Two incidents of infectious bursal disease in small holder poultry layer farms despite vaccination

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Introduction

Poultry industry plays a major role in uplifting the contribution of the livestock sector to GDP in Sri Lanka. With the increase of poultry population within the country, the disease burden has also grown significantly. Diseases can cause substantial economic losses in the poultry industry leading to a downfall of the progress made during recent years. Infectious bursal disease (IBD) is highly prevalent in Sri Lanka. According to the annual report of the department of animal production and health, 163,713 and 119,104 cases of IBD were reported in the country during the years 2017 and 2018 respectively. IBD is an immunosuppressive disease that mainly affects the bursa of fabricious (BF) of young birds causing high mortality. Immunosuppression leads to several other impediments in the affected flocks including, gangrenous dermatitis, inclusion body hepatitis-anaemia syndrome, *Escherichia coli* infections and vaccination failures (Eterradossi and Saif, 2020). Morbidity could go up to 100% within flocks between two to six weeks of age. Incubation period of the disease is 23 days (Mahgoub, 2012).

Infectious bursal disease virus (IBDV) of the genus *Avibirnavirus* in *Birnaviridae* family is the causative agent of the disease and its genome consists of two segments of double stranded RNA (Brandt *et al*., 2001). IBDV is classified into two serotypes, pathogenic serotype 1 and apathogenic serotype 2. Serotype 1 is further classified into three strains; classical virulent, very virulent and antigenic variant according to its virulence.

Vaccination of birds is a major aspect in preventing IBD. Layers are vaccinated with inactivated oil-emulsified vaccines in order to achieve high titre of maternally derived antibodies (MDA) that prolong over the entire laying period. Chicks are vaccinated using live vaccines which are categorized into three groups as, mild, intermediate, intermediate plus or hot vaccines according to their ability to overcome MDA (Müller *et al*., 2012). Interference of MDA with vaccine uptake is a major constrain in early vaccination of chickens with live vaccines (Müller *et al*., 2012).

Objectives of this study were, application of molecular diagnostic tools for rapid laboratory confirmation of IBD virus and to identify whether the disease is detected in vaccinated flocks.

Methodology

Two small holder poultry farms in Kegalle and Kandy districts were selected and flock history including the vaccination history and mortality pattern and clinical signs were recorded. Post mortem examinations were performed in birds died of clinical signs suggestive

of IBD and clinically normal birds from the same flock. Bursal samples were collected and stored in -20°C until RNA extraction.

Total RNA was extracted using GenElute mammalian total RNA miniprep kit (Sigma) according to manufacturer's instructions. Conventional RT-PCR was performed using a one-step RT-PCR kit (Qiagen) according to manufacturer's instructions using primers that amplify a 474 bp product (Lin et al., 1993) in the variable region of the VP2 gene. PCR products were analyzed in a 1% agarose gel containing ethidium bromide and visualized under a UV transilluminator.

Results

In the layer farm located in Kegalle, a 21 days old flock of 1000 birds was affected. The flock had only been vaccinated with an IBD vector vaccine on day 1. Five deaths were reported up to day 19 and rapid increase of mortality had been observed on following days. Number of deaths were 12 and 60 on day 20 and 21 respectively. In the layer farm located in Kandy, the affected flock was 35 days old with a flock size of 600 birds. The flock had been vaccinated with IBD live vaccine on day 7. Forty birds had died within 3 days of the onset of clinical signs. Continuation of high mortality was reported by both farmers in follow-up inquiries.

Birds in the affected flocks in both farms had showed signs of depression, ruffled feathers (Figure 1A) and reduced appetite. Multifocal ecchymotic haemorrages were observed in bursa (Figure 1B), thigh muscles (Figure 1C) and pectoral muscles of the dead birds.

RT-PCR of the bursal sample revealed the anticipated 474 bp VP2 gene specific fragment (Figure 2). When PCR products were purified, sequenced and aligned with available GenBank sequences using Nucleotide BLAST, it was revealed that the two strains in Kegalle and Kandy farms were different from locally available vaccine strains. RT-PCR gave negative results for clinically normal birds from both flocks.

