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An Analysis of Cross-Protection of Commercial IBV Vaccines against Locally Isolated Field Strains of Infectious Bronchitis Virus in Pakistan

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MS and AG conceived and designed the study. MS conducted while AG, MR and HM supervised the study and helped in data analysis and interpretation. MAK critically reviewed and contributed in finalizing the article.

Key words
Cross-protection, Commercial IBV Vaccine, Infectious bronchitis virus, Field strains, Pakistan

ABSTRACT

Vaccine failure against infectious bronchitis (IB) due to emergence of nephro-pathogenic strains in field is a major problem. This study evaluated cross protection of commercial IBV vaccines against field isolates. A total of 160, day-old chicks were equally divided into four groups. Group A was vaccinated with a single dose of H120 strain IBV vaccine, group B with two doses (days 02 and 15) of H120 strain, group C with two doses of heterologous strains (H120 on day 02 and 4/91 on 15) while group D kept unvaccinated. Antibody titer was evaluated using a commercial ELISA kit. The difference in antibody titer of vaccinated groups was non-significant (P value > 0.05) till two weeks post-vaccination. At 04 weeks of age, the highest antibody titer was observed for group B, followed by group C and A. Challenge with a field isolate of IBV induced a rise in antibody titer in all groups. Group D had the highest score (severe) of clinical signs and mortality (n=04/10) followed by group A, showing a moderate score of clinical signs and mortality (n=02/10). In contrast, group C was the most protected group showing mild signs and no mortality. Nephro-pathogenic gross lesions were predominant in all groups except group C which had the lowest score. It was concluded that nephro-pathogenic strains are involved in field outbreaks of IB. Further, a vaccination program only with a classical strain could not provide full protection while priming with a classical strain in first week and boosting by a variant strain vaccine after second week may provide better protection against the disease. Protective-typing is also recommended for development of vaccine from locally isolated strains.

INTRODUCTION

Infectious bronchitis (IB) is one of the major respiratory diseases of poultry having great economic impact on the industry. In breeders and layers, the disease has a negative effect on the productivity of laying birds while in broiler mortality, reduced performance, and secondary infections are of serious concerns (Muneer *et al*., 2000; Cavanagh and Gelb, 2008).

IB was first described by Schalk and Hawn in 1931 as a respiratory disease while its cause being established in 1936 (Cook *et al*., 2012). The disease is caused by IB virus (IBV) which is member of the family Coronaviridae. Until 1956, the Massachusetts was the only identified strain causing IB, then Jungherr with his colleagues demonstrated the Connecticut isolate and reported that these two viruses cause similar disease but are antigenically different from each other (Jungherr *et al*., 1956). IBV contains a 27.6 kb single-stranded, positive-sense RNA genome. In the infected cells, virus produce six mRNAs, out of which three are responsible for the production of the spike (S) and membrane (M) glycoproteins as well as an internal nucleoprotein (N) (Pasternak *et al*., 2006; Sawicki and Sawicki, 2005). The S protein comprises two or three copies of each of two glycopolypeptides named S1 and S2. S1 is supposed to be more immunogenic against which haemagglutination-inhibiting (HI) and most of the virus-neutralizing (VN) antibodies are being induced during the course of infection (Cavanagh and Gelb, 2008). IBV affect three main body systems in poultry i.e., respiratory, female reproductive and kidneys. Respiratory clinical signs include depression, coughing, head-shaking and nasal and ocular discharges while necropsy findings

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include tracheitis, inflammatory lungs, and thickened cloudy air sacs. Death occurs from asphyxiation resulting from accumulation of caseous plugs in bronchi which mostly results from secondary infections. Even from the beginning of IB demonstration, the laying hens were recorded to be affected.

