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Evaluating the pathogenicity of GI-23 genotype of Infectious Bronchitis Virus isolated in Egypt and assessment of the protective efficacy of the variant II vaccine against it

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ABSTRACT

Infectious bronchitis virus (IBV) is a critical poultry pathogen that causes major economic losses globally. IBV has high levels of genetic, serotype, and pathotypic variability. In Egypt, the predominant genotype identified is GI-23 (Var II), which persists in its dissemination and pathogenic impact within the avian population, notwithstanding the enforcement of rigorous vaccination protocols. This study investigates the pathogenicity of the GI-23 genotype of IBV, specifically the strain isolated in Egypt (IBV/CH/EG/Moshtohor-3/2023), using one-day-old specific pathogen-free chicks. The chicks were inoculated with 105 EID50 of the virus in 0.1 mL via the intranasal route. Clinical signs, mortality rates, histopathological changes, and viral shedding were systematically monitored over 14 days following infection. Furthermore, it evaluates the protective efficacy of a commercially available variant II vaccine against this particular strain. The findings of this study revealed a considerable level of pathogenicity, as evidenced by a 40% mortality rate in infected SPF chicks, accompanied by severe respiratory and renal lesions, as well as elevated viral loads in oropharyngeal swabs. Furthermore, the variant II vaccination conferred a high protection rate of 93.4% and achieved a ciliostasis score of 0, indicating valuable protection against ciliary dysfunction.

1. INTRODUCTION

Infectious bronchitis caused by the avian infectious bronchitis virus (IBV) poses a big threat to the global poultry industry, since it is an acute and highly contagious disease. This viral pathogen has the potential to infect chicks across all age groups; however, it is particularly virulent in young chicks, rendering them the most susceptible population. The implications of IB on poultry health and production underscore the importance of understanding and effectively managing this disease (Cavanagh, 2007; Cook et al., 2012).

IBV is classified within the order Nidovirales, specifically under the family Coronaviridae and the subfamily Orthocoronavirinae. It represents the sole member of the genus Gammacoronavirus, which is known to infect avian species, particularly chicks (Carstens, 2010).

The infectious bronchitis virus (IBV) possesses a linear, positive-sense, non-segmented, single-stranded RNA genome that is approximately 27.6 kilobases in length. IBV particles are structurally maintained by four main proteins: phosphorylated nucleoproteins (N), membrane proteins (M), spike glycoproteins (S), and small envelope proteins (E)(de Wit and Cook, 2020).

According to current classification systems, IBV is divided into 9 distinct genotypes, designated from I : IX, along with a total of 38 lineages. This classification framework is founded on the sequencing of hypervariable regions within the spike gene and/or complete sequencing of the S1 gene (Rafique et al., 2024).

Notably, Genotype I (GI) encompasses a significant number of lineages, amounting to 30, which are categorized as GI-1 through GI-30. Conversely, the remaining genotypes each represent a single viral lineage, namely Genotype II (GII-1), Genotype III (GIII-1), Genotype IV (GIV-1), Genotype V (GV-1), Genotype VI (GVI-1), Genotype VII (GVII-1), Genotype VIII (GVIII-1), and Genotype IX (GIX-1) (Domanska-Blicharz et al., 2017; Mendoza-González et al., 2022; Rafique et al., 2024)

IBV targets the epithelia of the respiratory, intestinal tracts, the renal and reproductive systems in poultry so it is considered an epitheliotropic virus. The infection often results in respiratory distress and may lead to renal complications. Furthermore, IBV is associated with a significant decline in egg production among both layer and breeder hens, indicating its profound impact on poultry health and productivity (Jackwood and De Wit, 2018).

