COMPARATIVE ANTIBODY RESPONSE AND PATHOGENICITY OF DIFFERENT INFECTIOUS BRONCHITIS VIRUS VACCINE STRAINS IN SPECIFIC-PATHOGEN-FREE CHICKENS

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ABSTRACT. Infectious bronchitis (IB) is recognised as an acute, contagious viral respiratory disease, and an important disease that causes severe economic losses in the poultry industry globally. The causative agent for IB is the avian infectious bronchitis virus (IBV), a coronavirus that commonly targets the respiratory, urinary, and reproductive tracts. Therefore, this study aims to evaluate and compare the antibody response as well as the pathological changes in chickens following inoculation with different IBV strains. Forty one-day-old chicks were inoculated once with either Mass H120 strain or with non-Mass strains (4/91, 1/96, MH5365/95 P88). Clinical signs were observed daily, and the sera were collected weekly for antibody response evaluation. Four chickens in each group were euthanised at 7 and 28 day post-inoculation (dpi) for ciliostasis tests, and both gross and histopathological examinations were conducted. Results showed that chickens inoculated with MH5365/95 P88 produced the highest antibody response compared to other IBV inoculated groups. No clinical signs associated with IB were observed in the inoculated and control groups during this experiment. Ciliostasis evaluation showed that the chickens were protected against ciliary damage following IBV vaccine virus inoculation. Gross and histopathological examinations of the respiratory, digestive, urinary, and reproductive tracts showed no distinct lesions. In conclusion, the IBV vaccines used in this study can stimulate the production of antibodies, although the level of antibodies produced varies. Hence, no undesirable complications were evaluated following vaccine inoculation in the chickens based on the clinical signs, ciliary activity, and both gross and histopathological lesions.

***Keywords*:** Infectious bronchitis virus, chickens, antibody, pathogenicity

INTRODUCTION

The coronavirus infectious bronchitis virus (IBV) is the cause of avian infectious bronchitis (IB), an acute and contagious viral respiratory disease in chickens. According to the scientific literature search, IB is widely common both globally and domestically. Hence, it is one of the most important poultry diseases, which results in numerous economic losses in the poultry industry globally (Ennaji *et al.,* 2019; Gallardo, 2021; Ignjatovic & Sapats, 2000). IBV is transmitted by contaminated aerosols, especially in direct chicken-to-chicken close contact, ingestion of contaminated feed and water, or indirectly through mechanical spread (e.g. machines, people, and material surfaces).

The IBV possesses a single-stranded positive-sense RNA genome that ranges from 27 to 32 kb in size (Lai & Cavanagh, 1997). The genome encodes several viral proteins which play a key role in antigenicity and immunologic reactions of the virus. The spike (S) protein that emanated in the virion exterior was identified as the main immunogenic antigenic structure of IBV. The S protein consists of two subunits, namely S1 and S2 (Yamada & Liu, 2009). The S1 mediated by the receptor-binding domain (RBD) is responsible for viral attachment, hence it is the main inducer of protective immunity and it carries a majority of the virus neutralising epitopes (Promkuntod

et al., 2014). On the other hand, the S2 plays a role in facilitating the fusion of the virion and cellular membranes (Lai & Cavanagh, 1997). The generation of new IBV genotypes mostly occurs due to the mutations in the S1 gene. The amino acid variation in the S1 domain is found as one of the main trigger factors of IBV diversity (Leow *et al.,* 2018; Wickramasinghe *et al.,* 2014). Hence, the RBD of the S1 is reported responsible for viral variations and recombination (Wang & Huang, 2000). Therefore, the S1 is widely used in genotyping and serotypic evolution of IBV strains (de Wit *et al.,* 2011). A report in another study showed that the majority of IBV serotypes differ from each other by 20 to 25% of amino acids in the S1 sequences (Jackwood *et al*., 2001). In addition, some new variant strains may differ by more than 50% in the S1 amino acid sequences (Gelb Jr. *et al.,* 2005). Previous study reported that cross-protection decreases when the amino acid differences are higher than 5%, indicating that the immune response against one serotype provides poor protection against other serotypes (Caron, 2010).

Infectious bronchitis is commonly related to respiratory diseases, nephritis in young chickens, and decreased egg production and quality in layers (Cavanagh, 2007; Ignjatovic & Sapats, 2000; de Wit *et al.,* 2011). IBV principally infects the epithelium of the respiratory tract, which causes respiratory distress, and predisposes it to secondary pathogenic bacterial infections (Matthijs *et al.,* 2003). In addition, several IBV strains can cause extra-respiratory tropism in other epithelial cells, including the renal tubules and oviduct (Cook *et al.,* 2012). This results in variable morbidity, and mortality as well as pathology in chickens.

