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CASE REPORT

Infectious bronchitis associated with *Escherichia coli* infection in commercial broiler chickens: a case report

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ABSTRACT

Objective: In this case report, we have investigated the infectious bronchitis (IB) virus (IBV) outbreak with the co-infection of *Escherichia coli* in 28–33-day-old broiler chickens in Malaysia.
Materials and Methods: A farmer complained that Cobb 500 chickens, raised in the open house, were having bloody diarrhea, open mouth breathing, non-uniform growth, and ruffled feathers. The mortality was about 100 birds (from about 7000 birds) per day. The sick birds were isolated and subjected to physical examination, postmortem, and histopathological analyses. Gross lesions were observed and recorded. The lung samples have proceeded with histopathological evaluations. The lungs, kidneys, trachea, air sac, and heart samples were collected to isolate bacteria and fungi through a series of conventional cultural methods, followed by molecular confirmation of the IBV.
Results: Postmortem examination revealed air sacculitis, hemorrhagic tracheitis, pulmonary congestion, fibrin deposition in the liver and air sac, hemorrhagic enteritis, and renomegaly. The bacterial culture and biochemical tests revealed *E. coli* in the lungs, trachea, liver, intestine, and kidney samples. However, no fungus could be isolated from those samples. Histological evaluation of lung samples demonstrated infiltration of inflammatory cells in the pulmonary tissues. Apart from this, reverse transcription-polymerase chain reaction confirmed the presence of avian coronavirus responsible for infectious bronchitis (IB).
Conclusion: The chickens were diagnosed with IB concurrent with *E.coli*. The chickens exhibited typical nephropathogenic strain of IBV infection, causing high mortality.

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KEYWORDS

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Introduction

Infectious bronchitis (IB), caused by infectious bronchitis virus (IBV), is a contagious disease of avian species that leads to considerable economic loss to farmers [1]. The prevalence of IB has not been well documented in Malaysia. However, the phylogenetic analysis demonstrated that the QX strains of IBV are the predominant (about 47%) strains in Malaysia [2]. Avian coronavirus belongs to single-stranded positive sense, enveloped RNA virus under the genus *Gammacoronavirus* of family Coronaviridae. The morbidity rate, especially in young chickens, can reach up to 100%. However, the mortality rate varies from 0% to 82%, depending on several factors, including the type of strain involved, viral load, and the bird's health status [1].

There are two forms of IBV: respiratory and nephrogenic. Birds of any age are inevitably susceptible to IB, but the infection is more severe in young chickens. The spike S1 glycoprotein present in the virus structure was believed to play a significant role in its virulence property [3]. In breeder and layer chickens, some strains can affect the kidney and oviduct, causing nephritis and a drop in egg production. The transmission of IBV occurred through aerosol and direct contact with infected chickens. Fecal shedding from the infected chickens and contaminated objects may contribute to the virus's spread [4]. The IB is

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an immunosuppressive disease. Colibacillosis caused by pathogenic *Escherichia coli* is often found as the secondary disease with the IB, severely affecting the chickens with the manifestations of acute septicemia, followed by death. This case report describes the outbreak of IB disease co-infection with *E. coli* in a commercial broiler farm in Malaysia.

Case Presentation

This study's protocols were reviewed and approved by the Animal Ethics Committee Faculty of Veterinary Medicine, Universiti Malaysia 2019 [No. UMK/FPV/ACUE/FYP/2/2019].

A farmer complained that the chickens had bloody diarrhea. Therefore, the farmer administered a coccidiostat. The clinical signs disappeared, but the mortality was still high. The mortality of 12-day-old chicks was 100 birds per day. The farmer has two houses, which he has been operating for 3 years. Each house was occupied with 6,640 and 7,500 birds, respectively. The breed was Cobb 500, and the age was around 28–33 days. Upon entering the house, a strong odor was smelt. The management system was "all-in-all-out." Vaccination against Newcastle disease and IB was administered at the hatchery.