Respiratory symptoms associated with this IBV infection involve discharge from the nostril, excessive lacrimation, sneezing, coughing, rales, and episodes of gasping for air. Conversely, renal symptoms may present as behavioral alterations such as lethargy, ruffled feathers, increased water intake, and the passage of wet, pale droppings characterized by a high concentration of urea(Jackwood and De Wit, 2018)

In layer hens, the condition decline in egg production and adversely affects egg quality. The eggs frequently exhibit significant shell abnormalities, including soft shells, rough surfaces, and instances of being shell-less. Furthermore, the eggs may be diminutive, misshapen, or contain watery albumin that lacks a clear distinction between the thick and thin layers (Bo et al., 2022; Jackwood and De Wit, 2018).

In current study assesses the pathogenicity of genetically characterized IBV GI-23 strain (IBV/CH/EG/Moshtohor-3/2023) in specific-pathogen-free (SPF) chicks. As well as, variant II vaccine protectivity against virus.

2. MATERIAL AND METHODS

Ethical approval

The materials and procedures employed in this research were approved by the Scientific Research and Animal

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Welfare Ethics Committee, Faculty of Veterinary Medicine, Benha University, Egypt, with approval number BUFVTM37-06-23.

Virus Titration in SPF-ECE:
The IBV GI-23 strain, designated as IBV/CH/EG/Moshtohor-3/2023, was initially isolated from a broiler chick and subsequently characterized genetically for HVR1,2 and 3 (Accession numbers: PV341021& PV345595). This strain was used to evaluate its virulence properties and protectotype in chicks. In the present study, the virus underwent two passages of propagation via the allantoic sac route in 9-day-old specific pathogen-free (SPF) embryonated eggs. The viral titer was determined using the Reed and Muench equation, with results calculated as the 50% embryo infectious dose (EID50) (Reed and Muench, 1938).

Pathogenicity experiment in SPF chicks
One day SPF chicks(n=30) were utilized to assess the pathogenicity of the IBV IBV/CH/EG/Moshtohor-3/2023 strain. The chicks were individually housed in HEPA-filtered isolators and categorized into 2 distinct groups: Group A and Group B (15 chick for each). All chicks within group A were inoculated with IBV/CH/EG/Moshtohor-3/2023 strain at a viral dose of 105 EID50 in 0.1 mL via the intranasal route (Lisowska et al., 2021) In contrast, the chicks in group B were received 0.1 mL of PBS intranasal route, serving as a mock group.

Clinical signs observation
To evaluate the pathogenic characteristics of the IBV/CH/EG/Moshtohor-3/2023 strain, all birds in group A were observed clinically for 14 days post-infection (dpi). The observations focused on various clinical signs, including depression, ruffled feather, diarrhea, respiratory issues (such as tracheal rales and gasping), and mortality. These signs were scored on a scale of 0 (absent), 1 (mild), 2 (moderate), or 3 (severe) (Wang and Huang, 2000; Zhao et al., 2019)

Histopathological Examination
Necropsy examinations were conducted on the 5th and 7th day post-inoculation (dpi). Two chicks were humanely euthanized, and subsequently, tissue samples were harvested from the trachea and kidney. kidneys and tissue paraffin sections were routinely prepared and stained with H&E according to Suvarna et al., (2018) ,

