





A Field Study on Infectious Bronchitis Virus in Broiler Chickens in Southern Iraq

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Received: June 26, 2025, Revised: July 28, 2025, Accepted: August 30, 2025, Published: September 25, 2025



ABSTRACT

Infectious bronchitis virus (IBV) is a highly transmissible avian Gamma-coronavirus that continues to pose a major challenge to poultry health and productivity worldwide, particularly in broiler production systems. The present investigation aimed to detect and characterize IBV infections in broiler flocks located in two districts of Southern Iraq between late 2024 and early 2025. A total of 200 clinically affected broilers (100 broilers from each flock) were sampled, with tracheal and kidney tissues collected for clinical evaluation, histopathological assessment, and viral isolation. Suspected IBV-infected chickens showed respiratory distress, increased mortality, and kidney lesions. The suggestive post-mortem lesions were caseous plug exudates at the tracheal bifurcation, as well as congested and hyperemic kidneys. The inoculation of tracheal and kidney tissue suspension in embryonated chicken eggs (ECEs) resulted in death, stunting, curling, dwarfism, congestion, and subcutaneous hemorrhages. The histopathological findings in tracheal tissues revealed epithelial desquamation, goblet cell depletion, and lymphocytic infiltration, while kidney findings exhibited tubular degeneration, glomerular disruption, and fibrin deposition. These findings emphasize the need for future studies to focus on the molecular identification of circulating strains, vaccine matching, and monitoring of post-vaccination protection levels in Iraq.

Keywords: Broiler chicken, Histopathology, Infectious bronchitis virus, Isolation, Kidney, Trachea

INTRODUCTION

The major difficulties threatening poultry production worldwide are viral diseases. Globally, infectious bronchitis (IB) is considered the second most economically damaging viral disease affecting the poultry sector, following the highly pathogenic avian influenza (De Wit and Cook, 2019). Infectious bronchitis is responsible for substantial economic losses to the poultry industry, particularly in broiler flocks where it reduces weight gain, feed efficiency, and survival rates. Losses may range from \$0.03 to \$0.10 per broiler, depending on strain virulence and flock age (Jackwood and de Wit, 2020; Rafique et al., 2024).

As reported by the International Committee on Taxonomy of Viruses (ICTV, 2024), the infectious bronchitis virus (IBV) belongs to the genus *Gamma-coronavirus*, subgenus *Igacovirus*, within the subfamily *Orthocoronavirinae* of the family *Coronaviridae*. The

virus possesses a pleomorphic, enveloped structure and contains a positive-sense, single-stranded, non-segmented RNA genome of approximately 27.6 kb. This genome encodes both non-structural proteins and the major structural proteins, namely the nucleocapsid (N), membrane (M), envelope (E), and spike (S) proteins (Dimitrov et al., 2019; Quinteros et al., 2022).

The IBV is commonly considered a respiratory pathogen that replicates in the tracheal mucosa (Amarasinghe et al., 2018), while some strains of the virus show broad tissue tropism to kidneys, reproductive tract, bursa of Fabricius, gastrointestinal tract (proventriculus and cecal tonsils), and spleen (Rafique et al., 2024). In laying hens, variant strains of IBV may cause damage to the reproductive tract (Cook et al., 2012; Ramsubeik et al., 2023). This broad tissue tropism emphasizes the complexity of IBV pathogenesis (Bande et al., 2016; Rafique et al., 2024).

The IBV mainly spreads systemically through tracheal macrophages and blood monocytes, leading to deep respiratory infections (De Wit and Cook, 2019). In some cases, the virus has also been detected in cloacal swabs and cecal tonsils, suggesting the possibility of retrograde viral ascent from the lower gastrointestinal tract to the kidneys via the ureters, particularly with nephropathogenic strains (Quinteros et al., 2022). Infection of the nasal passages and tracheal lining with IBV rapidly destroys the ciliated epithelium, leading to impaired mucociliary clearance and thereby increasing susceptibility to secondary bacterial infections (Cook et al., 2012). In addition, nephropathogenic IBV strains are capable of inducing marked renal pathology, including tubular epithelial cell necrosis, inflammatory infiltration, and renal dysfunction progressing to failure (Hoerr, 2021). The severity of IBV is influenced by the management practices, live virus vaccines, immunosuppressive conditions, and coexisting pathogens (Hoerr, 2021). Consequently, this study aimed to detect the presence of IBV in broiler chicken flocks in two districts of Southern Iraq. The investigation included viral isolation and histopathological examination of the trachea and kidneys to evaluate dual viral tropism, providing a comprehensive understanding of the pathological behavior of IBV under field conditions.

