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Case Report
Clinicopathological Features of Concurrent Outbreak of Gumboro Disease and Aspergillosis in Chicken in Hawassa City, Ethiopia

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Abstract

Background and Objective: The poultry industry in most developing countries is challenged by Gumboro and Aspergillosis. This study aimed to describe the major clinicopathological findings of concurrent outbreak of these diseases. **Materials and Methods:** An investigation was undertaken between March and April, 2018 following a report of an unknown disease outbreak in an intensively managed poultry farm located at Hawassa city. The farm was then visited by a team of veterinarians to collect valuable information and samples for further examination. Systematic postmortem examination and histopathology were then made in the Veterinary Pathology Laboratory of Hawassa University to characterize the lesions. **Results:** The bursas were swollen, edematous and/or hemorrhagic and the air sacs and lung had several white to yellow caseous plaques. Moderate to severe lymphoid depletion and granulomatous inflammation surrounding spores and branched hyphae were dominant microscopic lesions on the bursa and lungs, respectively. The collected information at different levels were then summarized and interpreted. Accordingly, concurrent outbreak of infectious bursal disease (IBD) and Aspergillosis were detected for the first time in Ethiopia. **Conclusion:** Possible failure of vaccine or emergence of highly pathogenic strains of IBD virus and poor ventilation were incriminated for the outbreak.

Key words: Gumboro disease, aspergillosis, concurrent outbreak, clinicopathology, white to yellow caseous plaques, severe lymphoid depletion, Ethiopia

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Chicken is important to subsistence, economic and social livelihoods of a large human population both in urban and in rural settings of developing countries. Chickens are especially important to women, children and aged individuals, who are the most vulnerable member of the society in terms of under-nutrition and poverty[1]. Ethiopia has huge poultry population which is predominantly reared under extensive/traditional type of management system. Over 96% of the country's chicken are local breeds and kept mainly in free-range system where the chicken scavenge for food around homestead during day time. In the recent decades, mainly to suffice the protein demand of the growing human population, the number of exotic chickens and intensive farms are relatively increasing[2].

The huge poultry population is challenged by several constraints including diseases. Infectious diseases are recognized as one of the major constraints because they pose enormous economical and societal impact to farmers[3]. Among these, Infectious Bursal Disease (aka IBD, Gumboro disease) and Aspergillosis are becoming serious threat and challenging to the emerging poultry industry in Ethiopia[4].

Infectious bursal disease is an acute and highly contagious viral disease of young chickens caused by a non-enveloped virus belonging to the *Avibirna virus* genus[5,6]. It is one of the major causes of economic losses to the poultry industry as a result of high mortality rates in its acute clinical form and of immunosuppression induced by subclinical infection[7]. Due to its immunosuppressive effect, affected flocks show increased susceptibility to infection with opportunistic pathogens, often leading to chronic disease situations and suboptimal vaccine responses[8,9]. It may also pose significant impact on public health by favoring the development of several poultry infection of zoonotic importance like Salmonellosis, Campylobacter and Avian influenza[9].

Avian aspergillosis (aka Brooder pneumonia, mycotic pneumonia, pneumomycosis) is one of the most common fungal infections affecting the lower respiratory tract of birds including chickens[10,11]. It can occur in sporadic as well as in epidemic form, resulting into high morbidity and mortality[12]. The causative agents, spores of *Aspergillus fumigatus* and other species are ubiquitous but thrive very well in warm and humid areas with poor ventilation and sanitation[13,14]. The prevalence and severity of aspergillosis is higher when infections with the spores or conidia happen concurrently with diseases or stressors known to compromise the immune system[15].

In Ethiopia, IBD and Aspergillosis were first reported in few commercial poultry farms situated at the central part of the country in 2005 and 2016, respectively[2,16]. In the past one decade, however, IBD was widely spreading to other parts of the country involving both commercial and backyard poultry production systems despite regular vaccination practices[17]. In contrary, further report was not made on the occurrence of Aspergillosis in the country at large.

Except few outbreak reports of IBD in northwest and central Ethiopia[2,18], there is still paucity of information about the prevalence of IBD from southern Ethiopia. To authors' knowledge, there is no publication on concurrent outbreak of IBD with Aspergillosis in the country. Therefore, this case report briefly summarized the clinicopathological findings of concurrent outbreak of IBD with Aspergillosis in Hawassa city.