IBV RNA shedding RT-qPCR
Tracheal and cloacal swabs were collected on days 3, 7, 9, and 14 post-inoculation. Subsequently, viral RNA was extracted directly from the collected swabs a Viral RNA xtraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The shedding of IBV is determined by use primer target highly conserved region within the nucleoprotein (NP) gene of IBV as follows: IBV-FP (5' – ATGCTCAACCTTGTCCCTAGCA – 3') and IBV-RP (5' – TCAAACTGCGGATCATCACGT – 3'). Additionally, the probe used is AIBV-TM (FAM-TTGGAAGTAGAGTGACGCCCAAACTTCA-BHQ1) (Meir et al., 2010)
Protective Efficacy of Variant II Vaccine
Forty-five one-day-old SPF chicks were categorized into 3 groups of 15 birds each. Group A vaccinated at one day old with IBV variant II commercial vaccine via the intraocular route, using a dose of at least 103.5 EID50/0.1 ml, as recommended by the manufacturer. Fourteen days after vaccination, this group was challenged intranasally with the 105 EID50 of the IBV/CH/EG/Moshtohor-3/2023 strain in 0.1 ml. Group B remained unvaccinated and was also challenged with the IBV/CH/EG/Moshtohor-3/2023 strain at the same age. Group C, which was neither vaccinated nor challenged, served as a negative control. All birds were individually housed in isolators under identical conditions.
Upon completion of the challenge, chicks were monitored daily for clinical signs, mortality rates, protection percentages, and tracheal ciliary activity. The trachea was necropsied from each chick and processed to assess ciliary activity using a scoring system based on the criteria established by Tamura et al. (1993). The ciliostasis score for each bird was determined by calculating the mode of the scores from each section and then taking the overall mode from the scores of the three segments. The ciliostasis was scored as follows: score 0 (75% - 100% of cilia is active), score 1(50% - 75% of cilia is active), score 2 (25% -50% of cilia is active), score 3 (0 %-25% of cilia is active)

3. RESULTS

The clinical findings
Fifteen Chicks subjected to the study were rigorously monitored for clinical symptoms over 14 days post-infection (DPI). The affected chicks predominantly exhibited signs of lethargy, huddling, feather ruffling, and mild watery diarrhea. Symptom severity was graded using a scale ranging from 0 (absent) to 3 (severe), as detailed in Table 1. Among the 15 chicks evaluated, a total of six birds died between 3 and7 days post-inoculation (DPI), resulting in a calculated mortality rate of 40%, as illustrated in both Table 1 and Figure 1. Notably, the negative control group displayed no discernible signs or deaths throughout the observation period.

Table 1: The clinical observations of one-day-old SPF chicks infected with the IBV/CH/EG/Moshtohor-3/2023 strain monitored daily for 14 days after infection.

groups		DPI	Observation records			Clinical score	Total mortality	Total mortality %
			Dead	Mortality % / Day	survived			
Group A	Infected group	3	2/15	13.3 %	13/15	1	6/15	40 %
		5	3/13	23 %	10/13	3		
		7	1/10	10 %	9/10	2		
		9	0/9	0	9/9	1		
		12	0/9	0	9/9	0		
		14	0/9	0	9/9	0		
Group B	Negative control	3	0/15	0	0/15	0	0	
		5	0/15	0	0/15			0
		7	0/15	0	0/15			
		9	0/15	0	0/15			
		12	0/15	0	0/15			
		14	0/15	0	0/15			

Clinical score: Score 0: No clinical signs; score 1: Lacrimation, slight shaking of head, watery feces; score 2: Lacrimation, presence of nasal exudate, depression, watery feces; score 3: Strong lacrimation, presence of nasal exudates.

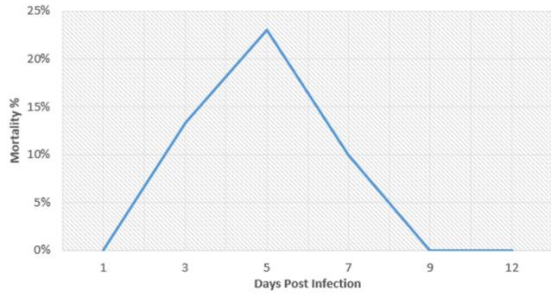

Figure 1: Diagram illustrating the mortality curve of one-day-old SPF chicks infected with the IBV/CH/EG/Moshtohor-3/2023 strain.

Histopathological Findings

The gross pathological examination of dead and necropsied chicks revealed significant lesions in both the trachea and kidney. Notably, from five days post-inoculation (dpi), petechial hemorrhage and catarrhal exudates were observed in the trachea. Additionally, by seven dpi, the presence of a caseous plug at the tracheal bifurcation was noted. The kidneys displayed pronounced swelling, accompanied by urate deposits in ureters, alongside evidence of nephrosis at both five and seven dpi. In contrast, normal gross feature were recorded in chicks within the control group, as illustrated in Figure 2.

