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Detection of Infectious Bronchitis Virus in Two Broiler Flocks

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Abstract | Infectious bronchitis virus (IBV) remains a major threat to poultry production, causing respiratory and renal disease with high mortality and significant economic losses. This study investigated suspected IBV outbreaks in ten broiler flocks from different districts of Babylon province, Iraq. Twenty tissue samples were collected from clinically affected birds aged 19–32 days that showed signs of respiratory distress, reduced feed intake, and watery diarrhea. Postmortem findings included tracheal congestion with caseous plugs and swollen, pale kidneys with urate deposits, which strongly suggested nephropathogenic IBV infection. Rapid antigen testing revealed positive results in 60% of the samples, while molecular assays identified two confirmed isolates: AR01 (Jebaleh) and AR02 (Al-Mahawil). Real-time PCR results supported these findings, with Ct values of 21.85 and 23.70, respectively. Both isolates were successfully propagated in embryonated chicken eggs, where embryo stunting and curling were observed. Isolate AR01 caused complete embryo mortality within 96 hours, while AR02 required 120 hours, and EID_{50} calculations confirmed higher virulence of AR01. Avian influenza virus (AIV) and Newcastle disease virus (NDV) were not detected in any samples. These results highlight the persistent circulation of IBV in Iraqi broiler flocks and emphasize the need for continuous surveillance and updated vaccination strategies to minimize economic losses.

Keywords | Infectious bronchitis virus, Broiler chickens, Iraq, RT-PCR, Respiratory disease

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INTRODUCTION

One of the most significant viral diseases affecting broiler chickens is infectious bronchitis virus (IBV), which causes significant financial losses for the poultry sector (Javadov *et al.*, 2020). The virus, which belongs to the family Coronaviridae and is categorized under the name Gammacoronavirus, spreads quickly through infected equipment, aerosols, and direct contact (Balasuriya *et al.*, 2022). IBV mostly causes acute respiratory disease in broilers, which is typified by coughing, sneezing, tracheal rales, and gasping. Growth retardation, low feed conversion efficiency, and increased mortality are frequently observed in conjunction with these symptoms (Hoerr, 2021). Additionally, urate deposition from nephropathogenic strains might result in pale, enlarged kidneys, which raises mortality and lowers carcass quality (Shrirang, 2018). Despite the widespread use of live attenuated and inactivated vaccinations, IBV outbreaks in broiler farms around the world persist. Due to the great genetic heterogeneity of the virus, widely used vaccines like H120, Ma5, and 4/91 frequently offer insufficient protection (Guzmán and Hidalgo, 2020). Numerous variants are produced by frequent mutations and recombination in the spike (S1) gene, many of which are unable to provide cross-protection with currently available vaccines. As a result, even with rigorous vaccination schedules, flocks frequently suffer from respiratory illnesses, secondary

bacterial infections, and productivity losses (Shosha *et al.*, 2025). The production of broilers in the Middle East, particularly Iraq, is still significantly hampered by IBV. Despite immunization, local variations like GI-23 (Variant-2 Middle East) and GI-13 (QX-like) have been connected to nephropathogenic lesions, severe respiratory illness, and higher mortality (Lisowska *et al.*, 2017).

MATERIALS AND METHODS

Sample collection and case history

A total of twenty (20) tissue samples were collected from ten (10) broiler flocks located in different districts of Babylon governorate, Iraq. The examined flocks age ranged from (19 to 32) days. Despite routine vaccination with commercially available vaccines against infectious bronchitis virus (IBV), Newcastle disease virus (NDV), avian influenza virus (AIV), and infectious bursal disease virus (IBDV). All investigated flocks exhibited clinical signs suggestive of infectious bronchitis virus (IBV) infection, such as respiratory distress, high mortality and some of flocks showed watery diarrhea. From each flock, two (2) samples were aseptically collected from tissues of trachea, lungs, and kidneys of clinically affected birds. These samples were immediately placed in sterile containers, stored on (4˚C) and transported to the laboratory for subsequent diagnostic evaluation.