MATERIALS AND METHODS

Description of the farm: The outbreak was observed in Hayo Doc poultry farm which is located at Cheffe Kote Jabesa village in Hawassa city in Southern Nations, Nationalities and Peoples Regional State (SNNPRS). The farm was established in September, 2017 by a cooperative comprising eight entrepreneurs and their employees with the intention of creating job and sustainable income for young graduates. The farm started the business with a total of 6,438 day old chicks. The chickens were managed intensively on a deep litter system. The farm was not properly fenced to separate it from residents in the village and prevent access of rodents, cats, dogs and local backyard chicken to the farm. As part of routine management practice, the chickens were vaccinated for Newcastle disease at the age of 7, 21 and 28 days. Moreover, they had also been vaccinated for Gumboro disease using attenuated IBDV D78 vaccine. The health and production issues were regularly supervised by one young veterinarian.

On mid of March 2018, a serious outbreak of unknown cause occurred in the farm and reported to the Faculty of Veterinary Medicine of Hawassa University. Then, the farm was visited by a team of senior veterinarians from the Faculty to gather all relevant information and collect sample for further laboratory based investigation.

Ethical approval and consent to participate: Ethical clearance was obtained from Hawassa University, Faculty of Veterinary Medicine, Research Ethical Review Committee. During the study, permission and oral informed consent were obtained from the farm owners. All the chickens included for postmortem examination were killed in a humane manner to

reduce their suffering and pain. This article does not contain any studies with human participants performed by any of the authors.

Antemortem and postmortem examination: A total of 14 representative sick chickens, of which two of them died during transportation were brought to the Veterinary Pathology Laboratory of Hawassa University for further examination. All chicks were depressed and had watery whitish diarrhea and ruffled feather. Following thorough physical examination, they were euthanized and necropsied as per standard procedure[19] and the gross lesions were recorded.

Histopathological examination: At necropsy, the bursa of Fabricius, liver, thigh muscle and lung tissues were collected and preserved in 10% buffered formalin for histopathological studies. The fixed tissue samples were further processed for paraffin technique, sectioned at 4-6 µm thickness and stained with Hematoxylin and Eosin (H and E) as per standard procedure[20]. The slides were then examined under light microscope and the lesions were recorded and interpreted accordingly.

RESULTS

History and circumstantial evidences: The outbreak affected young chicken at the age of 40 days. Within 36 h of the onset of the outbreak, a total of 1280 chicks /growers were died with the history of dramatic depression, dyspnea, watery whitish diarrhea, ruffled feather and anorexia. Mortality reached pick (40%) within two days of onset and then gradually declined but continued for 2 weeks. The team noted that the poultry premise was poorly ventilated with dusty litter; the birds were over-crowded and managed under poor hygienic conditions on a floor system.

Findings of postmortem examination: Swollen and edematous and/or hemorrhagic bursa (Fig. 1a) and petechial to ecchymotic hemorrhage of the leg, thigh and/or pectoral