IB is endemic in Pakistan and several classical (M-41, H120, H52) and variant (D-274, D-1466, Arkansas, 4-91 and JMK) serotypes have been reported from various regions of the country (Ahmed *et al*., 2007; Shabina *et al*., 2018; Umar *et al*., 2019). These studies mostly relied upon serological evidences rather than virus isolation or molecular detection methods, which were insufficient to draw conclusion about the molecular nature of the field virus and to differentiate the field viruses from the vaccine strains. Several tests have been employed to identify prevalent IBV serotypes, including virus isolation, VN, HI, hybridization, ELISA and RT-PCR. However, despite widespread vaccination, IB outbreaks are still very common indicating little or no cross-protection between different IBV serotypes (Cook *et al*., 2001; Liu *et al*., 2009). A combined or consecutive use of two or more serotypes as vaccines has been suggested to be a solution of the problem (Cook *et al*., 2001; Jackwood *et al*., 2005). A cross-protection study between the field isolates and the prevailing vaccine strains in the country could give us better understanding to design vaccine schedule and selection of vaccine strains. This study aims to evaluate the cross protection conferred by commonly used commercial IBV vaccines against field isolates of IBV.

MATERIALS AND METHODS

In this study, three different vaccination regimes were tested in different groups while keeping one group as a control receiving no vaccination. For this purpose, a total of 160 number of days old commercial broiler chicks were reared in Experimental Shed Facility at University of Veterinary and Animal Sciences, Lahore. The shed was properly cleaned and fumigated one week prior to the arrival of chicks. The chicks were equally divided into four groups and offered with feed and water ad libitum. In adherence to established ethical guidelines, this study involving poultry birds prioritized their welfare, minimizing distress and discomfort. Oversight and approval were obtained from the appropriate regulatory or ethical review body.

Vaccine strains and vaccination regime

A total of 160 chicks were equally divided to four groups (Group A, B, C and D). A representative number of chicks from each group was sacrificed and tested for maternal derived antibodies (MDA). Two commercial vaccines were used for evaluation in the current study i.e., one having classical (H120) strain and the other variant (4/91) strain. These vaccine strains were selected because these are most commonly used for vaccination against IB in Pakistan. Briefly, after checking MDA titer, the chicks in Group A, B and C were vaccinated on day 02 of age with a primary dose of commercial IBV vaccine having the classical (H120) strain, while Group D was kept unvaccinated (control). Two groups were given booster dose on day 15 i.e., Group B with the same IBV H120 strain vaccine, while Group C with commercial IBV vaccine having variant (4/91) strain. The aim of booster doses given in two groups with two different strains was to check the level of protection provided by different strains combinations. Vaccination was done through intranasal route.

Monitoring of antibody titer

MDA titer against IBV was tested in about 5% chicks before vaccination. After vaccination, the antibody titer in chicks of various groups was regularly monitored through indirect ELISA. For this purpose, blood was collected from birds of all groups on day 08, 15, 22, 29 and 36 of age and serum was separated. All the samples were processed for ELISA using commercial kit of AsurDX™ Infectious Bronchitis Virus (IBV) Antibody Test Kit Manual, Catalog # 10045-02 (192 Wells) of Biostone™ animal health. The procedure was performed according to the manufacturer's instructions.

Challenge with IBV field strain

On day 29, ten birds in each group were subjected to challenge with field strain of IBV. Challenge virus was received from Director University Diagnostic Laboratory, UVAS, Lahore. The challenge virus (CK/PAK/UDL/MS-04/MULT/2020) was previously isolated and genetically characterized as variant strain having Accession number as OL763344. A 0.2 mL of the propagated virus was administered through nasal drops. All the birds were kept under observation for 07 days for recording clinical signs, mortality and gross lesions as previously described (Ismail *et al*., 2020). The data was collected on a pre-designed proforma to record respiratory and other clinical signs, mortality and gross lesions. The antibody titer after challenge with field isolate was also tested in all groups through ELISA. For gross lesions, all the challenged birds were sacrificed on day 36 of age and their organs (trachea, lungs and kidneys) were examined for scoring of gross lesions. The data was compiled and analyzed through ANOVA for comparison of groups and time, whereas means were compared through LSD multiple comparison test by using Statistix software (8.1).