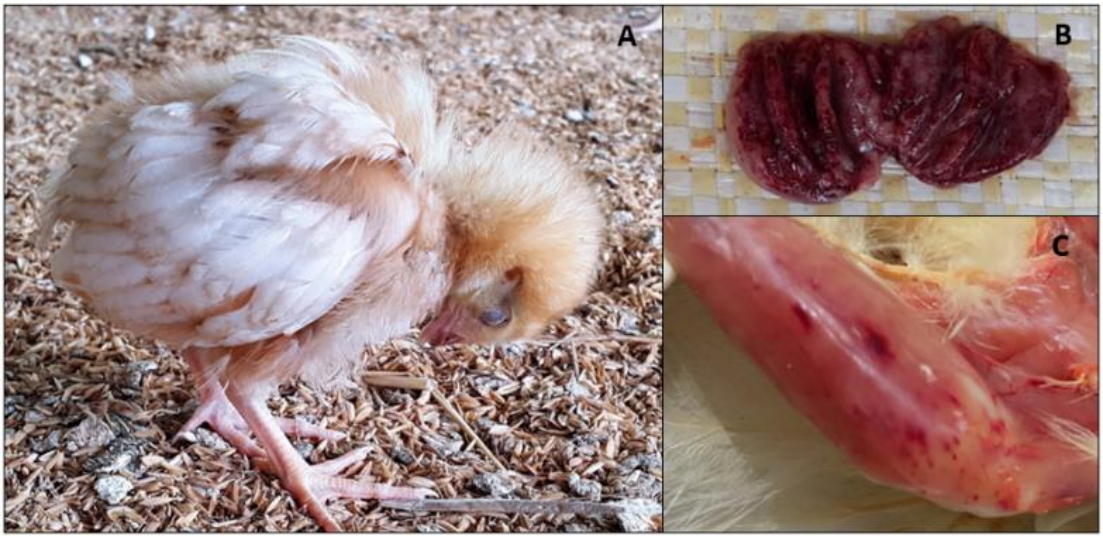

Figure 1: Clinical and postmortem lesions of the affected birds
Depressed bird with ruffled feathers (A), Multifocal ecchymotic haemorrhages in bursa (B) and thigh muscles (C)

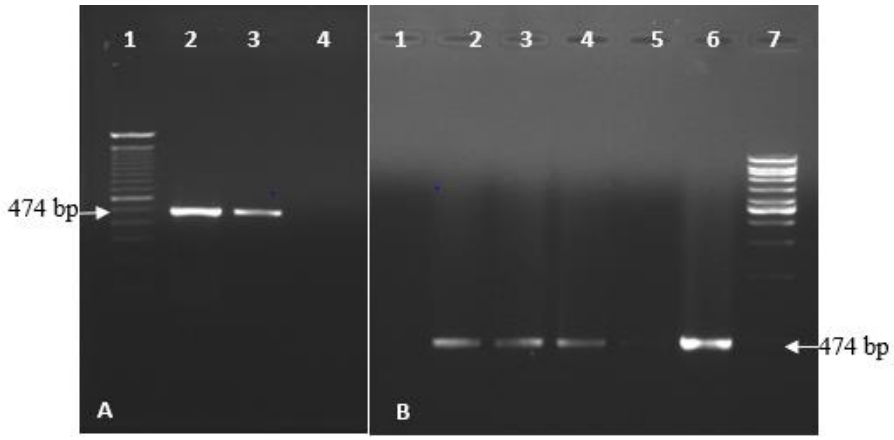

Figure 2: PCR gel image
Kandy Layer farm (A) Lane 1- 100 bp ladder, Lane 2- Positive control, Lane 3- Sample, Lane 4- Negative control
Kegalle Layer farm (B) Lane 1- Negative control, Lane 2- Positive control, Lane 3-5- Routine diagnostic samples not related to this case, Lane 6- Sample from Kegalle farm, Lane 7- 1 kb ladder

Discussion and conclusion

Birds in the affected flocks were within 3 to 5 weeks of age. Mahgoub, 2012 has stated that birds within two to six weeks of age are highly susceptible to the disease. In the present study, depressed birds with ruffled feathers and reduced appetite were observed in both affected flocks. In flocks affected with IBD, distress, depression, ruffled feathers, anorexia and diarrhoea are the major clinical signs observed (Mahgoub, 2012). Except diarrhoea, the clinical signs we observed are in accordance with the literature. During postmortem, multifocal haemorrhages were present in the BF of the affected birds. According to literature, changes in the BF varies throughout the course of the disease. Initially, bursa increase in size and weight due to edema and hyperaemia and then returns to the normal size and continues to atrophy. A gelatinous, yellowish transudate covering the serosal surface can be observed at the initial stages and extensive haemorrhages on the entire BF is observed towards the later stages (Eterradossi and Saif, 2020). We observed haemorrhages mostly in thigh muscles of the affected birds although haemorrhages in thigh and pectoral muscle are a frequently observed gross lesion (Eterradossi and Saif, 2020). Renal changes were observed in the birds from the Kegalle farm. According to Eterradossi and Saif, 2020, increased mucous in intestine and renal changes can be seen in advanced stages of the disease. Considering the flock history, clinical signs and the postmortem lesions observed, IBD was tentatively diagnosed as the cause of high mortality observed in these two flocks.

In the present study, primers that amplify a 474 bp product in the variable region of the VP2 gene was used. The molecular studies confirmed that the flocks were infected with IBD as the RT-PCR product gave the anticipated 474 bp IBDV VP2 gene specific fragment. Segment A of the bi segmented IBDV genome encodes viral protein 2 (VP2), a major structural protein (Brandt *et al*., 2001). The variable region in the VP2 gene is frequently used in phylogenic studies as it contains the determinants responsible for antigenic variation (Brandt *et al*., 2001). Layer flock at Kegalle farm had been vaccinated according to the recommended protocol. However, it was observed that the vaccination at Kandy farm is incomplete. IBD has occurred despite vaccination in these two farms. Vaccination failure could occur due to high level of MDA at the time of vaccination (Müller *et al*., 2012). If the circulating and

infecting wild type virus does not match with the vaccine virus strain there can be a failure in producing a protective immune response (Müller *et al*., 2012). In addition, improper administration of the vaccine could lead to a vaccination failure.

Initial sequencing results of isolates from both farms show highest nucleotide homology to Middle Eastern field IBDV isolates. However, further sequencing and phylogenetic studies are ongoing and, the findings will be communicated in the future as a separate scientific publication. Our study shows that despite vaccination IBD continues as a major threat to the local poultry industry. Therefore, further investigations to find the route course for this problem is a timely requirement.

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