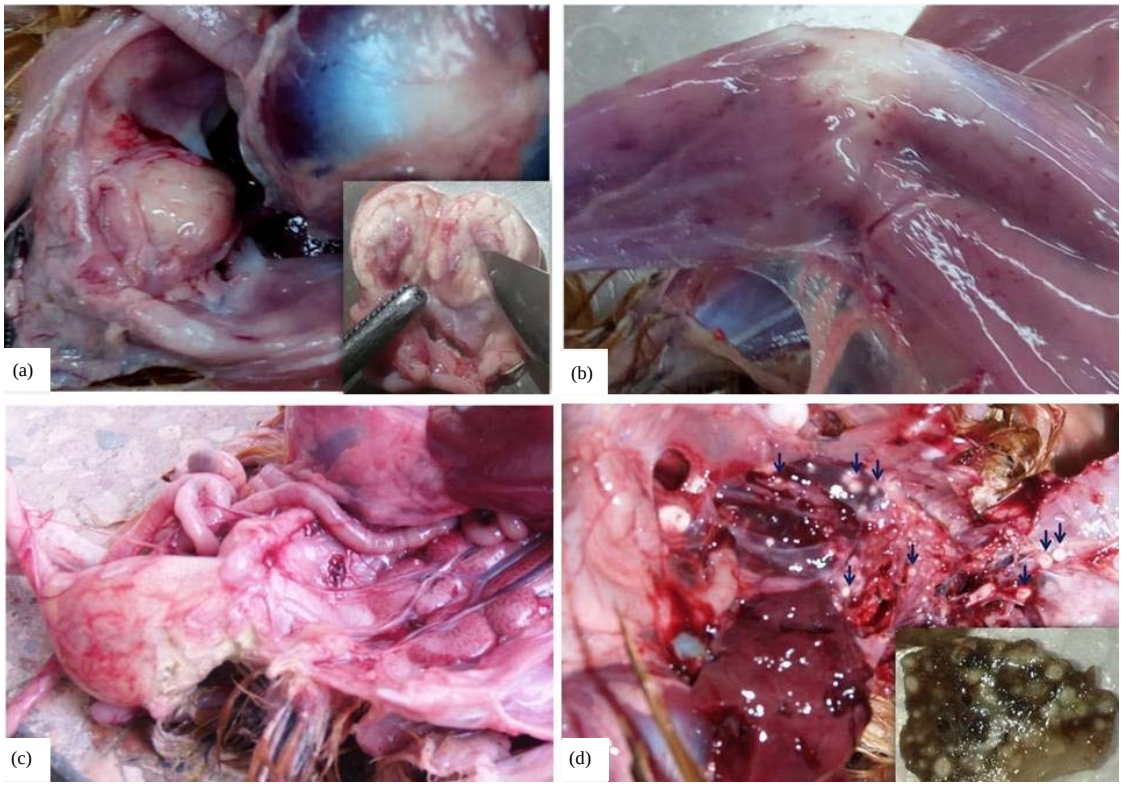

Fig. 1(a-d): Gross lesions of Gumboro representing, (a) Prominent swelling of the Bursa of fabricius (Inset represent cut surface), (b) Petechial to ecchymotic hemorrhage on the leg muscle, (c) Severely distended cloaca and kidneys. Gross lesions of Aspergillosis on the lungs and air sacs (d) characterized by caseous nodule of varying size (arrow). Inset represent formalin preserved tissue

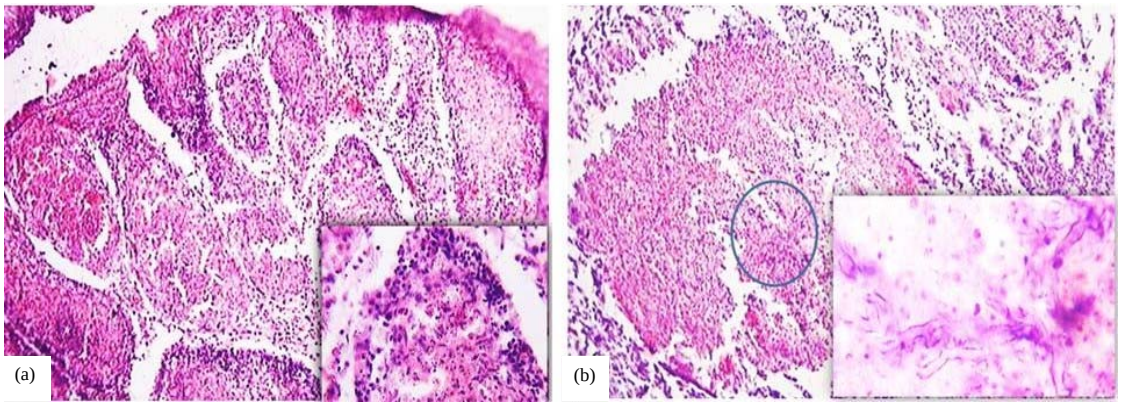

Fig. 2(a-b): Histopathology of gumboro revealing, (a) Moderate lymphoid depletion of Bursal follicles, central accumulation of cellular debris and infiltration by heterophils with cortical remnants (see the inset, 40x). Histopathology of Aspergillosis characterized by (b) Classic granuloma surrounding central branched hyphae (Inset, 40x). H and E stain, 10x magnifications

muscles (Fig. 1b) were seen in 9 chickens. In most cases the subcutaneous blood vessels were congested and the cloacae were filled with whitish diarrhea and urate (Fig. 1c). Upon incision, the bursas of five chickens were filled with cheesy mass in their lumen and longitudinal bursal laminae of six chickens were hypertrophied and had moderate hemorrhage (Fig. 1a). Spleens of most chicken were slightly enlarged. Liver and kidneys of four chicks were pale and moderately swollen. Moreover, white to yellow caseous plaques/nodules were observed on the lungs and thoracic air sacs of 6 chicks (Fig. 1d). All the chicks with such lesions were highly emaciated and had chronic respiratory difficulty.