MATERIALS AND METHODS

Ethical approval

This study was conducted following the ethical guidelines for animal research approved by the Institutional Animal Ethics Committee of the College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Sampling

According to farm records and veterinary interviews, two infected commercial broiler farms were included in this study: one located in Abu Khosib (approximately 3,000 chickens) and the other in Shat Al-Arab (approximately 7,000 chickens), Basra Governorate, Southern Iraq. The affected flocks were 14 and 20 days of age, respectively.

Both farms had received a single combined live vaccine via drinking water at 5 days of age, consisting of the La Sota strain of Newcastle disease virus (NDV) and the H120 strain of infectious bronchitis virus (IBV), provided by Boehringer Ingelheim, Germany. However, no booster IBV vaccinations or additional vaccines and drugs were administered before the outbreak, mainly due

to poor management practices and limited awareness of proper vaccination protocols among the farmers. From each farm, 100 clinically affected broilers were selected (200 broiler chickens in total) for pathological examination and virus detection.

This field-based pathological investigation was conducted between December 2024 and March 2025 in Basra Governorate, Southern Iraq. The study included two commercial broiler farms: one in Abu Khosib with approximately 3,000 broilers aged 14 days, and another in Shat Al-Arab with approximately 7,000 broilers aged 20 days. An active outbreak in the previous flocks was suggestive of IBV infection and was characterized by severe respiratory distress with gasping and a high mortality rate of average of about 40% both farms.

A total of 200 chickens (100 from each flock) showing severe respiratory manifestations were selected and delivered to the pathology laboratory at the College of Veterinary Medicine, University of Basrah, for further examination. A systematic necropsy procedure was performed, and the gross lesions of the respiratory and renal systems were recorded.

Viral isolation

The inoculation of IBV was performed using the allantoic cavity of 9-11-day-old specific pathogen-free (SPF) embryonated chicken eggs (ECEs) obtained from Nile S.P.F. Company, Giza, Egypt. Tracheal and renal tissues from clinically affected broilers showing typical gross lesions were aseptically collected, pooled, and homogenized in sterile phosphate-buffered saline (PBS) supplemented with antibiotics (1000 IU/mL penicillin and 1000 µg/mL streptomycin) to prepare a 10% (w/v) suspension. The homogenate was clarified by centrifugation at 3000 rpm for 15 minutes at 4°C using a refrigerated high-speed centrifuge (Sigma 3-30KS, Sigma Laborzentrifugen GmbH, Germany), and the supernatant was collected. Approximately 0.2 mL of the filtrate was then inoculated into the allantoic cavity through a 0.22 µm syringe filter under sterile conditions. The inoculated eggs were incubated at 37°C and monitored daily for embryo viability and lesions. Embryos that died within the first 24 hours were discarded as non-specific. After 4-7 days, embryos were chilled at 4°C for 6 hours, and allantoic fluid and embryo lesions were examined. Gross pathological changes, such as stunting, curling, subcutaneous hemorrhages, and dwarfing, were considered indicative of IBV replication. The inoculation and isolation procedures were designed according to the methods described by Hoan et al. (2023).

Histopathological examination

For histopathological examination, tissue samples from the tracheal bifurcation region and the posterior lobes of the kidneys, including both cortex and medulla, were collected. All collected tissues were promptly immersed in 10% neutral-buffered formalin and fixed for 48-72 hours. Following fixation, samples were processed through a standard histological protocol, which included dehydration in graded ethanol, clearing in xylene, and embedding in paraffin wax. Tissue blocks were sectioned at a thickness of 4-5 μm using a rotary microtome (Leica RM2125 RTS, Germany) and placed onto glass slides. Sections were subsequently stained with hematoxylin and eosin (H&E) according to established procedures to enable microscopic evaluation (Bancroft and Layton, 2019; Layton et al., 2019). A compound light microscope (Olympus CX23, Japan) was used for histopathological examination at magnifications of $\times 40$ and $\times 100$.

RESULTS

Broiler chickens from both examined flocks exhibited severe respiratory signs, including sneezing, tracheal rales, open-mouth breathing, and dyspnea (Figure 1a). Affected chickens had died on their backs after a period of labored breathing and agitation, suggesting acute anoxia (Figure 1b).