The histopathological analysis of the trachea at 5 days post-infection (dpi) in group A revealed significant damage to the tracheal epithelium, shown as loss of cilia and a moderate infiltration of lymphocytes in lamina propria. Meanwhile, the renal tubulointerstitial region illustrates abundant lymphocytic cellular infiltration.

At seven days post-infection (dpi), the mucosal layer of the trachea showed significant thinning, accompanied by a complete loss of cilia. Examination of kidney sections revealed necrosis and degeneration of epithelia of the renal tubules. Additionally, the renal tubular interstitium displayed signs of congestion, while the glomeruli exhibited occasional vasodilation and hyperemia. No histopathological changes were noted in any tissue examined from the control group B, as illustrated in Figure 3

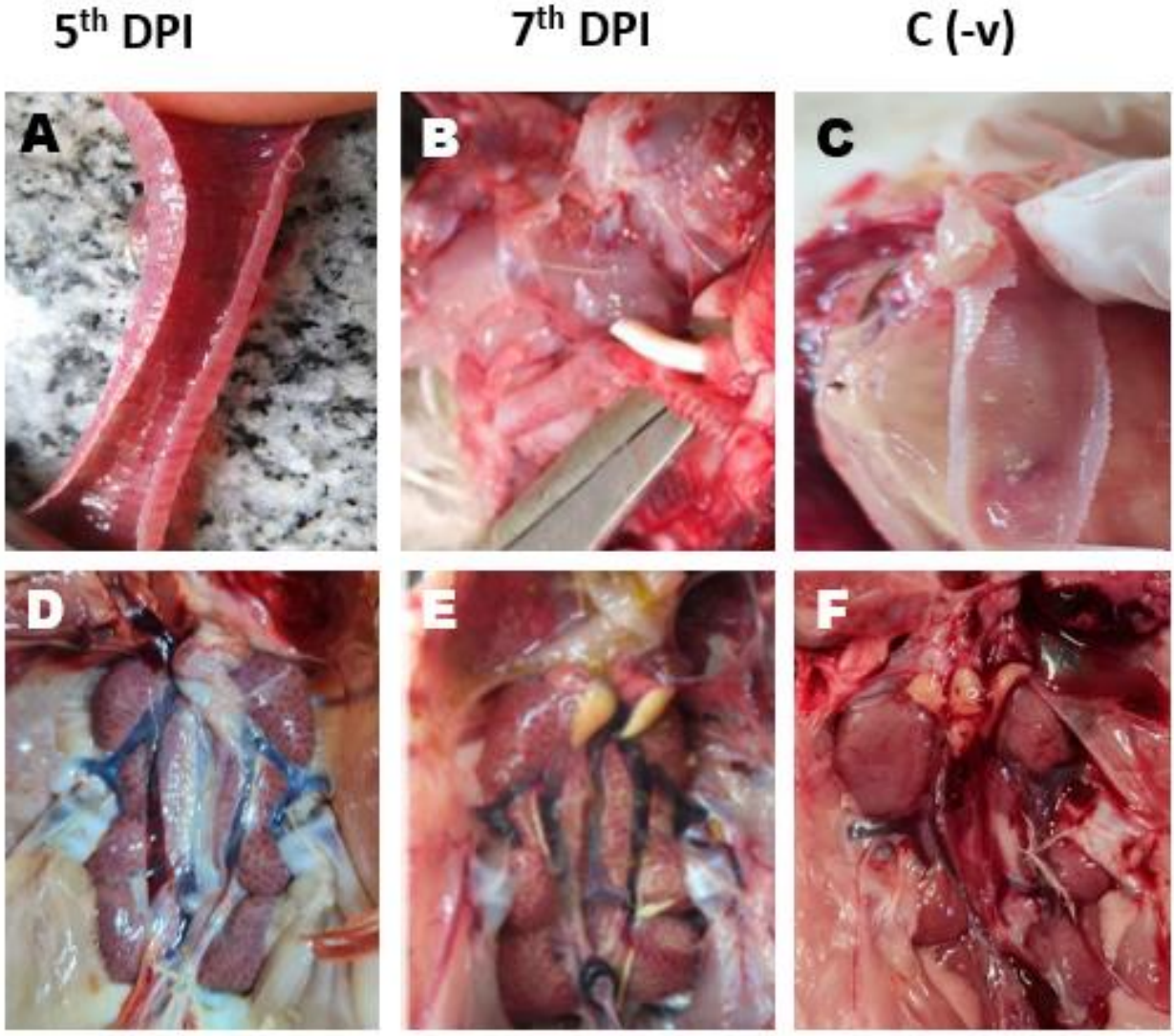

Figure 2: The macroscopic changes during the necropsy for the pathogenicity study in infected group with the IBV/CH/EG/Moshtohor-3/2023 strain and the negative control group. On the 5th day post-inoculation (dpi), A) the trachea exhibited mild mucoid tracheitis, D) the kidney appeared pale and slightly swollen, with mild urate deposits in the ureters. By the 7th day post infection, B) a caseous plug had formed at the tracheal bifurcation, and E) the kidneys showed increased swelling; significant urate deposits were visible in the ureters. C) and F) normal clear trachea and kidney of negative control group respectively.

