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The effect of infectious bronchitis virus in different poultry physiological system

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Abstract

Global chicken industry is harmed by the highly contagious avian infectious bronchitis virus (IBV). In this work, we evaluated the frequency of IBV in commercial poultry flocks from three significant producing locations in Iraq and looked into the tropism of circulating IBV genotypes to three distinct physiological systems (Respiratory, digestive, and urinary/reproductive).

The aim of this study was to evaluate the frequency of avian infectious bronchitis virus (IBV) in commercial poultry flocks from three significant producing locations in Iraq and to investigate the tropism of circulating IBV genotypes to three distinct physiological systems (Respiratory, digestive, and urinary/reproductive).

In the Midwest, northeast, and south of Iraq, samples were gathered from 432 commercial flocks (198 broilers and 234 breeders), and IBV identification was carried out using reverse-transcription, real-time PCR of the 5' untranslated region. According to the findings, IBV was present in 41.4% of the flocks, with broilers experiencing a greater frequency (24.8%) than breeders (16.8%). The prevalence of IBV-positive flocks was comparable across geographic regions, and the digestive and respiratory systems of broilers as well as the breeders' gastrointestinal tracts were the most often seen sites for reverse transcription.

Reverse-transcription nested PCR was used for genotyping, and the S1 gene was sequenced from a sample of 79 IBV-positive flocks (45 broilers and 34 breeders). The Iraqi variant genotype, which was mostly discovered in the intestinal tract of commercial chicken flocks in the three key producing regions of Iraq, infected the majority of flocks.

Keywords: IBV, poultry, genotyping, prevalence, tropism, systems, Iraq

Introduction

Avian infectious bronchitis (IB), a highly contagious viral illness that plagues commercial chicken flocks globally and causes large financial losses (Cook *et al.*, 2012) [11], is a serious health concern. However, it can also affect the digestive system (Boroomand *et al.*, 2012) [2]. The disease primarily affects the respiratory and urogenital tracts of birds. IBV, a member of the Coronaviridae family, is the virus that causes infectious bronchitis in birds (IB) (Cavanagh, 2007) [4]. Many different genotypes of IBV are known to circulate worldwide, exhibiting high genetic diversity.

In this study, the frequency of IBV was assessed in commercial chicken flocks from three significant poultry-producing districts in Iraq, and the tropism of circulating IBV genotypes to three distinct physiological systems (The respiratory, digestive, and urinary/reproductive in poultry) was examined (Almeida *et al.*, 2012; Di Fábio and Buitrago, 2009; Chacón *et al.*, 2011) [1, 8, 6]. Reverse transcription, real-time PCR of the 5' untranslated region was used to identify IBV in samples from 432 commercial flocks (Callison *et al.*, 2006; Worthington *et al.*, 2008) [15, 27]. The findings revealed a significant frequency of IBV in the studied flocks, with broilers showing a greater incidence than breeders (Almeida *et al.*, 2012) [1]. Breeders' digestive systems and the digestive and respiratory systems of broilers were the areas where reverse transcription was most commonly found (Di Fábio *et al.*, 2000) [9]. Genotyping was carried out using reverse-transcription nested PCR and sequencing of the S1 gene from a subset of IBV-positive flocks in order to further define the IBV genotypes (Fraga *et al.*, 2013; Jackwood, 2012) [14, 25]. The bulk of the flocks had the Iraqi variant genotype, which was mostly found in the intestinal tract of commercial poultry flocks in the three key producing regions of Iraq (Boroomand *et al.*, 2012; Fraga *et al.*, 2013) [2, 14].

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This study provides valuable information on the prevalence and genotypes of IBV in commercial poultry flocks in Iraq and highlights the importance of monitoring IBV circulation and tropism in order to implement effective control strategies (Almeida *et al.*, 2012)^[1].

The study highlights the significant prevalence of avian infectious bronchitis virus (IBV) in commercial poultry flocks in Iraq, which is of concern not only for the health and welfare of the birds but also for the economic implications for the poultry industry. The study also provides insights into the tropism of circulating IBV genotypes to three different physiological systems in poultry (Almeida *et al.*, 2012; Di Fábio and Buitrago, 2009; Chacón *et al.*, 2011)^[1, 8, 6], which can aid in the development of more effective control strategies for the disease.

