrade elastin on the blood vessel wall leading to intravascular thrombosis and dissemination of the fungus to other organs (Dagenais and Keller 2009; Álvarez-Pérez et al. 2010). In the brain, the necrotic caseous lesion was seen as granulomatous encephalitis (Abundis-santamaría 2003). Granulomatous pneumonia and encephalitis were localised in this case with manifestation of clinical signs such as gasping, torticollis, and incoordination. A study conducted by Schlam et al. 2016) found that gliotoxin secretion by a new hyphae growth can further suppress phagocytic defence system. The immunosuppressed chicks were then predisposed to secondary bacterial infection in which the lesions of omphalitis and yolk sac infection have been observed in this case. The cause of death is believed due to the granulomatous inflammation in the lung which caused the obstruction of the airway, pulmonary hypoxia, and respiratory failure.

Fungal culture and histopathological examination are the methods that can provide the definitive diagnosis for the disease (Fischer and Lierz 2015; Chung et al. 2020). These goal methods were used in the current case to diagnose aspergillosis. To identify the presence of fungal hyphae and mycelia, periodic acid-Schiff (PAS), Bauer's and Gridley's stains, and Grocott's and Gomori methanamine Silver stain (GMS) can be used (Girma et al. 2016; Chung et al. 2020). The best treatment in aspergillosis infection is by giving copper sulphate which was practised in this study. Copper sulphate is an inorganic compound that combines sulphur with copper which have antimicrobial activity against a wide range of aerobic, anaerobic bacteria, and fungi (Beckerman 2008). However, fungal granulomas are commonly difficult to penetrate even with the most potent antimicrobial drugs (Beernaert et al. 2010). The disease preventive measures should be conducted as early as the stage of hatchery and brooder by practising strict hygiene and sanitation procedure. Contaminated, dirty, and broken eggs must be eliminated before the eggs are set in the incubator. Fumigation using formaldehyde or thiabendazole at 120–360 g/m^3 can be applied in the contaminated hatchery (Pattisson et al. 2008). At the farm level, bedding and feed should be kept in clean, dry, and non-dusty condition to limit fungal development. The relative humidity control through proper ventilation in the chicken house should be regulated. Contamination of the farm environment can be managed by a periodic or repeated antifungal treatment (Arné et al. 2011). Fungistatic agents such as nystatin, thiabendazole, or copper sulphate can be sprayed for 3 consecutive days to minimise litter fungal contamination (Leishangthem et al. 2015). It is necessary to remove and cull infected and sick birds to reduce farm contamination with fungal spores (Gothami et al. 2017) which was advised to the farmer in this case study. In this case, concurrent treatment with antibiotic was conducted as some birds showed signs of bacterial infection of omphalitis and yolk sac infection. In this particular case, after being treated with copper sulphate and streptomycin for 5 days, we observed a decline in mortality rate from 5 to 2%; thus, the birds were responsive towards the treatment. Nevertheless, prolonged use of antibiotic regime needs to be avoided as it may lead to further immunosuppressive condition and hence favour the imbalance of intestinal flora and, thus, may promote growth of fungi (Velasco 2000; Dhama et al. 2003).

Conclusion

Aspergillosis is an infectious mycotic respiratory tract disease caused by *Aspergillus* spp. Diagnosis of aspergillosis can be done through clinical signs, postmortem lesion, fungal culture, and histopathological examination. Improving the hygiene and sanitation at the farm and hatchery levels together with proper disinfection protocol can help in reducing the load of the spores and spread of the disease.

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Declarations

Ethics approval All applicable international, national, and/or institutional guidelines were followed.

Conflict of interest The authors declare no competing interests.

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