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Molecular and Clinical Study of Caseaous Bronchopneumonia in Broilers

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ABSTRACT

The industrialization of poultry production and the application of intensive rearing systems have boosted the incidences and importance of respiratory diseases. Information regarding avian respiratory diseases has become more urgent as they are responsible for increasingly severe economic losses. In the present study, 50 cases showing caseaous bronchopneumonia were tested for the presence of four respiratory pathogens. Test results showed that of the test group 56% were positive for infectious bronchitis virus (IBV), 14% were positive for both IBV and Newcastle Disease Virus (NDV) , 8% were positive for NDV, 8% were positive for Avian Influenza Virus (AIV), 2% were positive for mycoplasma gallisepticum (MG) and 2% were positive for both IBV and MG. Additionally, 10% were found negative to all four tested pathogens. Our data shows that IBV and NDV contribute to 78% of infections, either mixed or alone. Furthermore, the clinical finding of caseaous bronchopneumonia cannot be depended on in the diagnosis of respiratory diseases as multiple diseases share the same finding.

KEYWORDS respiratory diseases, poultry, PCR, Infectious bronchitis

Introduction

Respiratory diseases are of increased importance due to their role in high economic losses in poultry industry (Malik, Patnayak, & Goyal, 2004). The rapid adoption of intensive poultry production systems in the 1950s increased incidence of respiratory disease. In these types of rearing systems, a large number of birds are kept in a small area. This led to increased stress and respiratory distress and made the birds more vulnerable to infection by respiratory pathogens(Bradbury, 1984). Moreover, the use of live virus vaccines has increased the incidences and susceptibility of birds to infection with pre-existing respiratory pathogens such as mycoplasma gallisepticum. These types of vaccines are proved to cause damage to the respiratory epithelium making the bird more susceptible to secondary infections(Glisson, 2013).In Iraq, the study of respiratory diseases in poultry are of increased importance as a recent study showed that 59.31% of broiler cases submitted to Babylon Veterinary Hospital were respiratory diseases (Almremdhy, 2014).

The etiology of respiratory illnesses is complex and often involves multiple pathogens that contribute to the final clinical outcome (Kleven, 2008). A wide variety of pathogens have been associated with the etiology of poultry diseases including: avian influenza virus (AIV), newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious laryngotracheitisvirus and avian pneumovirus (VILLEGAS, 1998; Roussan, Haddad, & Khawaldeh, 2008).Among bacterial pathogens, the most common pathogens include Mycoplasma gallisepticum (MG), Escherichia coli, Ornithobacterium rhinotracheale, Haemophilus paragallinarum,Bordetella avium and Pasteurellamultocida, (El-Sukhon, Musa, & Al-Attar, 2002; GLISSON, 1998). Environmental and other noninfectious stress factors as well as immunosuppressive agents have also been reported to trigger respiratory diseases (Glisson, 2013).

Materials and Methods

Sampling

The study included 50 farms submitted to a private veterinary clinic in Sulaymaniyah during the period from January 21, 2015 to May 4, 2015. The recorded cases were suffering from respiratory illness and manifested Caseaous bronchopneumonia during necropsy. Lungs, tracheas and tracheal secretions were collected aseptically from 3-5 birds per farm and placed in sterile petri dishes. A sterile surgical blade was then used to cut small pieces from each organ. These pieces were then processed to become paste-like in consistency. This process helped to liberate the pathogens' nucleic acids and facilitate DNA and RNA extraction.

RNA extraction

RNA extractions were done using a Total RNA Mini Kit (Tissue) developed by Geneaid Biotech Ltd. All of the manufacturer's recommended protocols were followed.

DNA Extraction

DNA extractions were done using a Genomic DNA Mini Kit (Tissue) developed by Geneaid Biotech Ltd. All of the manufacturer's recommended protocols were followed.

Oligonucleotides used for molecular detection

Primers used in the study were produced by Macrogen®, Korea. The sequences of each primer are listed in Table 1 along with their references.

