

Infectious Bronchitis Virus: a major cause of respiratory disease outbreaks in chickens in Ghana

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Abstract

Infectious Bronchitis Virus (IBV) causes respiratory diseases with significant economic losses in the global poultry industry. The possible presence of this pathogen in the commercial poultry industry in Ghana has not yet been evaluated and thus there is no control strategy in place. In the present study, the RT-PCR technique was used to detect IBV in respiratory disease outbreaks in 6 chicken farms in the Ga East district of Accra. RNA was extracted from tracheal swabs of birds with respiratory signs and used in an RT-PCR to amplify the S1 glycoprotein gene of IBV. Forty seven (47) samples were obtained and analyzed during a 3 month period from broilers, layers and broiler breeders. Thirty (30) of the samples were positive for IBV by RT-PCR, giving a prevalence of 64%. This study confirms the presence of IBV in the study area and provides initial evidence of IBV involvement in respiratory disease outbreaks. Additional studies including virus isolation attempts and characterization will provide necessary data to outline and implement an effective control strategy in the country.

Keywords: Infectious Bronchitis Virus, RT-PCR, chickens, Ghana

1. Introduction

Infectious Bronchitis (IB) is an acute, highly contagious disease of the upper respiratory and urogenital tract of chickens (Cavanagh & Gelb, 2008). The etiological agent of IB is Infectious Bronchitis Virus (IBV), a member of the group 3 Coronavirus genus. The viral genome is enveloped, single stranded, positive sense RNA with a size of about 27kb (Cavanagh & Gelb, 2008). The disease is worldwide in distribution and economically important to the poultry industry. Clinically, IB is characterised by tracheal rales, cough, sneeze, nasal discharge and gasping. Mortality is low but morbidity is high. Nephropathic strains can cause up to 30% mortality in young birds. Among laying flocks, egg production drops below 10% to 50% depending on the presence of other factors complicating the situation. IBV also severely affects the quality of eggs that are produced. Eggs may have thin shells, be misshapen, and have thin watery albumin. In broilers, there is reduced feed efficiency resulting in poor weight gain. IB is airborne and the mode of transmission of the virus is via the respiratory route through droplets expelled by coughing or sneezing (Cavanagh & Gelb, 2008). Horizontal transmission from farm to farm is possible through movement of people with contaminated footwear, clothes, equipment and vehicles. IBV can be controlled primarily by the mass application of modified live vaccines (Sellers *et al.* 2008) and is used routinely in many prone areas to control the disease and minimise its effect.

Respiratory diseases of unknown etiology continue to characterise the commercial poultry industry in Ghana contributing significantly to production inefficiencies and economic losses to farmers in the industry. Although IBV is worldwide and vaccines are available for their control, IBV vaccination are not carried out in Ghana. This is probably due to the lack of evidence of the presence of this pathogen and knowledge of the extent of the activity of the pathogen in the country.

The objective of our study was to detect the possible involvement of Infectious Bronchitis Virus in the outbreaks of respiratory diseases in chickens in Ghana using the Reverse-Transcriptase Polymease Chain Reaction (RT-PCR) technique.

2.0 MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the Ga East district, an urban community in the Greater Accra region of Ghana from November, 2011 to January, 2012. Small-scale commercial poultry production is intensively carried out in this district. In addition, livestock, particularly cattle, sheep and goats are also kept by farmers in this district.

2.2 Farm selection and sampling

Small scale commercial poultry farms in the district were visited and the study was explained to the farmers for their consent. Farmers were encouraged to voluntarily report outbreaks of respiratory diseases on their farms to the Animal Research Institute (ARI). Upon receipt of information from farmers, the diagnosis team visited the farm. The affected flock was examined by a Veterinarian and birds showing signs typical of respiratory diseases were isolated from the main flock and observed for at least 1 hr with feed and water provided. Characteristically, the birds were depressed and weak. Some birds gasped for air, coughed and sneezed with ocular and nasal discharge observed. Feed intake was poor among some birds and greenish to brown watery diarrhoea was seen at the anus of some birds.

2.3 Sampling

From each diseased flock on a farm, five (5) birds were randomly handpicked for sampling. A cotton swab was used to firmly swab the trachea of the birds using one (1) cotton swab per bird. Swab was immediately put in a labeled 2ml Eppendorf micro centrifuge tube containing 500µl of 0.1M PBS and firmly closed. Samples were immediately put in a cool box and transported to the Molecular Biology Laboratory of the Animal Research Institute (ARI) for further processing.