Preliminary diagnosis by using IBV antigen rapid test kit

Collecting swabs from (kidney, trachea and cloaca) of the 10 flocks and using IBV Ag Rapid Test Kit from (Bionote/ Korea). And by following the manufacturer's instructions, the operation was carried out, and the existence of control and test lines was used to interpret the results. A quick test for first diagnosis before confirmatory laboratory testing was made available by this assay.

Molecular identification of infectious bronchitis virus using conventional and real-time PCR

Viral RNA extraction and cDNA synthesis

Viral RNA was extracted from tissue samples using a commercial spin-column kit (Intron, Korea), following the manufacturer's protocol. The purified RNA was converted to cDNA with the AddScript kit (Addbio, Korea) in a 20 µl reaction containing RNA template, primers, nucleotides, and enzyme mix under standard reverse transcription conditions.

Conventional PCR

The S1 gene of IBV was amplified using GoTaq® G2 Green Master Mix (Promega, USA) and specific primers in 50 µl reactions. Thermal cycling included initial denaturation at 94 ˚C, 35 amplification cycles of denaturation, annealing (50–58 ˚C), and extension, followed by a final elongation step. PCR products were resolved on agarose gels and visualized under UV light.

Real-time PCR

For confirmation, real-time PCR was carried out with the Vetproof IBV RNA Test Kit (BioChek, Netherlands), targeting a conserved region of the S1 gene. Reactions were run on a Stratagene MX3005P system using an initial activation step at 95 ˚C followed by 40 amplification cycles. This method provided sensitive and reproducible detection of IBV RNA.

Virus propagation in embryonated chicken eggs and EID_{50} determination

After being diluted 1:10 in (Phosphate-Buffered Saline) PBS with antibiotics, two IBV Ag Rapid Test-positive samples from broiler flocks with high mortality in Al-Mahawil (27 days) and Jebaleh (21 days) were injected (0.2 mL) into the allantoic cavity of SPF embryonated chicken eggs that were 9–11 days old. Eggs were candled every day and incubated at 37˚C; those that died within 24 hours were thrown away, and those that died later were examined for IBV lesions. Allantoic fluids were titrated using tenfold dilutions following four serial passes, and the Reed and Muench method was used to determine the 50% egg infective dosage (EID_{50}). After being kept on ice, the inocula were moved for NDV, AI, and IBV PCR detection.

Molecular detection by real-time RT-PCR

One-step qRT-PCR (TaqMan assay) was used to test for Avian Influenza Virus (AIV), Newcastle Disease Virus (NDV), and Infectious Bronchitis Virus (IBV) in two isolates obtained as allantoic fluid from SPF embryonated chicken eggs, ARO1 (Jebaleh) and AR02 (Al-Mahawil). The QIAamp Viral RNA Mini Kit (Qiagen, Germany) was used to extract the viral RNA, and the AccuPower® RocketScript RT-qPCR Kit (Bioneer, South Korea) was used for amplification. The primer-probe sets were designed to target the M gene of AIV, the F gene of NDV, and the S1 gene of IBV. Reverse transcription was run for 10 minutes at 50˚C, denaturation for 2 minutes at 95˚C, and 40–45 cycles of 95˚C for 15 s and 60˚C for 30 s. Each run contained positive, negative, and internal controls. Results were interpreted by Ct values: ≤35 positive, 35–38 retested and >38 or undetected negative.

RESULTS

Clinical signs and gross lesion

Ten broiler flocks were investigated in the field, and the results showed significant mortality rates between 50% and 80%. In addition to depression, decreased feed intake,

distress, as evidenced by gasping, coughing, and nasal discharge. A postmortem examination revealed typical gross lesions, such as enlarged, congestion kidney or pale kidneys with urate deposits and tracheal congestion with catarrhal exudates and a plug at the tracheal bifurcation (Figure 1). Infectious bronchitis virus infection was strongly suggested by these morphological and clinical findings.