Table 1 Oligonucleotides used for molecular detection

pathogens	Primer	Sequence	Gene fragment	Amplicon	Reference
NDV	NDV F	5'-GGT GAG TCT ATC CGG ARG ATA CAA G-3'	F Gene	202bp	(Creelan, Graham, & McCullough, 2002)
	NDV R	5'-TCA TTG GTT GCR GCA ATG CTC T-3'			
MG	RNAG1	5'- TAA CTA TCG CAT GAG AAT AAC-3'	16S rRNA gene	330bp	(Kempf, Blanchard, Gesbert, Guittet, & Bennejean, 1993)
	RNAG2	5'- GTT ACT TAT TCA AAT GGT ACA G-3'			
AIV	M30F2/08	5`- ATGAGYCTTYTAACCGAGGTCGAAACG -3`	Matrix (M) Gene	244bp	(WHO, 2014)
	M264R3/08	5`- TGGACAAANCGTCTACGCTGCAG -3`			
IBV	f-IBV-S1	5`- GTT TAC TACTAC CAA AGT GCC TT -3`	S1 gene	448bp	(Rauf, 2014)
	r-IBV-S1	5`- GTG TAA ACA AGG TCA CCA TTT A -3`			
Housekeeping gene for poultry	foreword	5`- TGAGAACTACGAGCACAAAC -3`	12S RNA	183bp	(Rashid , Babashekh, Marouf, & Amin, 2014,)
	Reverse	5`- GGGCTATTGAGCTCACTGTT-3`			

RT-PCR amplification
For one-step RT-PCR the thermocycler was programmed for 42 °C for 60 minutes, an initial denaturation at 95°C for 5 minutes, 40 cycles consisting of denaturation at 95°C for 30s and annealing at 52°C for 30s for IBV and AIV primer. The annealing temperature for NDV primers, however, was 55 °C for 30 s with an extension at 72°C for 30s. The final extension phase ran at 72°C for 5 minutes.

PCR amplification
For amplification of MG, the thermocycler was programmed for an initial denaturation at 95°C for 5 minutes, 40 cycles consisting of denaturation at 95°C for 30s, annealing at 55°C for 30s and extension at 72°C for 30s. The final extension

Analysis of PCR product.
PCR products were mixed with Green-DNA Dye then loaded to 1% agarose gel. Electrophoresis were done using the Safe-Blue Illuminator/Electrophoresis System. The amplicon of PCR products (Table 1) were analyzed according to the migration pattern of a 100 bp DNA ladder.

Results and Discussion
Respiratory cases that manifested caseaous bronchopneumonia (Figure 2) during necropsy were tested for the presence of the nucleic acid of four respiratory pathogens. The polymerase chain reaction (PCR) test results showed that 56% of the cases were positive to infectious bronchitis alone, 14% were positive to both IB and ND and 8% were positive to ND alone. Avian influenza virus was detected in 8% of the cases and mycoplasma gallisepticum single infection was detected in 2%. In addition, 2% of cases were positive to both IB and MG. Of all studied cases, 10% were negative to the four tested pathogens (Figure 1). The electrophoresis of PCR product is shown in Figure 3.

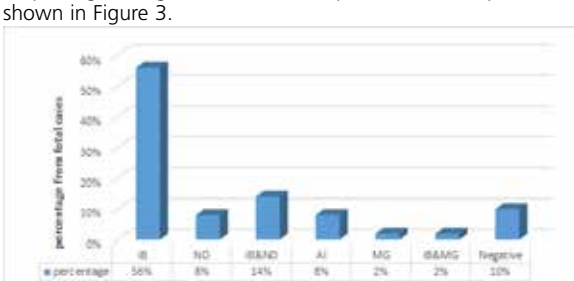


Figure 1 A bar chart showing the percentage of each diagnosis of all cases in the study

Furthermore, Infectious bronchitis was detected in 70% of the cases and 14% were mixed with NDV. NDV was detected in 22% of total cases. IB and ND either mixed or alone contributed to 78% of total cases.