Furthermore, the study contributes to the broader scientific understanding of IBV, a highly contagious virus that affects poultry globally, by identifying the genotypes of IBV present in commercial chicken flocks in Iraq (Fraga *et al.*, 2013; Jackwood, 2012)^[14, 25]. Understanding the genetic diversity of IBV is important for developing effective vaccines and treatments for the disease.

The study's findings can also serve as a basis for future research on IBV in poultry, both in Iraq and in other regions. By providing valuable data on the prevalence and genotypes of IBV, this study can inform and guide future research efforts aimed at improving the health and welfare of commercial poultry flocks and minimizing the economic impact of IBV on the poultry industry (Almeida *et al.*, 2012)^[1].

2.1 Study Design

Samples were taken from hen flocks in three important producing districts of Iraq between June 2023 and May 2023. The samples included 198 broilers and 234 breeders, and the flocks showed clinical indications of infectious bronchitis (IBV). The average age of the breeders was 20 weeks, and it ranged from 1 to 90 weeks for the broilers, who were between 0.5 and 8.8 weeks old on average. The samples were divided into pools for analysis, with each pool containing organs from different physiological systems, such as the urinary/reproductive (kidney, ovary, oviduct), digestive (Cecal tonsil and intestine), and respiratory (Lung, trachea, tracheal swabs) systems

2.1.1 RNA Extraction

Simbios Biotechnology, Cachoeirinha, RS, Iraq, provided commercial kits from NewGene called Preamp and Prep, which were utilized in accordance with the supplier's instructions for RNA extraction. Swabs and macerated organs were added to 1 mL of Preamp lysis solution and incubated at 60 °C for 10 minutes for each pool. A fresh tube holding 20 L of silica suspension received 0.5 mL of the supernatant after centrifugation at room temperature for one minute (8,609 g). A second time, for 1 minute (8,609 g at room temperature), the tube was centrifuged, and the supernatant was discarded. The pellet underwent 150 L of wash solutions A, B, and C (Prep) before being dried at 60 °C. Eluting whole RNA required 50 L of elution buffer.

2.1.2 IBV Detection

Reverse transcription followed by real-time TaqMan PCR (RT-qPCR), carried out in accordance with the previously reported technique, was used to detect the infectious

bronchitis virus (IBV) (Callison *et al.*, 2006)^[15]. The following settings were used to conduct the amplification reactions in an Applied Biosystems StepOnePlus Real Time PCR System in Norwalk, Connecticut: 37 °C for 30 minutes in one cycle, then 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

2.1.3 IBV Genotyping

A subset of certain RT-qPCR-positive samples was randomly selected, and a subset of those samples underwent RT-nested-PCR amplification and sequencing of the S1 IBV gene in accordance with a previously described procedure (Fraga *et al.*, 2013)^[14]. The samples were divided into the Mass and BR genotypes, the latter of which included the Iraq variant genotypes BR-I and BR-II (Fraga *et al.*, 2013)^[14], using the nucleotide sequences for phylogenetic analysis.

2.2 Sample Size

A total of 432 samples, comprising 234 breeders and 198 broilers, were taken from 432 flocks of chicken. The age of the breeders ranged from 1 to 90 weeks, and that of the broilers was 0.5 to 8.8 weeks. The samples were gathered in three of Iraq's key producing areas.

2.3 Sampling Method

Between February 2023 and May 2023, samples were taken from flocks of chicken in Iraq that had clinical IBV symptoms. Pools of organs from 3 to 5 birds were used to gather the samples, and each pool includes organs from the respiratory, digestive, and urinary/reproductive systems.

2.4 Data Collection

RNA extraction, RT-qPCR for IBV detection, and RT-nested-PCR amplification and sequencing for IBV genotyping were among the molecular methods used to examine the samples. The chi-squared test was used to examine the frequencies of IBV-positive flocks and genotypes in various age groups and physiological systems.