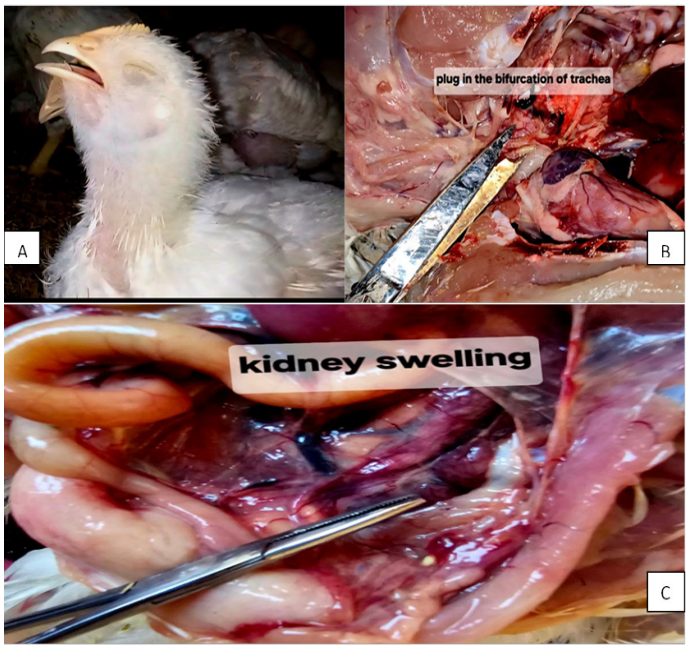

Figure 1: A: Clinical sign (respiratory distress) include gasping, open-mouth breathing, neck extension. B: Gross lesion showing a caseous plug at the tracheal bifurcation in a broiler chicken. C: Swollen and congested kidneys in broiler chicken showing characteristic nephropathogenic lesion.

IBV antigen rapid test

The IBV Ag fast test (Bionote, Korea) was used to analyze 20 samples from 10 broiler flocks spread over Babylon Governorate. Of these, 8 samples (40%) had negative results, while 12 samples (60%) had positive ones (Table 1). The initial diagnosis of an infectious bronchitis virus infection was confirmed by positive reactions, which were primarily seen in flocks with high mortality rates and severe respiratory signs.

Molecular detection of IBV S1 gene in field isolates by cPCR and rtPCR

Six samples from high-mortality flocks (80% in Jebaleh and 67% in Al-Mahawil) were tested. Conventional PCR detected the 420 bp S1 fragment in two isolates, AR01 (Jebaleh) and AR02 (Al-Mahawil) (Table 2), while the other four were negative. Real-time PCR confirmed these results with Ct values of 21.85 for ARO1 and 23.70 for ARO2. The PCR bands were also visualized on 1.5% agarose gel, where only ARO1 and ARO2 showed the expected productand.

Table 1: Location of the farms,age, IBV Ag rapid test results.

IBV Ag rapid test results	No. samples	Flock age/ days	Location
Positive	2	27	Al-Mahawil
Positive	2	21	Jbala
Positive	2	24	Al-Musayyab
Negative	2	30	Al-Hashimiya
Positive	2	22	Al-Shomili
Negative	2	26	Al-Qasim
Negative	2	29	Al-Neel
Negative	2	30	Al-Kifl
Positive	2	26	Al-Mashroo
Positive	2	32	Al-Hilla

Table 2: Detection of IBV virus by conventional PCR assay (targeting gene S1).

Sample ID	Target gene	Result
AR01	S1	Positive
AR02	S1	Positive

Virus propagation and EID_{50} determination

All embryos died within 96 hours of inoculation with strain AR01 (Jebaleh), while all embryos died within 120 hours with isolate AR02 (Al-Mahawil). Furthermore, both isolates caused noticeable embryo stunting, a condition that is typical of IBV replication in eggs (Figure 2). These results were further supported by the viral titration, which, according to the Reed–Muench method, revealed that isolate AR01 (Jebaleh) had an infectious dosage of EID_{50} = 10^–8.75 (≈1.78 × 10^{-9}/0.1 mL) and isolate AR02 (Al-Mahawil) had an infectious dose of EID_{50} = 10^–6.78 (≈1.66 × 10^{-7}/0.1 mL) (Table 3).