The post-mortem gross examination of the trachea in the suffering and dead chickens revealed severe mucosal congestion with the presence of diffuse catarrhal to fibrinous diphtheritic cheesy exudates or caseous plugs at the tracheal bifurcation region (Figure 2a). The kidneys were enlarged and congested (Figure 2b).

Viral isolation using SPF ECEs revealed embryonic mortality by the 5th day post-inoculation. Out of 30 inoculated embryos (15 from each flock), 24 embryos developed characteristic IBV-induced lesions, including growth dysplasia and dwarfism, curling and stunting of the body, congestion of internal organs, and subcutaneous hemorrhages (Figure 3).

The histopathological examination of the trachea is shown in Figure 4. Extensive epithelial damage, desquamation, and sloughing of epithelial cells were observed in 160 out of 200 examined samples (80%), accompanied by marked mucosal hyperplasia and irregular thickened epithelial zones. A markedly reduced number of goblet cells with vacuolated cytoplasm, along with submucosal edema, heterophilic infiltration, and vascular congestion, was detected in 140 samples (70%).

The histopathological findings in kidney tissue are illustrated in Figure 5. The tubule epithelium revealed multifocal to diffuse degenerative and necrotic changes in 150 samples (75%), with luminal obstruction by cellular debris and proteinaceous casts. Vacuolar degeneration, pyknosis, and karyorrhexis were also common. Atrophy of glomerular tufts, accompanied by infiltration of inflammatory cells such as neutrophils and mononuclear cells within the interstitium, was observed in approximately 100 out of 200 examined kidney samples (50%). In 60 out of 200 samples (30%), tubular epithelium was replaced by fibrinous material and necrotic debris, leading to narrowing of the lumina due to epithelial swelling. These gross and microscopic findings confirm the presence of IBV-induced pathological changes in both respiratory and renal tissues.

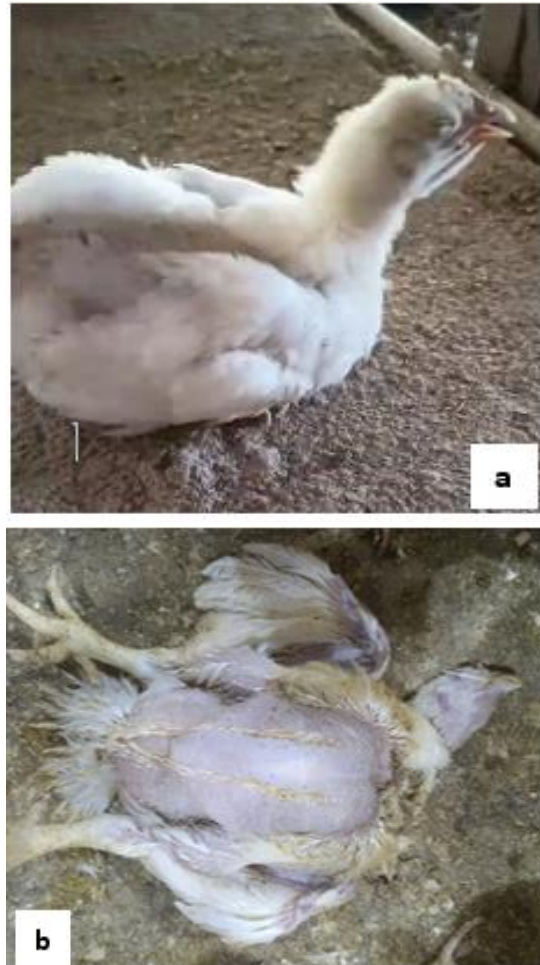


Figure 1. Broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. **a:** 14-day-old chickens show open mouth breathing, **b:** 20-day-old broiler chickens found dead and lying on their backs.