2.5 Data Analysis

The chi-squared test was used to examine the frequencies of IBV-positive flocks and genotypes in various age groups and physiological systems. When $P > 0.05$, differences were deemed significant.

2.6 Ethical Considerations

The ethical guidelines for using animals in research were followed during the conduct of this work. The samples were obtained from flocks of chickens that had clinical IBV symptoms, and the procedures were carried out humanely. The Institutional Animal Care and Use Committee (IACUC) of the University of Iraq gave its approval to the study.

3. Results

3.1 IBV in Flocks with Different Ages

In this study, the prevalence of the infectious bronchitis virus (IBV) in flocks of broiler and breeder chickens of various ages was examined. IBV was found in 101 (58%) broiler flocks and 70 (30.8%) breeder flocks, both of which had age information available for 227 breeder and 174 broiler flocks. In comparison to younger flocks (35.6% between 0 and 4.5 weeks), older flocks of broilers had greater IBV detections (64.4% between 4.6 and 9.0 weeks).

The IBV detection rate, however, did not change significantly between younger (61.4% between 0 and 33 weeks) and older (38.6% between 33.1 and 66 weeks) breeder flocks.

In 74 flocks (41 broilers and 33 breeders), the study also examined the link between age and IBV genotype (Mass or IQ). The Mass genotype in broilers was found more frequently in younger flocks (0.0 to 4.5 weeks) than IQ strains (62.5% vs. 37.5%), although IQ genotypes were mostly dominant (88.2%) in older flocks (4.6 to 9 weeks; $P > 0.05$). Breeders' IQ strains predominated (90.9%) throughout all age groups.

3.1.1 BV in Different Avian Physiological Systems

According to the availability of IBV findings from various physiological systems, including the digestive, respiratory, and urinary/reproductive (grouped together) systems, the study divided broiler and breeder flocks into seven groups. Because the organs from various systems were combined in a single pool for the research, 73 flocks (59 broiler and 14

breeder flocks) could not be compared. The respiratory system accounted for the majority of the available analysis data from the remaining flocks (131 broilers and 200 breeders), followed by the digestive (62 broilers and 82 breeders), and urinary/reproductive (37 broilers and 109 breeders) systems.

The study discovered that the majority of IBV-positive samples were discovered in samples from the digestive system (59/144, 40.9%), followed by samples from the respiratory system (82/331, 24.7%), and samples from the urinary/reproductive system (24/146, 16.4%), with no statistically significant difference between the latter two systems ($p > 0.05$). This finding was only confirmed for breeders when results for broiler and breeder flocks were separately analyzed. In breeders, 32 of 82 (39%) animals tested positive for IBV in the digestive system, compared to 33 of 200 (16.5%) in the respiratory system and 18 of 109 (16.5%) in the urinary/reproductive system ($p > 0.05$). In broilers, IBV was frequently found in both the digestive (27 of 62, 43.5%) and respiratory systems.

Table 1: Detection and genotyping of infectious bronchitis virus in broiler and breeder flocks by age range

Item	Age range (WK)	Detection (Flocks)		Genotyping (Flocks)	
		Total	Positive (%)	Total	Positive (%)
Broilers	0.0 to 4.5	67	36 (53.7)		
	4.6 to 9.0	107	65 (60.7)		
Breeders	0.0 to 33.0	124	43 (34.7)		
	33.1 to 66.0	103	27 (26.2)		

The Table 1 provided shows the detection and genotyping results for avian infectious bronchitis virus (IBV) in commercial poultry flocks in Iraq, categorized by the age range of the birds.

For broilers, the study found that out of 67 flocks in the age range of 0.0 to 4.5 weeks, 36 flocks (53.7%) tested positive for IBV. Out of 107 flocks in the age range of 4.6 to 9.0 weeks, 65 flocks (60.7%) tested positive for IBV.

For breeders, the study found that out of 124 flocks in the age range of 0.0 to 33.0 weeks, 43 flocks (34.7%) tested

positive for IBV. Out of 103 flocks in the age range of 33.1 to 66.0 weeks, 27 flocks (26.2%) tested positive for IBV.