Upon physical examination, it was observed that the chickens were having open mouth breathing, non-uniform growth, ruffled feathers, and wet droppings. The sick chickens were isolated from the flock. The postmortem was carried out from 10 moribund and 10 dead chickens. Gross lesions were observed and recorded. Samples of lungs, kidneys, trachea, air sac, and hearts were collected for bacterial and fungal isolations. Nutrient and MacConkey agar were used for bacterial isolation, while sabouraud dextrose agar was used for fungal isolation. There was bacterial growth on nutrient and MacConkey agars, and no growth was found on fungal agar. Then, bacterial identification and isolation were made based on colony morphology, Gram stain, catalase production, and biochemical tests [5,6]. Colony morphology showed a large, moist, and greyish white colony on the nutrient agar, while on the MacConkey agar (lactose-fermenter), flat, dry, and pink colonies appeared. Gram stain characteristics showed Gram negative (red) with a rod in shape. It also produces bluish black colonies with dark centers on Eosin Methylene Blue agar. Biochemical tests were displayed as positive for catalase production, methyl-red, indole test, Hydrogen sulfide production, and negative for oxidase test, Voges–Proskauer test, citrate, and urease test.

The postmortem findings revealed the presence of hemorrhage with excessive deposition of mucus in the trachea, suggestive of a hemorrhagic tracheitis, as shown in Figure 1a. The air sac was cloudy with deposition of opaque and yellowish material indicating caseous exudate, as shown in Figure 1b. The lungs were congested, suggestive of pulmonary congestion, as shown in Figure 1c. The kidneys and liver were enlarged, and there was a presence of fibrin deposition in the liver and peritoneum cavity, as shown in Figure 1d and Figure 1e, respectively. Additionally, mucosa of the intestine had petechial hemorrhages, suggesting hemorrhagic enteritis, as shown in Figure 1f. Differential diagnosis was made with infectious bronchitis, infectious laryngotracheitis, infectious coryza, chronic respiratory disease, and aspergillosis.

Furthermore, a standard diagnostic work-up with histopathology and reverse transcription-polymerase chain reaction (RT-PCR) was conducted. Samples of lungs, trachea, and kidneys were subjected to RT-PCR for avian coronavirus. An amount of 50 ng/µl of extracted RNA was used as a template and nuclease-free water was used as a negative control. At first, 5 µl of RNA was pre-heated at 95°C and added to 20 µl of one-step RT-PCR reaction master mix containing 12.5 µl of 2× Access Quick buffer, 0.5 µl of AMV reverse transcriptase, 0.5 µl of *Taq* polymerase, 0.2 µl of RNasin ribonuclease inhibitor, and 20 pmol of both forward primer UTR-1 GCT CTA ACT CTA TAC TAG CCT AT and reverse primers UTR-2 AAG GAA GAT AGG CAT GTA GCT T. The reaction was carried out in a thermal cycler (Biorad, T100). The samples in the reaction mixture were incubated for 1 h at 42°C, followed by 5 min at 65°C, 35 cycles of amplification (denaturation: 30 sec at 92°C, annealing: 30 sec at 50°C, elongation: 30 sec at 72°C), and a final extension for 5 min at 72°C.

Results and Discussion

The samples from lungs, kidneys, trachea, intestine, and liver were positive for *E. coli*, but negative for fungus. The presence of *E. coli* in organs other than the gastrointestinal tract is unusual. This phenomenon commonly happens when the bacteria enter the bloodstream and cause septicemia and spread into other internal organs [7].

The histopathological assessment demonstrated the infiltration of inflammatory cells and the lungs congestion, as shown in Figure 1g. This correlates with the clinical signs, such as gasping and open mouth breathing of chickens, indicating that the virus is localized in the trachea and lungs. Renomegaly, congestion, and nephritis are suggestive of nephropathogenic IBV strains.

Molecular detection of IBV using RT-PCR showed that the chickens were positive for avian coronavirus, which caused IB (Fig. 2). RT-PCR assay is a reliable test for the confirmation of IBV, and it was comparable to virus isolation assay targeting the S1 glycoprotein gene [8].

