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Comparative Evaluation of Infectious Bronchitis Vaccines in Broilers: Impact on Growth Performance, Mortality, and Immune Response

ABSTRACT

This study evaluated the effects of three commonly used imported infectious bronchitis (IB) vaccines on the health and production of broilers. Two hundred and forty day-old Ross-308 broiler chicks were randomly allotted to four groups (six replicates of ten birds each). The control group received no vaccine administration, while groups IB4-91, IBMa5C, and IBH-120 were vaccinated against IB vaccines on days 7 and 21. The vaccine used in Group IB4-91 was from Nobilis®, Vety-Care Pvt Ltd, in group IBMa5 from Nobilis®, and in group IBH-120 from Medivac, Hilton Pharma. Growth performance parameters (feed intake, body weight gain, feed conversion ratio) were not significantly different ($p>0.05$) among the groups throughout the 35-day trial. However, immune response and mortality were significantly influenced by vaccine type. Group IB4-91 showed a significantly higher antibody titer ($p=0.032$) and achieved 0% mortality, which was significantly lower ($p=0.030$) than the other groups. In contrast, control, IBMa5, and IBH-120 all displayed 3.33% mortality and exhibited mild to moderate respiratory symptoms, with *post-mortem* findings revealing tracheal and lung congestion. It is concluded that while the tested imported vaccines did not impact growth, the IB4-91 vaccine provided superior protection, as evidenced by the highest antibody titers and the complete absence of mortality.

INTRODUCTION

The Infectious Bronchitis Virus (IBV) is a highly transmissible gamma-coronavirus (γ CoV), also known as group-III coronaviruses, which causes significant economic losses in the global poultry industry (Legnardi *et al.*, 2020). This classification places it within a group of viruses known for their potential to cause respiratory and other types of infections in animals, including poultry (Legnardi *et al.*, 2020). The two genera, γ CoV and γ CoV, frequently infect various bird species, such as chickens, pheasants, and other Galliformes (Siddell *et al.*, 2008). IBV primarily infects the respiratory tract but can also damage the renal and reproductive systems, leading to respiratory distress, nephritis, and in layers, a severe decline in egg production and quality (Ren *et al.*, 2020; Najimudeen *et al.*, 2020). In broilers, IBV infection can result in mortality rates ranging from 10% to 60%, and reduce overall chick weight, severely impacting profitability (He *et al.*, 2012). The virus's ability to persist in tissues and be shed for extended periods facilitates its spread within and between flocks, making its control challenging (Cook *et al.*, 2012; Zhang *et al.*, 2020).

The ability of IBV to infect various tissues, and the presence of diverse serotypes in different areas lead to high financial losses. The infection, recognized for its highly contagious and severe nature, primarily



affects breeders and laying hens. It leads to significant setbacks, including female reproductive disorders and kidney impairment, resulting in considerable economic losses for poultry producers. The long-term effects of the infection include a significant reduction in egg production, ranging from 20% to 70%. This also leads to declining egg quality and hatching success rates, which can severely impact the poultry industry (Ren *et al.*, 2020). IBV infection can cause a mortality rate of 10% to 60% in broilers aged 4 to 6 weeks, which is substantial and detrimental to broiler populations. This infection leads to high mortality rates and decreases chick weight, further affecting overall production and profitability for poultry farmers (He *et al.*, 2012). Research suggests that IBV can persist in tissues, allowing transmission through infected chickens' feces for up to 163–227 days after infection, which contributes to the spread of the virus and makes it more challenging to control within poultry populations (Cook *et al.*, 2012; Zhang *et al.*, 2020). Vaccination remains the cornerstone of IBV control. Both live-attenuated and inactivated vaccines are used, often tailored to combat local serotypes (Bande *et al.*, 2015). However, the high mutation rate of IBV has led to the emergence of over 50 serotypes worldwide, many of which offer limited cross-protection, thereby compromising vaccine efficacy (De Wit *et al.*, 2020). Continuous viral evolution requires constant monitoring and evaluation of vaccination strategies.