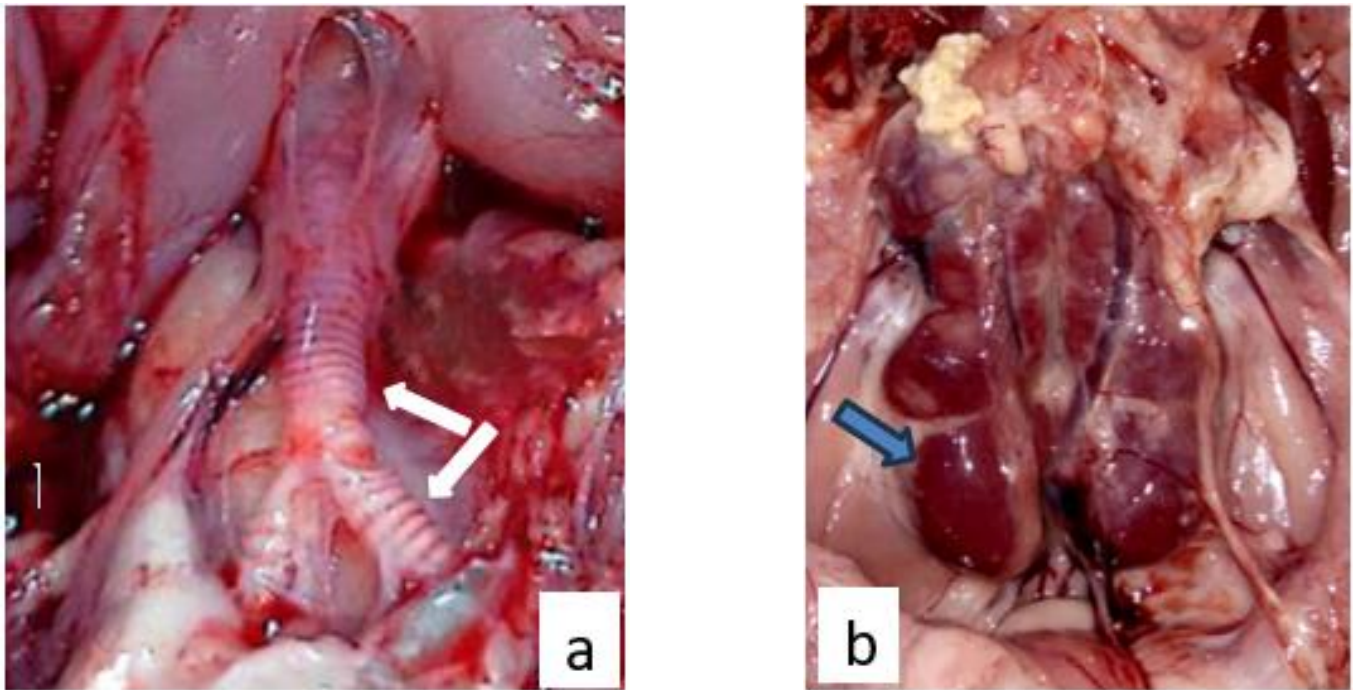


Figure 2. Gross lesions of 14-day-old broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. **a:** Yellow caseous material in trachea and tracheal bifurcation (white arrows). **b:** Swollen and congested kidney lobes (blue arrow).

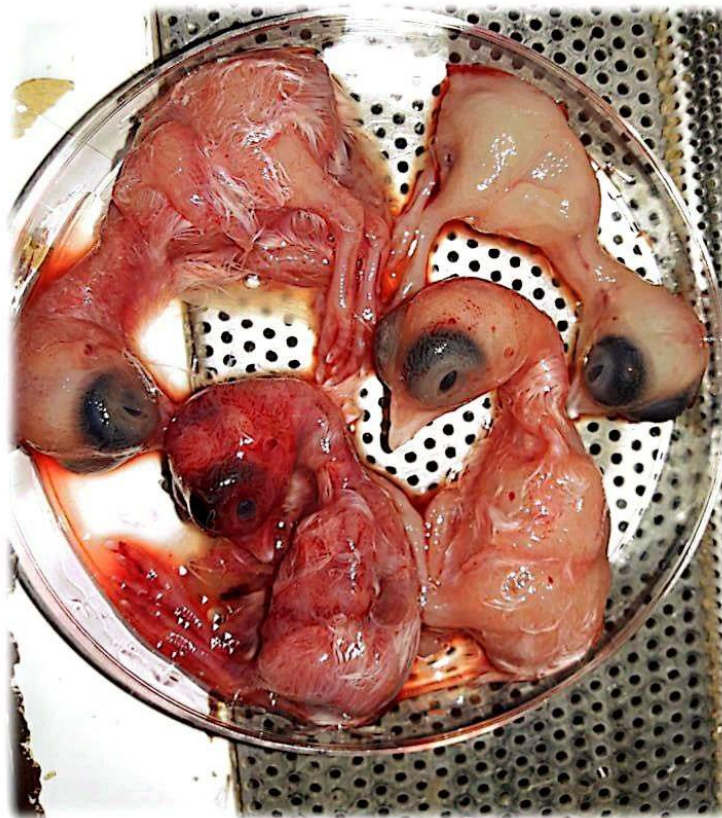


Figure 3. Specific pathogen-free chicken embryos at day 5 post-inoculation with infectious bronchitis virus suspected tissue suspension. Embryos show stunting, curling, and hemorrhages (typical lesions of IBV infection).