2.4 Viral RNA extraction

The tube containing the swab was vigorously vortexed for about 20sec. Sixty microlitres (60µl) of this sample was pipetted with a 200µl barrier pipette tip into a pre-labeled 2ml sterile micro centrifuge tube. The Qiagen RNeasy Mini Kit was used to extract RNA from all the samples following the manufacturer's instructions. RNA was eluted in 60µl RNase free water. The extracted RNA was stored at -20°C until needed. The Massachusetts antigen (M-41) (GD, Health Services, Deventer, Netherlands) and PBS (1X) were added as a positive and negative extraction controls respectively.

2.5 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed using the IBV-LC3 and IBV-LC5 primer pair that targets a 383bp segment of the S1 gene common to all Infectious Bronchitis Virus in a 200µl thin walled PCR tube in a final reaction volume of 25µl. The One-Step RT PCR kit from Qiagen was used following the manufacturer's instructions with some slight modifications. Briefly into each tube, 13µl of RNase free water was added. This was followed by the addition of the following: 5µl of 5X One-Step RT-PCR buffer, 1µl dNTP mix (10mM), 1.25µl of each primer (10pmol of each primer) and 1 µl enzyme mix(5U/µl). 2.5µl RNA extract was added. The amplification was carried out in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems). For the cycling conditions; Reverse Transcription for synthesis of complimentary DNA was carried out at 45°C for 60min, followed by 94°C for 5 mins. This was followed by 40 cycles of 94°C for 45sec, 51°C for 1min and 72°C for 90sec and a final extension at 72°C for 5min. Four controls were added in each reaction. An extraction negative control (1X PBS), extraction positive control (M-41), PCR positive control (M-41) and PCR negative control (nuclease free water). The RT-PCR products were analyzed by gel electrophoresis on a 1% agarose gel containing ethidium bromide, visualized by a High Performance UV trans-illuminator (UVP) and photographed.

3.0 Data analysis

Data was entered in Microsoft Excel, 2007 and analysed using descriptive statistics. The prevalence of IBV among flock was calculated as follows:

$$\text{Prevalence} = (\text{Number of positive samples} / \text{Total number of samples analysed}) \times 100$$

4.0 RESULTS AND DISCUSSION

A total of six (6) farmers in the study district voluntarily reported outbreak of respiratory disease on their farms during the 3 month period. Forty seven (47) samples were collected and analysed from ten (10) affected flocks. The flocks were made up of broilers, layers and broiler breeders. All the farms except one (broiler only), had at least two flocks of multiple ages (layers and/or broilers on the same farm) present at the same time. The ages of the birds ranged from 5 to 64 weeks of age. As shown in Table 1, the broilers were 5- 8 weeks old and broiler

breeder flock was 64 weeks. Among layers, the highest age recorded was 52 weeks and the minimum was 41 weeks. The flock size varied from 150 birds in broilers to 850 birds for layers (Table 1).

Table 1: Characteristics and age distribution of flocks sampled in study

Bird type	Number of flocks tested	Number of samples analysed	Age of birds (weeks)	Flock size
Broilers	3	15	5-8	150 - 400
Layers	6	30	41-52	300 - 850
Broiler breeder	1	2	64	500
Total	10	47		

The primer pair LC3 and LC5 amplifies segment of the S1 glycoprotein gene of IBV given a product size of 383bp (Figure 1). The S1 gene was detected in 30 of the test samples. Two-thirds (2/3) of the broiler samples were positive for IBV. More than 50% of the layers tested positive. Seventeen (17) samples tested negative to IBV. The overall prevalence of IBV in the total samples analysed was 64% (Table 2).

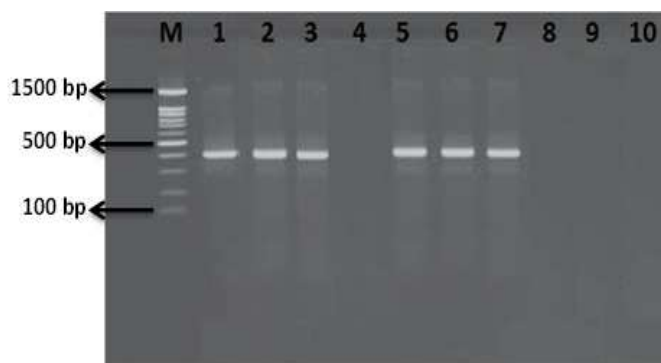


Figure I: 1% agarose gel stained with Ethidium bromide. Lane M: 100bp ladder, 1: positive extraction control (M-41); 2: positive PCR control (M-41); 9: negative extraction control (PBS); 10: negative PCR control (nuclease free water); 3, 5, 6, 7: positive samples; 4, 8: negative samples

Table 2: Reverse Transcription polymerase chain reaction results for Infectious Bronchitis Virus in tested samples

Type of bird	Number of birds tested	Number of positives detected	Prevalence (%)
Broiler	15	10	66.67
Layer	30	18	60.0
Broiler breeder	2	2	100.0
Total	47	30	63.8

Respiratory diseases are caused by a host of pathogens that elicit similar clinical signs. Laboratory confirmation is therefore needed to identify the specific pathogen(s) involved in a disease. Diagnosis of poultry diseases in Ghana is based mainly on clinical signs and gross lesions with limited laboratory investigations used for confirmatory diagnosis.

The Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) method is extensively used in the rapid and accurate diagnosis of IBV in many advanced laboratories. The method is sensitive and rapid and can be applied directly on samples from many sources including tracheal swabs (Rameneek *et al.* 2005). Tracheal swabs were used in the present study due to the high titers of virus that exist in the acute phase of IBV infection making it a suitable sample for viral detection in the live bird (Chandra 1987; Lucio & Fabricant 1990).

The S1 subunit of the Spike (S) glycoprotein of IBV has been the target for many RT-PCRs for IBV identification as well as for genotyping of IBV isolates by both sequencing and Restriction enzyme analysis (Jack & Jackwood 2008). The primer pair used in this study detects a region within the S1 gene without discriminating between IBV genotypes and has been used in several IBV molecular detection assays (Jackwood *et al.*, 2003; Roussan *et al.* 2009). Commercial chickens in Ghana are not vaccinated against Infectious Bronchitis Virus. Therefore, the detection of the S1 gene by RT-PCR is an indication of the presence of field IBV isolates excluding the possibility that the detected IBV could be a vaccine strain (Cavanagh *et al.* 1999). The detection of the S1 gene in test samples confirms the presence of IBV in the district and provides indirect evidence for at least contributing to the respiratory disease outbreaks on the poultry farms.

The high prevalence of IBV is likely a result of a lack of control interventions due to limited knowledge on the pathogen and extent of its activity in the country. Most of the farms typically have multiple aged birds an indication that chicks are brought in at different times in a production season. This may affect the spread of the pathogen on the farm and hence the prevalence of the disease. With the exception of two farmers, all the other farmers primarily kept layer flocks. Farmers have intensified poultry production and included broilers to take advantage of the festive Christmas season (study period). However, the numbers of broilers kept are low (Table 1) probably among other factors is the continual flooding of the Ghanaian market with relatively cheaper broiler meat from other countries (GAINS 2013) which makes local broiler production uncompetitive and a significant contributor to the decline in broiler production (Aning *et al.* 2008).

The high prevalence of IBV detected (Table 2) in this study indicates IBV is endemic in the district. The poor to complete absence of Biosecurity in the farms (data not shown) could aid in the entry and spread of the infection between and within flocks (Sellers *et al.* 2008). The absence of control interventions as a result of limited knowledge of the pathogen and extent of its activity in the country could also account for the high prevalence observed. Cook (2008) proposed that a careful and correct administration of live attenuated and inactivated vaccines can be effective in controlling infection among flocks. Vaccines stimulate the production of antibodies in the upper respiratory tract of the birds and protect them against IBV and reduce viral shedding. The high occurrence in the broiler flock could be attributed to the continual shedding of the virus by infected older layer flocks on the farms and possible transmission to broiler flocks aided by farm attendants. Infectious Bronchitis Virus may be shed through the respiratory tract and faeces and may even continue for several weeks after clinical recovery. It could also be attributed to the high susceptibility of broiler flocks to IBV compared with layers and breeders (Cavanagh & Gelb, 2008). The lack of IBV detection in some of the test samples may suggest the involvement of other respiratory pathogens in the respiratory disease outbreaks in chickens in the study area. This agrees with studies elsewhere, where *Mycoplasma gallisepticum*, Avian Influenza and others have acted alone or in concert with each other to cause respiratory disease in chickens (Roussan *et al.* 2008). The present study was however not designed to identify other pathogens that may be involved in the respiratory disease outbreaks.

5. Conclusion

This study shows that Infectious Bronchitis Virus is highly involved in respiratory disease outbreak in commercial poultry farms in the Ga East district of Greater Accra region. The RT-PCR method can be used as a diagnostic tool to conduct epidemiological studies of IBV in the country. The virus should be isolated and characterized for a suitable control strategy to be adopted to protect the birds against the disease and its associated losses to the poultry industry.

Acknowledgement

Authors are grateful to Management of CSIR- Animal Research Institute for sponsoring the study. The following Drs: F. Y. Obese, E. K Adu and K. Boa-Amponsem are deeply appreciated for their diverse support.

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