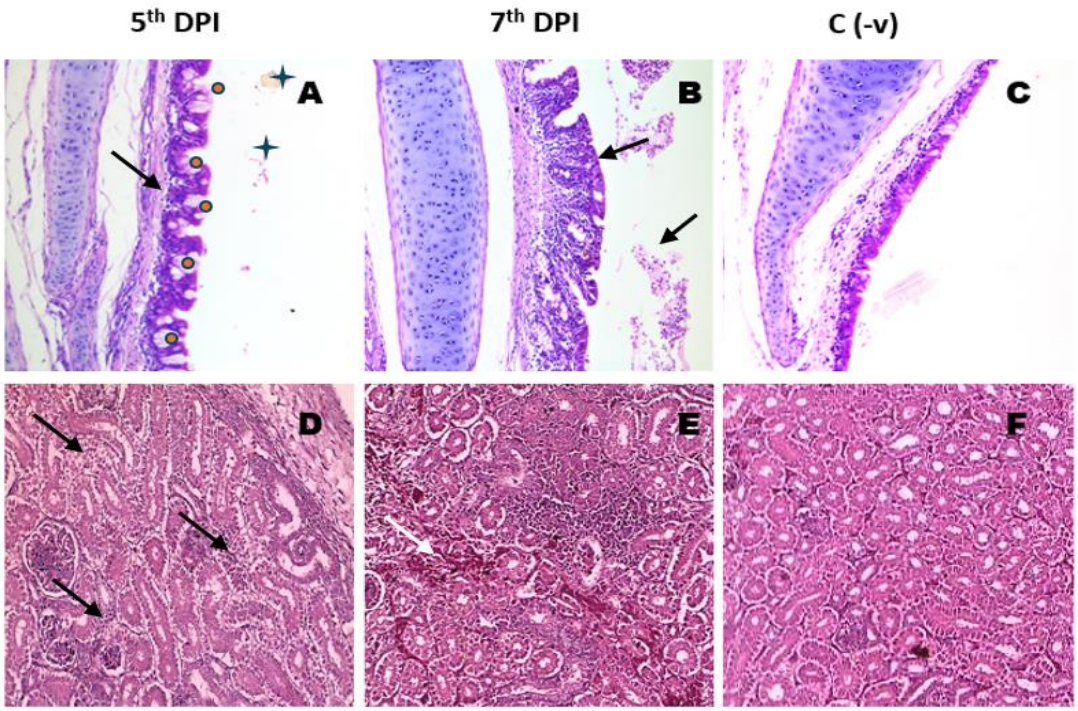

Figure 3: Photomicrograph of tracheal and kidney sections of chicks infected with the IBV/CH/EG/Moshtohor-3/2023 strain and chicks in negative control group. At 5th dpi, A) the tracheal mucosa showed lymphoplasmacytic infiltration, epithelial hyperplasia (black arrow), loss of cilia, degeneration of epithelial cells (black star), and activation of goblet cells (red circles). D) In the kidneys, pronounced lymphocytic infiltration was exhibited within the tubulointerstitial region (black arrow). By 7th dpi, B) the tracheal mucosa exhibits a degree of thinning, accompanied by a complete loss of ciliated epithelial cells and desquamated epithelium in lumen (black arrow). E) Kidneys showed evidence of necrosis and degeneration in the epithelial cells of the renal tubules. The renal tubular interstitium also displayed signs of congestion, while the glomeruli occasionally demonstrated vasodilation and increased blood flow (white arrow).C) and F) normal clear trachea and Kidney of negative control group respectively.

Viral Shedding

The quantification of IBV shed from the trachea and cloaca was conducted, as illustrated in Figure 4. In a comprehensive analysis of viral shedding dynamics, prolonged virus shedding was observed in one-day- old chicks across nearly all times assessed.

IBV shedding reached its peak on the 7th day post-infection (DPI), followed by a gradual decline until the lowest levels of viral copies were recorded at the 14th DPI.

The findings from this investigation indicated that the shedding of the virus from trachea was a significantly higher rate compared to its shedding from the digestive tract.

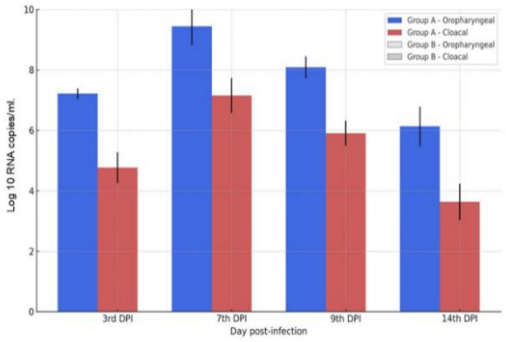