Table I. ELISA antibody titer (Mean± SEM) of various groups at different weeks of age.

Group	Maternal antibody titer	Mean antibody titer post-vaccination				Mean antibody titer post-challenge
	Day 0	Week 1	Week 2	Week 3	Week 4	Week 5
A	4699^{aa}±1284	3093^{ba}±226	2090^{da}±99	2683^{cc}±233	3342^{bc}±163	4542^{ac}±173
B	4626^{ba}±1083	3192^{ca}±201	2137^{da}±105	3646^{ca}±165	4451^{ba}±215	5620^{aa}±759
C	5078^{aa}±1123	3266^{ca}±167	2110^{da}±121	3058^{cb}±164	4016^{bb}±154	5038^{ab}±166
D	4843^{aa}±1390	2370^{bb}±293	953^{db}±102	164^{ed}±35	90^{ed}±11	1787^{cd}±196

1st Superscript along the row; 2nd Superscript along the column.

Following scoring of clinical signs was used:

1 = No signs
2 = Lacrimation/nasal exudate
3 = Lacrimation/nasal exudate + ruffled feathers
4 = Lacrimation/nasal exudate + ruffled feathers + depressed

Scoring of respiratory lesions:

1 = No Lesions
2 = Tracheal exudate
3 = Tracheal exudates + tracheal hemorrhages
4 = Tracheal exudates + tracheal hemorrhages + air plug + air sacs damage.

Scoring of kidneys lesions:

1 = No Lesions
2 = Mild swelling of kidneys
3 = Moderate swelling of kidneys
4 = Sever swelling + hemorrhages in kidneys

RESULTS

Immune response of various groups

The level of MDA titer against IBV was checked on day 02. However, no significant difference (P value > 0.05) was recorded in the mean maternal antibody titer on day 02 of age (Table I). After vaccination till two weeks post-vaccination, it was observed that the mean antibody titer of group A, B and C was almost similar in both weeks and no significant difference (P value > 0.05) was found in mean titer of vaccinated groups while the titer of group D (unvaccinated) was recorded significantly (P value <0.05) lower than those of vaccinated groups. A decline was observed in the mean titer in group D (control) as compared to other (vaccinated) groups. However, the control group was found sero-positive for maternal antibodies till day 15 of age (Table I).

After booster doses given on day 15 of age to Group B and C, the difference in mean titer of various groups was found more diverse on day 22 of age (three weeks post-vaccination) and highest value (3646) was recorded for group B, followed by group C (3058) and group A (2683). During this week, the overall difference in titer of all groups was significant (P value < 0.05) with group B showing the highest titer (3646).

The same pattern in the rise of mean titer on day 29 of age (four weeks post-vaccination) was recorded with highest value (4451) recorded for group B, followed by group C (4016) and group A (3342). The mean titer of all groups was significantly different (P value < 0.05) from each other. Group D (control) was found sero-negative for antibody titer (Table I).

Further, one-week post-challenge (day 36 of age), the mean antibody titer was observed as 4542 for group A, 5620 for group B, 5038 for group C and 1787 for group D. There was significant difference (P value < 0.05) in mean titer of all groups. Group B was recorded for highest antibody titer (5620), followed by group C (5038) and group A (4542). The birds of group D (unvaccinated) were found sero-positive after challenge resulting in a mean antibody titer of 1787 (Table I).

Post challenge clinical signs and mortality

All the challenged birds of all groups were carefully examined for development of any signs of IB and the data recorded accordingly. The data was recorded twice daily till 7 day of challenge. The clinical signs developed in 10 (100%) birds in group A, 06 (60%) ingroup B and 04 (40%) in group C while in group D all 10 (100%) birds showed clinical signs which increased in severity. The average score of clinical signs was 2.75 for group A, 2.1 for group B, 1.7 for group C and 3.83 for group D (Table II, Fig. 1).