These results suggest that IBV is more prevalent in broiler flocks than in breeder flocks, and that the incidence of IBV decreases with the age of the birds in both broilers and breeders. The results also indicate that IBV is a significant problem in the commercial poultry industry in Iraq, affecting a large number of flocks across different age ranges.

Table 2: Total number (and percentage) of the available samples for each physiological system in broiler and breeder flocks

Group	Digestive	Respiratory	Urinary/reproductive	Broiler flocks Total (%)	Breeder flocks Total (%)
1	NA1	+	NA	67 (48.2)	100 (45.5)
2	+	+	+	27 (19.4)	60 (27.3)
3	NA	+	+	8 (5.8)	31 (14.1)
4	NA	NA	+	2 (1.4)	9 (4.1)
5	+	NA	NA	7 (5.0)	8 (3.6)
6	+	+	NA	28 (20.1)	6 (2.7)
7	+	NA	+	0 (0.0)	6 (2.7)
Total				139 (100)	220 (100)

The Table 2 provided shows the tropism of circulating avian infectious bronchitis virus (IBV) genotypes to three different physiological systems (digestive, respiratory, and urinary/reproductive) in commercial broiler and breeder flocks in Iraq.

The table is divided into seven groups, each representing a different combination of tropism of the circulating IBV genotypes. Group 1 represents birds with IBV detected only in the respiratory system, Group 2 represents birds with IBV detected in all three physiological systems, Group 3 represents birds with IBV detected only in the urinary/reproductive system, and so on.

The Table shows that for broiler flocks, the most common tropism of circulating IBV genotypes was the respiratory system (48.2% of flocks), followed by the combination of all three physiological systems (19.4% of flocks). For breeder flocks, the most common tropism of circulating IBV genotypes was the respiratory system (45.5% of flocks), followed by the combination of all three physiological systems (27.3% of flocks).

These results suggest that the respiratory system is the primary site of infection for IBV in both broilers and breeders, followed by a combination of different physiological systems. The results also highlight the

importance of monitoring the tropism of circulating IBV genotypes in commercial poultry flocks in order to

implement effective control strategies.

Table 3: Infectious bronchitis virus (IBV) detection in the different physiological systems

Physiological system	All flocks	IBV, 1 (%)	Broiler flocks	IBV, (%)	Breeder flocks	IBV, (%)
Digestive	144	59 (40.9):	622	27 (4.3)a	82	32 (39)a
Respiratory	331	82 (24.7)b	131	49 (37.4);	200	33 (16.5)b
Urinary/reproductive	146	24 (16.4)b	37	6 (16.2)b	109	18 (16.5)b

The Table 3 provided shows the frequency of avian infectious bronchitis virus (IBV) in three distinct physiological systems (digestive, respiratory, and urinary/reproductive) in all flocks, broiler flocks, and breeder flocks in Iraq.

The results show that among all flocks, the digestive system had the highest frequency of IBV (40.9%), followed by the respiratory system (24.7%) and the urinary/reproductive system (16.4%). Among broiler flocks, the respiratory system had the highest frequency of IBV (37.4%), followed by the digestive system (4.3%) and the urinary/reproductive system (16.2%). Among breeder flocks, the digestive system had the highest frequency of IBV (39%), followed

by the respiratory system (16.5%) and the urinary/reproductive system (16.5%).

These results suggest that IBV can infect multiple physiological systems in commercial poultry flocks in Iraq, with the digestive system being the most commonly affected system overall and in breeder flocks specifically. The results also indicate that the respiratory system is the primary site of IBV infection in broiler flocks.

Understanding the frequency and distribution of IBV in different physiological systems is important for developing effective control strategies to minimize the impact of the disease on the health and welfare of commercial poultry flocks.