Figure 4: Shedding of IBV in the respiratory and digestive tracts of SPF chicks infected with IBV/CH/EG/Moshtohor-3/2023 strain. The graphs show the mean copy numbers of viral RNA per mL. Quantitative polymerase chain reaction (qPCR) data for viral loads were systematically collected from three individual birds in each experimental group at specified time points: 3-, 7-, 9- and 12-days post-challenge (dpc).

Protective Efficacy of Variant II Vaccine

In this study, the efficacy of the commercial Variant II vaccine to protect against the IBV/CH/EG/Moshtohor-3/2023 strain infection we assessed. For 12 days post-challenge (DPC), no clinical signs in the vaccinated-challenged group (Group A), except a single mortality case recorded on the third DPC. The protective efficacy of the vaccine was determined, revealing a protection rate of 93.6% in comparison to the unvaccinated challenged group (Group B). This latter group exhibited a significant mortality rate of 26.6%, and clinical manifestations were assessed with a score of 1 on the 3rd DPC, escalating to a score of 3 on the 7th DPC. No obvious clinical manifestations or fatalities directly linked to IBV were noted within the unvaccinated, unchallenged control group (Group C). As illustrated in Table 2.

The observed difference in ciliostasis between the category A and category B was significant on the 3rd and 5th DPC. Specifically, the vaccinated-challenged group demonstrated a ciliostasis score of 0 at both the 3rd and 5th DPC, as shown in Table 3 and Figure 5. In contrast, the positive control group exhibited a ciliostasis score of 2 on the 3rd DPC, which increased to a score of 3 by the 5th DPC. These outcomes reflect the efficacy of vaccination in mitigating ciliostasis in the context of the challenge.