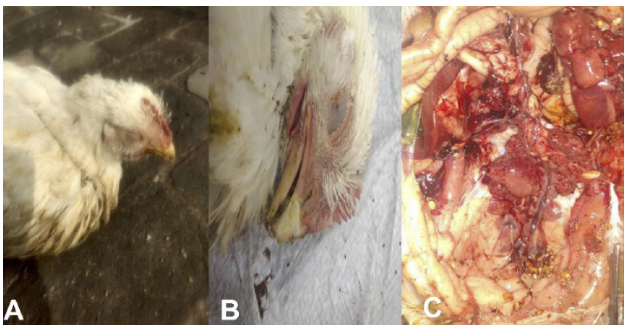

Fig. 1. Clinical signs (A), mortality (B), organs' lesions (C) in experimental birds.

Table II. Clinical signs scoring (CSS) in various groups as recorded after challenge with IBV field isolate.

Group	Day 1 PC		Day 3 PC		Day 5 PC		Day 7 PC	
	Affected birds	CSS	Affected birds	CSS	Affected birds	CSS	Affected birds	CSS
A	03	1.3	05	1.9	07	2.4	10	2.75
B	0	1	03	1.3	04	1.7	06	2.1
C	0	1	02	1.2	03	1.5	04	1.7
D	05	1.5	08	2.7	10	3.4	10	3.83

PC, post-challenge; CSS, clinical signs scoring.

These findings showed the highest number of affected birds and score of clinical signs in group D (unvaccinated/control) followed by group A which was vaccinated with a single dose of H120 strain vaccine. Group C showed the lowest score of clinical signs and number of affected birds which was vaccinated with two doses of heterologous strains (H120 and 4/91) vaccines. Further, the mortality was found to be 02 (out of 10) in group A and 04 (out of 10) in group D while no mortality was recorded in group B and C (Table III).

Table III. Post-challenge mortality of birds in various groups.

Group	Post-challenge mortality rate		
	Total birds	Death (s)	%
A	10	02	20
B	10	0	0
C	10	0	0

Table IV. Post-challenge scoring of gross lesions in different organs of birds in various groups.

Group	Total birds examined	Respiratory lesions score	Nephro pathogenic lesions score
A	10	2.6	2.9
B	10	1.9	2.1
C	10	1.7	1.4
D	10	3.2	3.7
P value		< 0.001	< 0.001

Scoring of post-mortem gross lesions

Post-mortem examination of dead birds was carried out on the day of mortality and the score of gross lesions in different organs was recorded (Fig. 1). The remaining live birds were sacrificed after seven days of challenge and subjected for necropsy findings. The average score for respiratory organs lesions was 2.6 for group A, 1.9 for group B, 1.6 for group C and 3.2 for group D. The score for nephro-pathogenic lesions was recorded as 2.9 for group A, 2.1 for group B, 1.4 for group C and 3.7 for group D. The score of lesions was higher in all groups as compared to group C (Table IV).

DISCUSSION

During the present study, cross protection of commercial IBV vaccines using different regimes was evaluated against nephron-pathogenic field isolate. Antibody response of various groups was compared on different intervals of time. All the groups were challenged with IBV field isolate and evaluated for protection against clinical signs development, mortality and gross lesions in various organs. Maternally derived antibody titer was also evaluated before vaccination and no significant difference was observed in MDA titer of various groups. However, after vaccination a decline in titer of various groups was observed. The findings of our study regarding decline in MDA titer are supported by the previously published data (Darbyshire and Peters, 1985; Awad *et al*., 2016a, b). In similar studies high MDA titer and its decline was recorded till three weeks of age (Shao *et al*., 2020; Kutle *et al*., 2020). However, it showed no correlation with the overall protection of birds against IBV. In all previously reported studies, the decline in antibody titer despite vaccination was observed. They concluded that maternally derived antibodies have very little or no effect on the efficacy and protection conferred by the vaccine. In our study, we found higher titer in group B vaccinated with two doses of homologous strain (H120 strain) vaccines as compared to group C which was vaccinated with heterologous strains (H120 and 4/91). These findings were in similar line with previous study (Boelm *et al*., 2018) in which higher antibody titer was recorded in birds vaccinated with two doses of homologous classical strain (H120). This was also recorded by other researchers in similar studies (Ismail *et al*., 2020) who reported good titer against H120 strain as compared to 4/91 strain. Further, higher titer was observed

for birds vaccinated with two doses of homologous classical strain vaccines as compared to those vaccinated with heterologous strains vaccines.