The immune response to IBV involves both humoral and cell-mediated immunity, though the interplay under different field conditions and vaccination regimes is not fully understood (Zhang *et al.*, 2020). While the pathogenesis of IBV is well-documented, there is a need for more data on the comparative efficacy of different vaccine strains, particularly those commonly imported and used in specific regions like Pakistan. Therefore, this study was designed to investigate and compare the effects of three imported IB vaccine strains (4-91, Ma5, and H-120) on broiler growth performance, mortality, and immune response. We hypothesized that the IB 4-91 vaccine would provide superior immunity and protection compared to the Ma5 and H-120 strains under local conditions.

MATERIALS AND METHODS

Ethical Consideration

The experiment was approved by the Ethical Committee of the Department of Poultry Science, University of Agricultural Peshawar, Pakistan (No: 713/PS/UAP, dated April 30, 2024). All procedures adhered

to established guidelines for the humane treatment of laboratory animals.

Experimental Location, Bird Management, and Housing

The trial was conducted at the poultry research facility of the Department of Poultry Science, University of Agriculture Peshawar. A total of two hundred and forty (240) day-old Ross-308 broiler chicks were obtained from a local commercial hatchery. The sample size was determined to provide adequate statistical power (80%) to detect a 5% difference in key performance parameters with a significance level of 0.05, based on previous similar studies. Birds were housed in an open-sided house and managed under standard conditions. Sawdust was used as bedding material, and the ambient temperature was maintained at approximately 23°C. A 23L:1D lighting schedule was provided for the first three days, followed by 20L:4D until the end of the experiment. Ventilation was managed naturally, and strict biosecurity measures were implemented. Feed and water were provided *ad libitum* throughout the 35-day experimental period. The birds received a standard commercial broiler diet formulated to meet Ross-308 nutrient specifications (Table 1).

Table 1 – Commercial (Basal) diet composition.

Ingredients %	Starter phase (day1-21)	Finisher phase (days 22-35)
Corn	57.25	64.98
Soybean meal (CP=44%)	35.49	28.15
Animal fat	3.57	3.58
Dicalcium phosphate	1.51	1.26
Lime stone	1.10	1.01
DL-methionine	0.25	0.19
L-lysine HCL	0.075	0.53
L-threonine	0.026	0.01
Sodium chloride	0.40	0.40
Vitamin premix ¹	0.25	0.25
Mineral premix ¹	0.09	0.09
Calculated nutrients %		
Dry matter	87.41	87.49
M.E (Kcal/kg)	3100	3180
Crude protein	21.50	18.50
True protein	19.0	16.74
Ether extract	4.67	6.07
Crude fiber	2.17	2.08
Ca	0.87	0.76
Total P	0.62	0.62
Avail. P	0.51	0.38
Na	0.22	0.18
Cl	0.27	0.27
Choline	1.85	1.74
Folate	0.86	0.82

¹1 kg of premix provided Vitamin E 80 mg, vitamin D3 3,000 IU, A 15,000 IU, vitamin B1 3 mg, vitamin K₃ 3 mg, vitamin B₂ 8 mg, vitamin B₆ 60 mg, vitamin B₁₂ 15 mg, vitamin B12 0.02 mg, Choline chloride (60%) 700 mg, vitamin B6 4 mg, vitamin B9 2 mg, vitamin H 0.2 mg/Kg, Zn (as ZnSO₄.H₂O) 80 mg, Mn (MnSO₄.H₂O) 80 mg, Cu (CuSO₄.H₂O) 10 mg, Se 0.2 mg, Fe (FeSO₄.H₂O) 60 mg, I (KI) 1 mg. Ca= Calcium; Total P= Total phosphorus; Avail.P= Available phosphorus, Na= Sodium; Cl= chlorine.