The yellow, cheesy exudate covered the liver, and the air sac was with fibrin. It is formed from lipopolysaccharide endotoxins released from *E. coli*. However, IBV infection caused immunosuppression in the chickens, thus triggering pathogenic *E. coli* to overhaul. The bacteria entered the

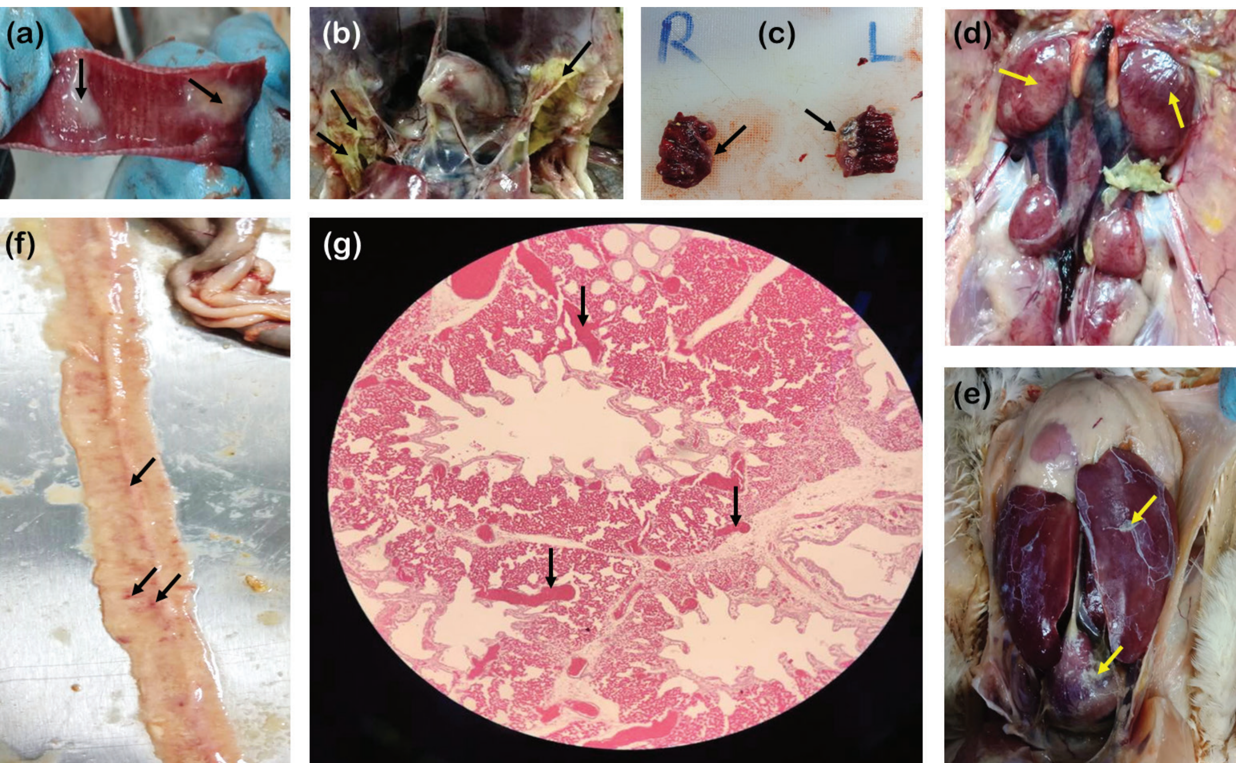

Figure 1. Gross histological features recorded in this study. (a) Gross lesions in the trachea of infected chicken. Note the hemorrhagic tracheitis with excessive mucoid deposition (arrow). (b) Gross lesions in the air sac of the infected chicken. Note the yellowish, cheesy appearance suggestive of air sacculitis with caseous exudation (arrow). (c) Gross lesion in lungs of infected chickens. Note the dark red area suggestive of pulmonary congestion (arrow). (d) Gross lesion in the kidney of infected chickens. The kidneys were swollen and dark-red (arrow), suggestive of interstitial nephritis. (e) Gross lesions in the liver of infected chickens. The liver was enlarged with fibrin deposition at the surface. Note the presence of fibrin at the peritoneum area (arrow). (f) Gross lesion in the intestine of infected chicken. Thinning of the intestinal wall was noticed with petechial hemorrhages on the mucosa of the jejunum, indicating hemorrhagic enteritis (arrow). (g) Infiltration of inflammatory cells and congestion of the lung (arrow).