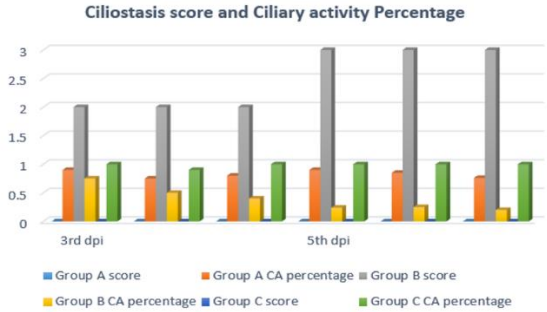

Figure 5: A diagram illustrating the tracheal ciliostasis score and ciliary activity of the trachea in SPF chicks subjected to experimental infection with the IBV/CH/EG/Moshtohor-3/2023 strain.

Table 2: The findings derived from the data collected during the vaccine challenge study

groups	DPC	Observation records						Protection percentage
		Dead	Mortality % / Day	Total deaths	Total deaths %	survived	Clinical score	
Group A Vaccinated challenged group	3	1/15	6.6 %	1/15	6.6 %	14/15	0	93.4 %
	5	0/14	0 %			14/14	0	
	7	0/14	0 %			14/14	0	
	9	0/14	0 %			14/14	0	
	12	0/14	0 %			14/14	0	
Group B Not vaccinated challenged group	3	1/15	6.6 %	4/15	26.6 %	14/15	1	-
	5	2/14	14.2 %			12/14	3	
	7	1/12	8.3 %			11/12	2	
	9	0/12	0 %			12/12	1	
	12	0/12	0 %			12/12	0	
Group C Not vaccinated, not challenged group	3	0/15	0 %	0/15	0 %	0/15	0	-
	5	0/15	0 %			0/15	0	
	7	0/15	0 %			0/15	0	
	9	0/15	0 %			0/15	0	
	12	0/15	0 %			0/15	0	

Clinical score: Score 0: No clinical signs; score 1: Lacrimation, slight shaking of head, watery feces; score 2: Lacrimation, presence of nasal exudate, depression, watery feces; score 3: Strong lacrimation, presence of nasal exudates

Table 3: The ciliostasis score of the group that received vaccination, followed by exposure to a challenge, in comparison to the positive infected group and the negative mock control group.

Groups		3rd dpi		5th dpi	
		score	CA percentage	score	CA percentage
Group A	Vaccinated challenged	0	90%	0	90%
		0	75%	0	85%
		0	80%	0	76%
Mean score and percentage		0	81.6%	0	83.6%
Group B	Positive control	2	75%	3	24%
		2	50%	3	25%
		2	40%	3	20%
Mean score and percentage		2	55%	3	36.7%
Group C	Negative control	0	100%	0	100%
		0	90%	0	100%
		0	100%	0	100%
Mean score and percentage		0	96.7%	0	100%

CA: ciliary activity was scored as zero (100 to 75% ciliary activity), 1 (<75 > 50% ciliary activity), 2 (<50 > 25% ciliary activity), and 3 (25 to 0% ciliary activity).

4. DISCUSSION

Infectious Bronchitis Virus is a significant pathogen that contributes to substantial economic losses in the world poultry industry. The virus is characterized by its variable tissue tropism and diverse virulence criteria. As a result, immune prophylaxis has emerged as the principal strategy for mitigating IBV infections and their associated impacts on poultry health and productivity(de Wit and Cook, 2020).

In this investigation, the genetically characterized strain GI-23 IBV/CH/EG/Moshtohor-3/2023 was utilized for in vivo experimentation. Initially, the pathogenic characteristics of the strain were evaluated utilizing one-day-old SPF chicks. The results obtained from this assessment indicated that the strain possesses virulent properties, ultimately resulting in a discernible disease outcome.