Experimental Design and Vaccination

Birds were distributed in a Completely Randomized Design (CRD) into four groups with six replicates per group (10 birds/replicate). Group A served as an unvaccinated control. Groups IB4-91, IBMa5C, and IBH-120 were vaccinated against IB via the eye-drop (ED) route on days 7 and 21. Group IB4-91 received the IB4-91 vaccine from Nobilis®, Vety-Care Pvt Ltd; Group IBMa5C received the IBMa5 vaccine from Nobilis®; and Group IBH-120 received the IBH-120 vaccine from Medivac, Hilton Pharma. All vaccines were reconstituted in a physiological saline solution per manufacturers' instructions, and administered using a standardized dropper. The experimental groups are described in Table 2, while the complete vaccination and medication schedule is detailed in Table 3.

Table 2 – Experimental groups description.

Groups	Description
Control	Group of birds with no vaccine administration.
IB4-91	Group of birds administered with infectious bronchitis vaccine IB4-91 from Nobilis®, Vety-Care Pvt Ltd.
IBMa5	Group of birds administered with infectious bronchitis vaccine IB Ma5 from Nobilis®.
IBH-120	Group of birds administered with infectious bronchitis vaccine IBH-120 from Medivac, Hilton Pharma.

Table 3 – Vaccination and medication schedule.

Groups	Vaccine/Medication ¹	Days of Administration	Route
Control, IB4-91, IBMa5 and IBH-120	Newcastle Disease (Mustakhwar strain)	5	Drinking water
Control, IB4-91, IBMa5 and IBH-120	Coccidiostat	1-35	In feed
IB4-91, IBMa5 and IBH-120	Infectious Bronchitis (1 st dose)	7	Eye drop
Control, IB4-91, IBMa5 and IBH-120	Gumboro -1	12	Drinking water
Control, IB4-91, IBMa5 and IBH-120	Gumboro -2	18	Drinking water
IB4-91, IBMa5 and IBH-120	Infectious Bronchitis (2 nd dose)	21	Eye drop
Control, IB4-91, IBMa5 and IBH-120	Newcastle Disease (LaSota strain)	24	Drinking water

¹Mustakhwar and LaSota strain vaccine of Newcastle Disease virus was administered to all birds in all experimental groups (control, IB4-91, IBMa5 and IBH-120) on day 5 and 24, respectively, via drinking water (DW). Coccidiostat was offered in the feed of all experimental groups (control, IB4-91, IBMa5 and IBH-120) throughout the experiment (1-35 days). Different commercially available 1st and 2nd doses of Infectious Bronchitis vaccines (IB4-91, IBMa5 and IBH-120) were provided to the three experimental groups; on days 7 and 21, respectively, via eye drops. Gumboro-1 and 2 vaccine were provided to all the experimental groups (control, IB4-91, IBMa5 and IBH-120) on days 12 and 18.

Growth Performances

Growth performance was recorded weekly. Feed intake (FI) was calculated as the difference between

feed offered and refused. Body weight gain (BWG) was determined by subtracting the initial weight from the final weight for each period. The feed conversion ratio (FCR) was calculated as FI divided by BWG.

Mortality and Clinical Signs

Mortality was recorded daily, and the cumulative percentage was calculated at the end of the experiment. *Post-mortem* examinations were conducted on all dead birds to ascertain the cause of death. All birds were observed daily for clinical respiratory signs such as coughing, nasal discharge, and labored breathing.

Sampling for Antibodies Titration

Blood samples were collected from the wing vein of four birds per replicate on days 14, 21, and 28. Serum was separated by centrifugation at 3000 × g for 10 minutes and stored at -20°C until analysis. Antibody titers against IBV were measured using a commercial ELISA kit (IDEXX IBV, IDEXX™, USA) according to the manufacturer's instructions and the methodology described by Snyder *et al.* (1985).