Findings of histopathological study: The major bursal lesions encountered were moderate to severe lymphoid depletion, infiltration by heterophils, intrafolicular hemorrhage, accumulation of cellular debris and interfollicular edema (Fig. 2a). There was also moderate lymphoid depletion in the lymphoid nodules of spleen and in some bursa of fabricius (Fig. 2a) and hemorrhage in muscle and kidneys. Moreover, tissue sections from lungs revealed classic granulomatous inflammation surrounding spores and branched hyphae suggestive of fungus of the genus *Aspergillus* (Fig. 2b). The granulomas were characterized by central necrotic area with infiltration of heterophils, macrophages, epithelioid cells and some giant cell.

DISCUSSION

Based on the history gathered (sudden onset, high morbidity, spiking mortality and rapid recovery from clinical signs), clinical signs and gross and microscopic lesions observed, the cause of the outbreak was concurrent infection of IBD and Aspergillosis. The clinical signs observed in sick chicken were in agreement with the previous reports of Gumboro disease (IBD)[21-24] and Aspergillosis[25-28]. Similarly, the gross and microscopic lesions observed on the bursa, liver, spleen, pectoral and thigh muscles and proventriculus were consistent with the previous reports made for Gumboro disease[2,22,29,30] and for Aspergillosis[16,27,28].

The poor biosecurity measure in the farm, with the concomitant free roaming of mice, dogs and wild birds, might be the reason for the introduction and rapid dissemination of the virus in the farm. In line with this, some studies proved that dogs and mice fed poultry products contaminated with IBDV potentially shed the virus in their faeces for 1-2 days after ingestion, which in turn resulted clinical disease and death in chickens[31,32]. The high mortality recorded in this outbreak may be due to the fact that it was the first outbreak of Gumboro in the farm and due to complication with Aspergillosis which is also known to induce severe loss (up to 15%) in a flock by itself[33]. Moreover, the highly pathogenic strain which is emerging in recent years because of mutation and genetic assortment[6,17,34] might have been introduced in the area. However, this pre-sumption needs to be substantiated by future studies.

In general, Aspergillosis in young chicken and pullets is commonly associated with overwhelming exposure to large numbers of conidia from heavily contaminated feed, litter or the hatchery environment[35]. Based on the age of the chicks affected in the current outbreak, the hatchery environment could not be incriminated as the source of infection. Rather,

the warm and humid environment of the farm sheds, overcrowding, inadequate ventilation, poor hygienic condition of the floor and feed storage conditions could be the major reasons for the growth of *Aspergillus* spp. as these conditions are known to favor its growth[13,16]. To identify the actual source of the conidia, however, representative samples from the litter, feed and floor should be collected and checked for the presence of the fungus using appropriate laboratory technique including culture.

The hardy nature of both pathogens (*Aspergillus* spp. and *Birna virus*) can cause their long and persistent survival in the poultry house even when thorough cleaning and disinfection procedures are followed. Therefore, if the source of *Aspergillus* is not identified and the necessary control and preventive measures are not timely implemented, the established IBD infection can cause catastrophic loss in the farm and in the area at large.

CONCLUSION

In any disease outbreak, especially concurring with Aspergillosis, systematic postmortem and histopathologic examinations should be taken as ancillary diagnostic technique. Since both Gumboro and Aspergillosis are known immunosuppressant poultry diseases, the occurrence of one can predispose the chicks for other prevalent diseases in the area. Possible failure of vaccine or emergence of highly pathogenic strains of IBD virus and poor ventilation were incriminated for the outbreak.

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SIGNIFICANCE STATEMENT

This study discovers the clinico pathological features of concurrent outbreak of Gumboro and Aspergillosis that can be beneficial for poultry producers and researchers. This study will help the researcher to uncover the critical areas of Gumboro outbreak in a vaccinated population that many researchers in the area were not able to explore. Thus a new theory on the emergence of highly pathogenic strain of *Birna virus* in the region may be arrived at.

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