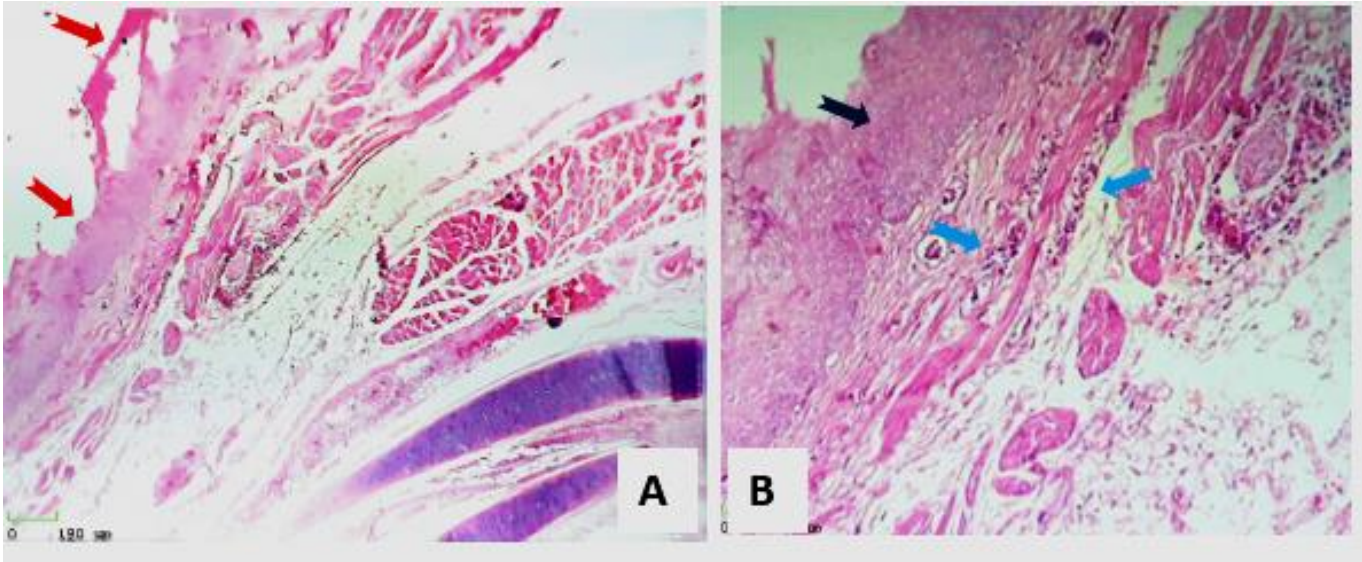


Figure 4. Tracheal tissue of broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. **A:** Extensive epithelial damage, sloughing, and desquamation (red arrow). **B:** Thickened epithelial areas (black arrow) with infiltration of inflammatory cells (blue arrows; H&E stain, $\times 100$).

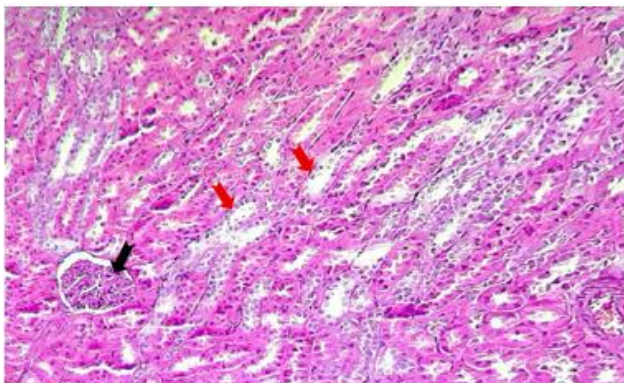


Figure 5. Histological alterations of the kidney of broiler chickens (*Gallus gallus domesticus*) naturally infected with infectious bronchitis virus in Basrah, Southern Iraq, during 2024-2025. There is epithelial cell degeneration, necrosis, and vacuolation of glomerular endothelial cells (black arrow), as well as dilatation of renal tubules (red arrow; H&E stain, $\times 40$).