A post-challenge rise in antibody titer in both vaccinated and unvaccinated groups was also observed. This pattern of post-challenge rise in antibody titer has also previously documented by various authors (Sasipreeyajan *et al*., 2012; Awad *et al*., 2016b). Other similar studies also reported similar findings for antibody titer increase till 21-days post infection (Boelm *et al*., 2018; Shao *et al*., 2020).

The challenged birds were also observed for development of clinical signs. It was recorded that highest score of clinical signs was observed in challenged birds unvaccinated (control) group D, followed by group A. Lowest score of clinical signs was recorded in birds of group C. These findings are supported by previously published data which report that combining of one classical and a second genetically related variant vaccine could provide better and broader protection against field isolates (Sultan *et al*., 2019; Sun *et al*., 2011). Significant difference in protection of vaccinated birds and unvaccinated birds has also been recorded (Sasipreeyajan *et al*., 2012). In a vaccine efficacy trial following different polyvalent strain approach, better protection was found for more heterologous polyvalent vaccine (Shao *et al*., 2020). In similar studies, it was suggested that the traditional vaccination program may be replaced with a different one for better protection of birds (Karimi *et al*., 2018; Helena *et al*., 2009).

The dead birds were examined for post-mortem gross lesions. Sever gross lesions were observed in organs of unvaccinated birds (group D) followed by single dose vaccinated group (A). Interestingly, the birds vaccinated with two doses of homologous strains vaccine (group B) and showing highest titer were clinically less protected as compared to birds vaccinated with two doses of heterologous strains (H120 and 4/91) (group C). Our results are in similar line with previously published data. In a similar study, the efficacy of classical and variant strains vaccines was evaluated against a field isolate which induced gross lesions in kidneys of vaccinated birds after challenge showing poor protection against their field isolate vaccinated with classical (H120) strain as compared to the variant one (Sun *et al*., 2011). In another study, kidneys lesions in birds challenged with field isolate were observed while recording better protection in birds vaccinated with a combination of H120 and Ma5 strain at one-day of age (Susan *et al*., 2012). Similarly, homologous strains along with other strains vaccine had been used in a clinical trial showing similar results. The researchers recorded lower level of clinical signs and gross lesions in vaccinated as compared to unvaccinated birds. They also observed good protection in birds receiving a booster dose of heterologous strain vaccine (Helena *et al*., 2009; Karimi *et al*., 2018). The lower protection level provided by homologous vaccine combination in comparison to heterologous combination might be due to genetic variability. Our findings about gross lesions and protection conferred by heterologous vaccine are supported by the data published by various other authors (Hesham *et al*., 2019; Shao *et al*., 2020; Kutle *et al*., 2020; Chen *et al*., 2020; Ismail *et al*., 2020). The findings of the current study contributed in investigating the cause of vaccine failure against IB and provided grounds for vaccine development using local strains. However, the study was limited to a few parameters. Inclusion of more strains for protecto-typing and local vaccine production may be considered in future studies.

CONCLUSION

It was concluded from the current study's findings that prevalent vaccine strains and regime cannot provide full protection against the nephro-pathogenic strains isolated from filed outbreaks. In light of the findings of the current study, a vaccination schedule using heterologous strains of IBV is suggested for better control of the disease.

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IRB approval and ethics statement

The current study was approved by the Ethical Review Body of the Institution (No. DAS/9025) and all the procedures were performed according to standard guidelines.

Statement of conflict of interest

The authors have declared no conflict of interest.

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