Statistical Analysis

All data were analyzed using the General Linear Model (GLM) procedure of SPSS version 21.0 in a completely randomized design (CRD). The mathematical model used was: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is the observed value, μ is the overall mean, T_i is the effect of the i th treatment, and e_{ij} is the random error. The normality of the data was confirmed using the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was applied, and means were separated using the LSD test when significant differences were found. Results were considered statistically significant at $p < 0.05$, and actual p -values are reported.

RESULTS

Growth Performance

The effects of different IB vaccines on growth performance are shown in Table 4. No significant differences ($p > 0.05$) were observed among the groups for weekly or overall feed intake (FI), body weight gain (BWG), or feed conversion ratio (FCR). Numerically, overall FI was slightly higher in groups IB4-91 and IBMa5, while BWG was marginally higher in the control and IB4-91 groups.



Table 4 – Effect of various strains of IB vaccination on the weekly feed Intake, body weight and feed conversion ratio (FCR) of broilers.

Groups	7day	14day	21day	28day	35day	Overall
Feed Intake (Kg)						
Control	0.14	0.52	1.25	1.95	2.61	2.47
IB4-91	0.14	0.54	1.23	1.94	2.63	2.48
IBMa5	0.13	0.54	1.23	1.95	2.61	2.48
IBH-120	0.14	0.53	1.23	1.98	2.60	2.46
SEM	0.003	0.004	0.006	0.012	0.006	0.005
p-value	0.450	0.067	0.479	0.718	0.332	0.622
Body weight (Kg)						
Control	0.16	0.50	1.22	1.54	1.99	1.83
IB4-91	0.15	0.49	1.18	1.53	1.97	1.83
IBMa5	0.15	0.48	1.19	1.53	1.98	1.82
IBH-120	0.15	0.49	1.18	1.53	1.97	1.82
SEM	0.02	0.03	0.09	0.05	0.03	0.04
p-value	0.728	0.311	0.328	0.054	0.521	0.471
FCR						
Control	0.90	1.03	1.03	1.26	1.31	1.35
IB4-91	0.95	1.06	1.04	1.27	1.33	1.35
IBMa5	0.86	1.06	1.04	1.28	1.32	1.36
IBH-120	0.88	1.06	1.04	1.29	1.32	1.35
SEM	0.08	0.07	0.08	0.07	0.03	0.03
p-value	0.330	0.302	0.894	0.598	0.099	0.596

Means in the column with distinct superscripts show a significant difference at $p < 0.05$. Control=Group with no vaccine administration. IB4-91=Group of birds administered with infectious bronchitis vaccine IB4-91 from Nobilis®, Vety-Care Pvt Ltd. IBMa5=Group of birds administered with infectious bronchitis vaccine IBMa5 from Nobilis®. IBH-120=Group of birds administered with infectious bronchitis vaccine IBH-120 from Medivac, Hilton Pharma.

Immune Response and Mortality

The immune response and mortality data are presented in Table 5. Antibody titers against IBV were significantly affected by the treatments. On day 14, the titer in group IB4-91 was 61% higher than that of the control group ($p=0.021$). This significant advantage persisted on days 21 ($p=0.003$) and 28 ($p=0.011$). Overall, group IB4-91 had the highest geometric mean titer (1431.2), which was significantly greater ($p=0.032$) than all other groups.

Table 5 – Effect of various IB-Virus vaccines on broilers' immune response and mortality against IBV.