While other workers also reported diagnose of the same respiratory disease (Roussan, Haddad, & Khawaldeh, 2008; Al-Dabhawe, Kadhim, & Samaka, 2013; Ahmed, et al., 2009; Hadipour, Habibi, Golchin, Hadipourfard, & Shayanpour, 2011; Al-Mohana, et al., 2013), our results do not match any of the previously published papers. The percentages of each disease diagnosis of total cases were different from the results of other researchers. The difference in the results could be due to the variation in geographical areas, a differing epidemiological status and a differing season in which the studies were conducted.

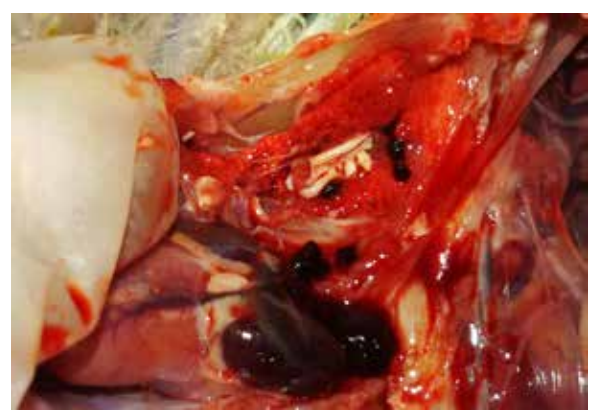
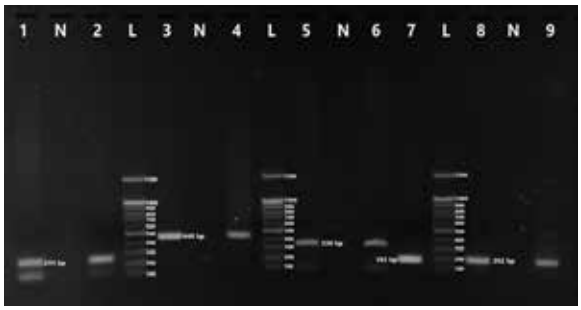


Figure 2 A photograph showing causational exudate appear in lung section

Similar studies focused on respiratory disease in general without special reference to caseaous bronchopneumonia. Roussan et al (2008) reported more mixed infections than our study. They reported 15.7% of respiratory cases mixed between AI and IB, 9.6% between IB and ND, 11.3% between AI and ND, 10% between IB and MG and 5.2% between ND and MG.

The cases that were found negative to the four tested pathogens could possibly be caused by other viral or bacterial pathogens. Escherichia coli, Mycoplasma synoviae Ornithobacterium rhinotracheale, Haemophil us paragallinarum, Bordetella avium, Chlamydophila psittaci and Staphylococcus aureus are among the bacterial pathogens that are reportedly causing respiratory diseases (GLISSON, 1998; Ferguson-Noel, 2013; Andreasen, 2013). Furthermore, Aspergillus fumigatus, a fungal disease is reported to cause caseao us pneumonia in brooding chicks (Abdulrahman , Saeed, & Muhammad, 2013).

From our data, we concluded that caseaous bronchopneumonia is an important clinical manifestation in the four studied pathogens. However, these clinical findings cannot be relied upon in the diagnosis for a respiratory disease. Further studies are necessary in order to further examine other possible causes, as 10% of cases in this study were negative to all studied pathogens.



1) Figure 3 NOT 2
2) the figure is too small and it contains lots of details , could you please put it as wide as possible to reveal the details of DNA analysis

Analysis of some positive and negative samples in 1% agarose gel. Lanes L; DNA ladder, lanes N; negative controls, lane 1; sample positive for AI, lane 2; positive control for AI, lane 3; sample positive for IB, lane 4; positive control for IB, lane 5; sample positive for MG, lane 6; positive control for MG, lane 7; housekeeping gene, lane 8; positive sample for ND, lane 9; positive control for ND.

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