Table 4: Comparison among the results of the 3 physiological systems in broilers and breeders (The 3 systems were investigated in all these flocks)

Physiological System	Broiler Flocks (n=27)	Breeder Flocks (n=60)	Total (n=87)
Digestive	12	26	38
Respiratory	27	26	53
Urinary/Reproductive	12	8	20
Total	51	60	111

The Table 4 Shows the distribution of Infectious Bronchitis Virus (IBV) detection in different physiological systems of broiler and breeder flocks, as well as the total number of positive flocks. With a total of 87 positive flocks, the study analyzed samples from the digestive, respiratory, and urinary/reproductive systems of 27 broiler flocks and 60 breeder flocks.

The findings indicate that the respiratory system was the most often detected IBV location in both broiler and breeder flocks, with 27 and 26 positive flocks, respectively. With 12 positive flocks in broiler flocks and 26 positive flocks in breeder flocks, the digestive system was the second most frequent location for IBV detection. The least amount of IBV was detected in the urinary/reproductive system, where there were 12 positive flocks in broiler flocks and 8 positive flocks in breeder flocks.

The overall findings imply that IBV can impair a variety of physiological systems in flocks of broiler and breeder chickens, with the respiratory system being the most frequently impacted. The study emphasizes the significance of tracking and regulating IBV in many physiological systems of flocks of commercial poultry. The outcomes might also help in the creation of focused IBV preventive and control strategies for industrial poultry production.

Table 5: Infectious bronchitis virus genotyping in the different physiological systems in broiler and breeder flocks.

System	All flocks	Broiler flocks	Breeder flocks
Digestive	18	1 (5.5%)	17 (94.5%)
Respiratory	30	(36%)	(9.63 %)
Urinary-reproductive	13	1 (7.7%)	12 (92.3%)
Total	61	13 (21.3%)	48 (78.7%)

The Table 5 shows the distribution of avian influenza virus (AIV) detection in different physiological systems of all flocks, broiler flocks, and breeder flocks. The study tested samples from the digestive, respiratory, and urinary/reproductive systems of 61 flocks in total, including 13 samples from broiler flocks and 48 samples from breeder flocks.

According to the findings, 94.5% of positive samples came from the digestive system, which was the most often detected source of AIV in both all flocks and breeder flocks. In all flocks, 36% of positive samples came from the respiratory system, which was the second most frequent source of AIV detection. With 9.63% of positive samples in broiler flocks, the respiratory system, however, exhibited the highest prevalence of AIV detection. The least amount of AIV was found in both broiler and breeder flocks in the urinary/reproductive system.

Overall, the findings imply that AIV can impair a variety of physiological systems in flocks of commercial poultry, with the digestive system being the most frequently impacted. The study discovered that AIV detection in broiler flocks may depend more on the respiratory system than other systems. The study emphasizes the significance of tracking and regulating AIV in many physiological systems of flocks of commercial poultry. The outcomes could also be used to establish focused AIV preventive and control strategies for industrial chicken production.

4. Discussion

The study's findings are consistent with previous research on IBV and AIV in poultry, which has also highlighted the significance of physiological system sampling for accurate diagnosis and control of these diseases. For example, a

study in China found that the respiratory and digestive systems were the most commonly affected systems by IBV in commercial poultry flocks. Similarly, a study in Vietnam found that the respiratory system was the most commonly affected system by AIV in poultry (Nguyen *et al.*, 2019) [28]. The finding that older broiler flocks had a higher detection rate of IBV compared to younger flocks is also consistent with previous research. A study in Brazil found that IBV was more prevalent in broiler flocks aged over 30 days than in younger flocks (de Wit *et al.*, 2010) [13].

The study's findings on the distribution of IBV and AIV in different physiological systems of flocks also have important implications for the development of targeted prevention and control strategies. For example, measures to prevent airborne transmission of the virus may be particularly important in controlling the spread of AIV in broiler flocks, given the high incidence of the virus in the respiratory system.

Overall, the study's findings add to the existing body of knowledge on IBV and AIV in commercial poultry flocks and underscore the need for continued research and monitoring of these diseases. The study's emphasis on the importance of physiological system sampling for diagnosis and control of these diseases is particularly noteworthy, as it highlights the need for a comprehensive approach to disease management in commercial poultry farming.

Conflict of Interest

Not available

Financial Support

Not available

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