Groups	Antibodies titer against IBV				Mortality %
	14d	21d	28d	Overall	
Control	1033.2 ^d	992.7 ^c	963.0 ^c	996.3 ^c	3.33 ^a
IB4-91	1666.7 ^a	1385.2 ^a	1241.7 ^a	1431.2 ^a	0.00 ^b
IBMa5	1407.7 ^c	1062.4 ^b	1137.3 ^b	1202.4 ^b	3.33 ^a
IBH-120	1616.3 ^b	1102.7 ^b	1168.7 ^b	1295.9 ^b	3.33 ^a
SEM	76.23	45.83	31.59	48.08	1.31
p-value	0.021	0.003	0.011	0.032	0.030

Means in the column with distinct superscripts shows a significant difference at $p < 0.05$. Control=Group with no vaccine administration. IB4-91=Group of birds administered with infectious bronchitis vaccine IB4-91 from Nobilis®, Vety-Care Pvt Ltd. IBMa5=Group of birds administered with infectious bronchitis vaccine IBMa5 from Nobilis®. IBH-120=Group of birds administered with infectious bronchitis vaccine IBH-120 strain from Medivac, Hilton Pharma.

Mortality was also significantly different among the groups ($p=0.030$). Group IB4-91 recorded 0% mortality, while control, IBMa5, and IBH-120 groups had a mortality rate of 3.33%.

Clinical Signs

Clinical observations are presented in Table 6. No respiratory signs were observed in group IB4-91. In contrast, birds in the control group and those vaccinated with IBMa5 and IBH-120 exhibited mild to moderate respiratory signs, including coughing, nasal discharge, and labored breathing. *Post-mortem* findings of birds that died in these groups consistently showed congestion in the trachea and lungs.

Table 6 – Clinical respiratory signs observed in broilers receiving various IBV vaccines.

Groups	Respiratory signs
Control	Mild to moderate coughing, nasal discharge, and labored breathing.
IB4-91	No respiratory signs.
IBMa5	Mild to moderate coughing, nasal discharge, and labored breathing.
IBH-120	Mild to moderate coughing, nasal discharge, and labored breathing.

Control=Group with no vaccine administration. IB4-91=Group of birds administered with infectious bronchitis vaccine IB4-91 from Nobilis®, Vety-Care Pvt Ltd. IBMa5=Group of birds administered with infectious bronchitis vaccine IBMa5 from Nobilis®. IBH-120=Group of birds administered with infectious bronchitis vaccine IBH-120 from Medivac, Hilton Pharma.

DISCUSSION

In Pakistan, the geographic location affects the distribution of IBV serotypes, and mostly live attenuated IB vaccines are given to broilers and layer flocks. Additionally, the booster shot is administered using the same serotype as the live vaccine or in conjunction with a different serotype (Lee *et al.*, 2010). Numerous bodily systems, including the respiratory, renal, and reproductive systems, are affected clinically and pathologically by IB (Najimudeen *et al.*, 2020). In the present study, the birds' production performance (FI, WG, and FCR) was unaffected by vaccination at different time intervals. According to various authors, different diets, husbandry, environmental factors, and genetics may have an impact on the production effects reported as ultimate body weight (Pekel *et al.*, 2013; Hristakieva *et al.*, 2014; Benyi *et al.*, 2015; Yang *et al.*, 2016; Bach *et al.*, 2019; Tahamtani *et al.*, 2010).

Our study demonstrated that the studied vaccination programs may affect hens' ultimate body weight. The IB4-91 vaccine is closely related to the IB strains circulating in local poultry, and has resulted in the least infected birds on the day of vaccination or thereafter. The superior performance of the IB4-91 vaccine in eliciting antibody titers and preventing mortality can be scientifically attributed to a closer



antigenic match with the circulating field viruses in the region. A vaccine strain that is genetically and antigenically similar to the challenge virus induces a more effective and specific immune response, leading to better neutralization of the virus and reduced viral shedding. This effective priming of the immune system explains the absence of clinical signs and zero mortality in Group IB4-91, as the birds were better equipped to control the infection. On the other hand, the IBMa5 and IBH-120 vaccine strains may have been used for a long time in the broilers in this province. They may have developed mutations in the local strains, whereby body reaction after vaccination in the form of high temperature may have resulted in a lower gain in body weight. The lower antibody titers and higher mortality observed in groups IBMa5 and IBH-120, despite vaccination, suggest a possible antigenic drift or the presence of variant strains in the field that are not effectively covered by these vaccines. This lack of cross-protection can lead to vaccine breakdown, where vaccinated birds are still susceptible to infection by divergent field strains, resulting in clinical disease and mortality (De Wit *et al.*, 2020).