DISCUSSION

Infectious bronchitis virus (IBV), a highly transmissible avian Gammacoronavirus, continues to represent a major global threat to poultry production, especially in broiler flocks. The infection is characterized by a notably short incubation period of only 18-36 hours, with the upper respiratory tract serving as the primary site of viral replication (Falchieri et al., 2024). IBV infection causes acute respiratory distress, renal damage, reproductive failure, and high mortality with considerable economic losses (Hoerr, 2021; Rafique et al., 2024).

The history of the affected broiler chicken flocks revealed up to 40% mortality rate by day 14 and 20 of age in both examined flocks, reflecting the high virulence and renal tropism (nephron-pathogenic potential) of the circulating field strains.

The mentioned respiratory signs and lesions, including gasping, rales, dyspnea, and the caseous plug at the tracheal bifurcation, are characteristic of IBV infections. These findings are aligned with those reported by Falchieri et al. (2024) in the United Kingdom and El Nemr et al. (2025) in Egypt. Moreover, the enlargement and congestion of the renal lobes are consistent with the pathogenesis of nephro-pathogenic IBV strains that are reported previously (Grgić et al., 2008; Hasan et al., 2020; Quinteros et al., 2022). The observed mortalities, stunting, curling, dwarfism, and hemorrhages of the inoculated embryos validate the diagnostic utility of ECEs for IBV isolation (Hoan et al., 2023; Berhanu et al., 2025).

The microscopic tracheal lesions reflect both direct viral cytopathic effects and the host's immune response. The combinations of deciliation, epithelial desquamation, goblet cell loss, and hyperplasia suggest both destructive and reparative tissue dynamics. These findings, together with epithelial apoptosis and regenerative hyperplasia in IBV-infected tracheal tissue, are described by Han et al. (2017) and El Nemr et al. (2025). Moreover, fibrin exudation within the lamina propria may reflect vascular leakage due to local cytokine storm induced by the viral replication (El Nemr et al., 2025).

The renal tubular necrosis, epithelial desquamation, and glomerular alterations represent hallmark lesions of nephron-pathogenic IBV strains and are consistent with their established pathogenic mechanisms (Hoerr, 2021). Moreover, previous studies have emphasized the role of pro-inflammatory cytokines in exacerbating tissue injury during systemic IBV infections (Quinteros et al., 2022; El Nemr et al., 2025).

In this field outbreak, the frequency of tracheal pathology occurrence, along with the renal damage, confirmed the dual tropism of IBV in the affected flocks. This pattern is consistent with the circulation of nephropathogenic IBV variants, which have been known to induce both respiratory and renal diseases (Rafique et al., 2024). The marked histopathological lesions and associated mortality confirmed the importance of timely diagnosis, effective vaccination programs, and tailored immune prophylaxis in endemic regions such as Southern Iraq.

CONCLUSION

Under field conditions, the concurrent respiratory and renal lesions, along with elevated mortality rates, suggest the circulation of a highly virulent nephron-pathogenic IBV strain in broiler farms in Southern Iraq. Such findings correlate with the observed clinical signs and histopathological damage in tracheal and renal tissues. In addition, the severe histopathological changes in the trachea and kidney tissues provide a clear explanation for the clinical signs and mortality observed during the outbreak. These results emphasized the importance of early diagnosis and continuous monitoring of IBV strains, especially in regions with intense poultry production and variable biosecurity standards. Therefore, vaccination strategies based on the use of the local virus strains, along with the application of high biosecurity levels, are crucial to limit the spread and consequences of IBV. Overall, these findings reinforce the urgent need for ongoing surveillance, genotype-based vaccine selection, and strict biosecurity in regions endemic with virulent IBV strains. Future studies should focus on the molecular identification of circulating IBV strains, vaccine matching, and monitoring of post-vaccination conditions, especially in regions facing mixed infection pressures and variable management practices.

DECLARATIONS

Acknowledgements

The authors would like to thank the Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Basrah, Iraq, for technical

support. Special thanks to field veterinarians and farm owners for their cooperation in sample collection.

Authors' contributions

Muhammadtaher Abdulrazaq Abdulrasol contributed to field sampling, histopathological examination, data collection, and writing the initial draft. Wafaa A. Abd El-Ghany participated in conceptualization, supervision, critical review, and editing. All authors read and approved the final manuscript.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors. All procedures involving animals were performed according to internationally accepted welfare standards, and samples were collected only from clinically diseased broiler chickens suspected of IBV infection.

Funding

No specific funding was received for this study.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have not declared any competing interests.

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