bloodstream and caused sepsis. During sepsis, inflammatory cytokine, chemokine, neutrophil, macrophage, and platelets were released massively. This lead to an increase in vascular permeability and stimulation of platelets to release prothrombin activator and Ca^{2+}. The clotting factor could activate the clotting pathway of fibrinogen to fibrin formation. Neutrophil and platelets adherence to the endothelium of blood vessel resulted in a massive efflux, thus causing fibrin and exudative fluid deposited onto the peritoneal cavity and other organs. In this case, the chickens were infected with IBV even though they were vaccinated during 1 day old. This might be on account of insufficient lifelong protection or improper vaccination. Antibody started to deplete 2 weeks after the initial vaccine injection. The second vaccination is required to prime up the antibody level in the body. Another possibility is that IBV serotypes from the available vaccine are not antigenically similar to field strain; thus, the vaccine does not ensure complete cross-protection [3]. Being an RNA virus, IBV quickly mutates and persists as numerous serotypes and strains. Hence, it would be challenging to provide comprehensively holistic protection. Currently, there are five groups of IBV strains detected in Malaysia [2]. According to Amarasinghe et al. [9], vaccination against IBV is not a guaranteed control method. This is in agreement with Feng et al. [10]. Hence, it is reported that a new generation of immunization is needed to cover various emerging strains of IBV. Other methods, including necessary biosecurity measures, are essential to prevent IBV outbreaks in poultry farms [10].

To date, there is no treatment available to eliminate IBV. However, control strategies may be applied to reduce the mortality rate and alleviate the effects of secondary bacterial infection by providing antibiotics, improved ventilation, and control of the farm's stocking density. Multivitamin or vitamin C (5 gm/l) is recommended to be given in drinking water for 3 days as supportive treatment. In the prevalent case, we did not recommend antibiotics administration because the chickens were close to market age.

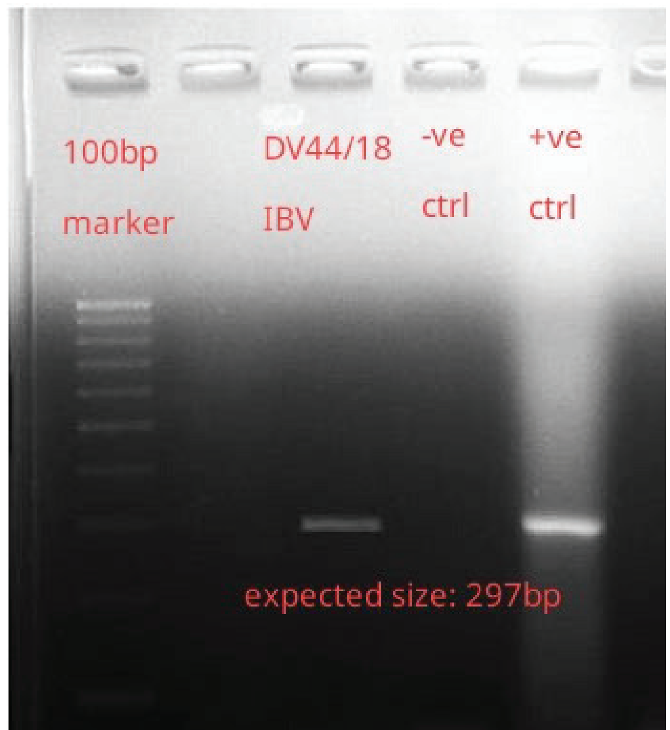

Figure 2. RT-PCR of IBV showing sample ID number DV44/18 as positive since a prominent band appeared as the same size corresponding to a positive control (known sample of IBV virus).

Conclusion

Based on the clinical signs, postmortem, histopathology, and RT-PCR, it was concluded that the broiler chickens were affected with IB concurrent with *E. coli*. The chickens exhibited a typical nephropathogenic strain of IBV infection, which caused a high mortality rate resulting in high financial setbacks for the farmer.

List of abbreviations

IB = Infectious bronchitis; IBV = Infectious bronchitis virus; RT-PCR = Reverse transcription polymerase chain reaction.

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Conflict of interest

The authors declared no conflict of interest regarding this case report paper.

Authors' contribution

Muhammad Luqman Nordin handled the case. Norhanizam Nordin, Nani Izreen Mohd Sani, Rumaizi Shaari, Maizan Mohamed, and Mohd Farhan Hanif Reduan conceived, interpreted the results, and drafted the manuscript. Arifah Abdul Kadir reviewed the manuscript.

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