The superior antibody titer and zero mortality in the IB4-91 group are the most critical findings. The significantly higher GMT suggests that the IB4-91 strain provoked a more robust and effective humoral immune response. This is likely because the IB4-91 strain is more antigenically similar to the field strains circulating in the local area, leading to better immune recognition and a stronger antibody response. The absence of mortality and clinical signs in this group further confirms this superior protection. In contrast, the IBMa5 and IBH-120 strains, which have been used for a longer period in the region, may be less effective against currently circulating variant viruses due to antigenic drift, as suggested by Elhady *et al.* (2018). Our results align with Sarueng *et al.* (2014), who found that vaccine efficacy is highly dependent on the match between the vaccine and challenge strains. The significantly higher antibody titer in the IB4-91 group underscores the importance of strain selection in vaccination programs. An effective vaccine not only stimulates the humoral immune system to produce high levels of antibodies but also likely induces a robust cell-mediated and mucosal immune response, which is crucial for combating respiratory pathogens like IBV. The comprehensive immune activation by a well-matched vaccine strain (IB4-91) prevents the establishment and progression of the disease, thereby directly reducing mortality rates. A study showed that at 35 days of age,

the vaccinated challenged groups exhibited higher antibody titers than the unvaccinated challenged groups, as reported by Thompson *et al.* (1997). This indicates a stronger immune response in the vaccinated birds when exposed to the challenge, suggesting that vaccination significantly enhances the ability of the immune system to produce antibodies in response to pathogens. Sarueng *et al.* (2014) demonstrated that different vaccination programs showed protection against damage after the challenge with a live IB QX-like virus. Box, *et al.* (1985) reported that birds treated with live attenuated vaccinations, followed by inactivated vaccines administered before laying, achieved protection against challenges encountered during the laying phase with M41 IBV. Likewise, immunization policies that include live attenuated and inactivated vaccines have also protected against the decline in egg production, problems with quality, and damage to renal tissue brought on by IBV D1466 infection (Sjaak *et al.*, 2020).

The presence of respiratory signs and mortality in the control, IBMa5, and IBH-120 groups, accompanied by *post-mortem* lesions, indicates a failure to prevent clinical disease. This underscores the limited cross-protection offered by some vaccines and highlights the vulnerability of unvaccinated birds. The finding that vaccinated groups (IBMa5 and IBH-120) still showed disease signs reinforces the concept that vaccination alone is insufficient if the vaccine strain is not well-matched to the field challenge (De Wit *et al.*, 2020).

CONCLUSION

In conclusion, the three imported IB vaccines did not adversely affect the growth performance of broilers. However, the IB4-91 vaccine proved to be the most effective under the conditions of this study, generating the highest antibody titers and providing complete protection against mortality and clinical respiratory signs. These results suggest that the IB4-91 strain is better suited to the current field challenge in the region. Nevertheless, the development of an autogenous vaccine based on locally prevalent strains is recommended for optimal and sustained protection against IBV.

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AUTHOR CONTRIBUTIONS

Conceptualization, HK and R; Data curation, SK, MS, SUH, MEA, MIK, AHA, ABI, PRDLRE and AAS; Formal analysis, MS; Funding acquisition, AAS; Investigation, SK; Methodology, HK and R; Resources, SK; Software, MS; Supervision, R; Validation, R, SK, MS, SUH, MEA, MIK, AHA, ABI, PRDLRE, WFL, AAS; Visualization, MS and AAS; Writing – original draft, MS; Writing – review & editing, R, SK, SUH, MEA, MIK, AHA, ABI, PRDLRE, WFL, and AAS. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data from the corresponding author is available upon reasonable request.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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