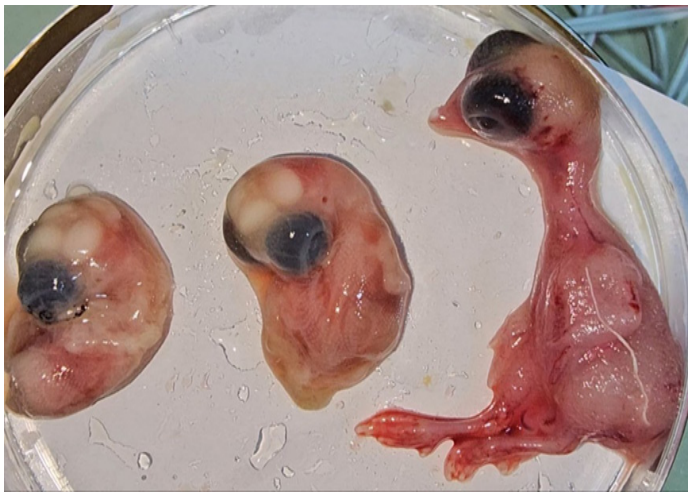

Figure 2: Embryonic lesions following IBV inoculation in SPF eggs, showing dwarfing, curling, stunting compared with control at right.

Table 3: EID_{50} values of IBV isolates in embryonated chicken eggs (Reed– Muench metho).

Isolate	Source farm	log10 EID_{50} (per 0.1 mL)	EID_{50} (per 0.1 mL)
A1	Jebalah	8.75-	1.78×10^{-9}
A2	Al-Mahawil	6.78-	1.66×10^{-7}

RT-PCR detection of AIV, NDV, and IBV

RT-PCR analysis of harvested allantoic fluids from both field samples (ARO1 - Jbala, ARO2 - Al-Mahawil) revealed that AIV and NDV were not detected, whereas IBV was confirmed with Ct values of 28.6 and 30.2, respectively, indicating IBV as the sole viral agent present (Table 4 and Figure 3)

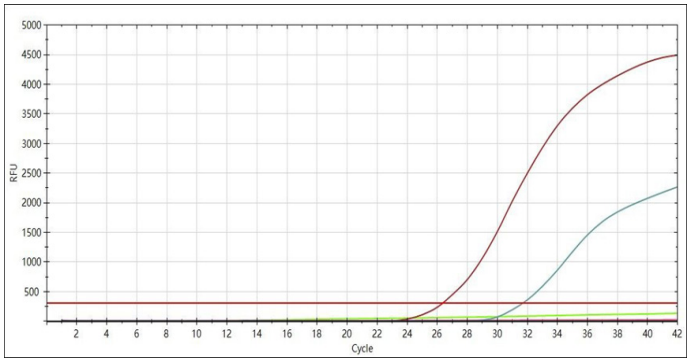

Figure 3: qPCR detection of IBV: Both AR01 and AR02 positive.

Table 4: Results of RT-PCR Detection of IBV, NDV and AIV.

AIV (CT)	NDV(CT)	IBV(CT)	Sample
Not detected	Not detected	28.6	A1-Jebalah
Not detected	Not detected	30.2	A2-Al-Mahawil