The infected SPF chicks exhibited both respiratory and renal tissue tropism; however, the lesions were more pronounced in the respiratory system. The clinical signs observed included depression, ruffled feathers, lethargy, respiratory distress, and mild watery diarrhea. The histopathological findings, which include damage to the tracheal epithelium, loss of ciliated cells, and necrosis of renal tubules, provide further evidence of the strain's virulence. These lesions are indicative of infections caused by IBV, as the virus predominantly targets epithelial cells within the respiratory and renal systems, as indicated by(Abou El-Fetouh et al., 2016; Zanaty et al., 2016; Lisowska et al., 2021).

Infection in one-day-old SPF chicks results in a mortality rate of 40%. The virus exhibits persistent shedding from both the respiratory and gastrointestinal tracts, with peak viral titers occurring seven days post-infection. Notably, throughout the study, the viral load detected in oropharyngeal swabs was significantly greater than that observed in cloacal swabs. These findings, which are consistent with the virulence character of GI-23 IBV strains (Zanaty et al., 2016; Lisowska et al., 2021)

It is essential to note that the virulence of IBV strains is influenced by many factors, including the virus titer, inoculation route, age and breed of the avian hosts, as well as the properties of the strain itself (Lisowska et al., 2021).

IBV exhibits substantial genetic and antigenic variability (de Wit et al., 2011), which poses considerable challenges for processing and implementation of an effective program of vaccination. This variability is particularly critical, as vaccination may not consistently confer adequate cross-protection against viruses that fall outside the targeted lineage. The existence of diverse vaccine formulations commercially available on the market allows for the strategic selection of vaccine strains that demonstrate antigenic similarities to prevalent strains circulating in the field.An integral component of the paper purpose was the assessment of the protective efficacy of the commercially available Variant II vaccine against the IBV/CH/EG/Moshtohor-3/2023 strain infection.

The experimental trial was conducted utilizing specific-pathogen-free (SPF) broiler chicks. The findings from this study indicate that SPF birds immunized with the Variant II vaccine, when subsequently challenged with the IBV/CH/EG/Moshtohor-3/2023 strain, demonstrated a protection efficacy of 93.4%. Furthermore, no signs or gross lesions were recorded in the vaccinated subjects throughout the observation period following the challenge.

The scoring of ciliostasis serves as a critical metric for evaluating the host response to infection by IBV. This methodology enables an accurate assessment of bird immunity in response to IBV challenges (Cavanagh et al., 1997).

The ciliostasis scores exhibited a significant reduction in the Variant II-vaccinated group, evidenced by a mean score of zero on both the 3rd and 5th days post-

challenge (DPC) with the IBV/CH/EG/Moshtohor-3/2023 strain. In contrast, the unvaccinated group demonstrated higher mean scores of 2 on the 3rd DPC and 3 on the 5th DPC. These findings suggest that the Variant II vaccine confers effective protective immunity to the respiratory tract against this specific strain.

The observed partial protection may be attributed to genetic divergence between the vaccine strain and the GI-23 field strain, as cross-protection among different IBV genotypes is frequently incomplete (Bande et al., 2016). Nevertheless, the findings suggest that the variant II vaccine may serve as an effective tool in controlling GI-23 outbreaks. There remains, however, potential for enhancement through the development of strain-specific vaccines.

5. CONCLUSIONS

The findings of this study emphasize the considerable pathogenicity associated with the GI-23 IBV IBV/CH/EG/Moshtohor-3/2023 strain, as well as the protective efficacy offered by the variant II vaccine. These results underscore the crucial need for ongoing surveillance, rigorous vaccine evaluation, and the development of strain-specific vaccines to mitigate the challenges posed by IBV's evolution effectively. Future research endeavors should focus on elucidating the molecular mechanisms underlying the virulence of GI-23, as well as exploring innovative vaccine platforms to enhance protection against this and other emerging genotypes.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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