DISCUSSION

The results of the present study confirm that infectious bronchitis virus (IBV) continues to represent a serious challenge to poultry production in Iraq and remains one of the major causes of respiratory disease in broiler chickens which are in agreement with (Ali and Allawe, 2023). The postmortem examination revealed tracheal congestion with caseous plugs and swollen, pale kidneys with urate deposition, findings that are strongly indicative of nephropathogenic IBV infection. These manifestations are in close agreement with descriptions in previous studies that have reported similar pathological changes in field outbreaks where nephropathogenic strains played a dominant role (Chen *et al*., 2024). The rapid antigen test employed in this work revealed that 60% of the samples were positive, which illustrates its value as an immediate screening tool for flock-level diagnosis. However, the discrepancy between the percentage of positive results by antigen detection and the subsequent molecular confirmation reflects the limitations of such rapid methods. In contrast to (Al-Mahmoudi, 2015), who reported a 93.33% positivity rate, the present study demonstrated a substantially lower detection rate, indicating that the sensitivity of the rapid test may be limited under practical field conditions. The confirmatory molecular assays used in the present study, including both conventional PCR and real-time PCR targeting the S1 gene, demonstrated that only two isolates, AR01 and AR02, were positive, producing an amplicon of 420 bp and Ct values of 21.85 and 23.70, respectively. These results are consistent with the molecular detection ranges expected for acute infections and highlight the importance of PCR as the gold standard in IBV diagnosis (Legnardi *et al*., 2020). which has been confirmed in multiple epidemiological surveys in the region and worldwide. The propagation of these isolates in embryonated chicken eggs provided further biological confirmation of their pathogenic activity. Both isolates induced embryo stunting and curling were in agreement with findings of (Bhuiyan *et al*., 2025), but AR01 from Jebaleh proved to be more virulent, causing complete embryo mortality within 96 hours, compared to 120 hours for AR02 from Al-Mahawil. The EID_{50} values also demonstrated this difference, with AR01 calculated at 10^–8.75 and AR02 at 10^–6.78, confirming a higher infectivity of AR01. Such differences in virulence between field isolates are well documented and reflect the heterogeneous nature of IBV populations circulating in commercial poultry, where some strains are associated with rapid embryo lethality and high pathogenicity, while others demonstrate comparatively slower replication and lower mortality, in agreement with the observations of (De Wit and Cook, 2014). These biological assays are of great diagnostic and epidemiological significance because they provide insights into the pathogenic potential of isolates, which cannot be determined by molecular methods alone. Another important finding in this study was the exclusion of avian influenza virus (AIV) and Newcastle disease virus (NDV) using multiplex RT-PCR, which confirmed that IBV was the sole etiological agent responsible for the observed clinical signs and high mortality in agreement with (Behboudi, 2022; Gerilovych *et al*., 2021). The absence of AIV and NDV in the tested samples strengthens the conclusion that IBV alone was sufficient to cause the severe outbreaks investigated here, which is consistent with other recent studies conducted in Egypt, Eithiopia, and Turkey, where IBV was identified as the primary pathogen in flocks (Amer *et al*., 2024; Ardiçli *et al*., 2022; Berhanu *et al*., 2025). From an epidemiological perspective, the persistence of IBV in broiler farms in Babylon province illustrates the endemic nature of the virus in Iraq. Similar findings have been reported in previous investigations where IBV was consistently detected in commercial flocks across north, middle and southern

Iraq, often associated with nephropathogenic lesions and substantial economic losses (Alabadi and Jasim, 2022; Almayahi *et al*., 2022; Sehry *et al*., 2023). In conclusion, the current study confirms that IBV continues to circulate actively in Iraqi broiler flocks and is capable of producing severe respiratory and nephropathogenic disease with high mortality, independent of other major respiratory viruses. The detection of AR01 from Jebaleh and AR02 from Al-Mahawil as distinct isolates with variable virulence underscores the heterogeneous nature of IBV and the challenges this poses for poultry health management. The integration of clinical observation, antigen screening, molecular confirmation, and embryo propagation represents a comprehensive diagnostic approach that provides clarity in outbreak investigation. These results emphasize the urgent need for ongoing surveillance, genetic characterization of circulating strains, and detailed field studies to better understand the epidemiology of IBV in Iraq and to mitigate its substantial economic impact on poultry production.

CONCLUSION

This work highlights the ongoing problem of IBV in chicken flocks despite vaccination campaigns by confirming that the isolated field strains were IBV-positive by RT-PCR, with no detection of AIV or NDV.

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NOVELTY STATEMENT

This study reports the detection and molecular characterization of two IBV isolates from broiler flocks in Babylon province, Iraq, highlighting their virulence and propagation in embryonated eggs. The findings provide updated data on the circulation of nephropathogenic IBV strains, which is crucial for improving surveillance and vaccination strategies in Iraqi poultry production.

AUTHOR'S CONTRIBUTION

ARAY designed and performed the experiments, analyzed the data, and drafted the manuscript. FHKA supervised the study, contributed to data interpretation, and revised the manuscript. Both authors read and approved the final version of the manuscript.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest regarding the publication of this paper.

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