Due to the occurrence of many different IBV strains, and the restricted cross-protection across different serotypes, vaccination to control and eradicate IB in the poultry industry remains a big challenge. Hence, suitable vaccines and proper vaccination are highly needed to control the spread of the disease. Therefore, this study aims to evaluate and compare the antibody response as well as the pathological changes in chickens following inoculation with different IBV vaccine strains.

MATERIALS AND METHODS

Ethical statement

All experimental procedures were performed according to the Institutional Animal Care and Use Committee (IACUC), Department of Veterinary Services (DVS) approval with reference number IACUC-DVS-008-2022.

Virus preparation

One IBV vaccine strain of Mass H120 (Merial, France), and two IBV vaccine strains of non-Mass; 4/91 (Intervet, Holland), and 1/96 (CEVA Sante, France) were commercially purchased. The local IBV isolates MH5365/95 P88 was initially isolated and serially propagated in embryonated specific-pathogen-free (SPF) eggs in VRI, Ipoh.

Chickens

One-day-old White Leghorn SPF chickens (*Gallus gallus domesticus*) were reared in stainless steel cages in the experimental animal facility of the VRI, Ipoh. The chickens were supplied with feed and water *ad libitum*.

Experimental design

A total of 50 one-day-old SPF chickens were divided into four groups (groups 1, 2, 3, and 4; n = 10 chickens per group) and one control un-inoculated group (group 5; n = 10). Chickens in groups 1, 2, and 3 were inoculated via the intranasal route with 0.05 ml of medium containing IBV vaccine Mass H120, 4/91, and

1/96 strains, respectively. The procedures were performed according to the manufacturer's instructions. Meanwhile, chickens in group 4 were inoculated via the intranasal route with 0.05 ml medium containing 1 x 10^5 EID_{50} of MH5365/95. Chickens in group 5 were kept as control.

Sampling Procedures

Chickens were inoculated with IBV vaccines at one-day-old. Clinical signs were observed every day, and the sera were collected every week throughout the experiment. At 7 and 28 day post-inoculation (dpi), four chickens in each group were euthanised for ciliostasis evaluation and gross and histopathological examination.

Sera Sampling

Sera samples were collected from all chickens at 7, 14, 21, and 28 dpi. The collected sera were used to measure the level of antibody by commercial ELISA kit (IDEXX Laboratories Inc., USA), and expressed as antilog of log_{10} titre according to manufacturer's instructions. Sera with titre level above the cut-off level more than 396 were considered serologically positive for IBV antibody titre.

Histopathological Examination

Trachea, lungs, and liver from humanely euthanised chickens were collected and fixed in 10% buffered-formalin. The tissues were embedded in paraffin wax and sections were cut to 5 µm thickness, and stained by haematoxylin and eosin (H&E) for microscopic evaluation. The histopathological severities were determined by scores recommendation described in a previous study by Nakamura *et al.* (1991). The lesions were scored as; (1) no lesions; (2) mild lesions; (3) moderate lesions, or (4) extensive or severe lesions.

Ciliostasis Estimation

To evaluate tracheal ciliostasis, the tracheas from four randomly selected chickens from each inoculated and control group at 7 and 28 dpi were removed and cut into sections. Three sections of the upper, middle, and lower parts of the trachea were cut and analysed. Ciliary movement in each tracheal section was analysed microscopically using optical microscopy at x200 magnifications (Olympus Corporation, Japan). The ciliostasis was scored as previously described by Cook *et al.* (1999) as follows: (0) all cilia in the complete tracheal section are beating; (1) 75-100% cilia beating; (2) 50-75% cilia beating; (3) 25-50% cilia beating; or (4) no or less than 25% cilia beating.

Protection Score Estimation

Assessment of protection was evaluated using ciliostasis estimation. The scores were estimated using the formula previously described by Jackwood *et al.* (2015) as follows: protection score = 100 – [(total of an individual score for the group/number of individuals in the group × 20) × 100]. A bird was considered protected if average ciliostasis score of >50%, with the maximum reachable protection score being 100%.

Statistical Analysis

Data of antibody profiles, ciliostasis scores, and lesion scores were summarised using basic descriptive statistics (simple counts and means). The ELISA antibody data were analysed using one-way ANOVA followed by the post hoc Tukey's HSD tests. All statistical calculations were performed using IBM SPSS version 22 (IBM Corporation, USA) software and the significance level was set at $p<0.05$.

RESULTS

Antibody Response

Chickens inoculated with commercial vaccines of Mass H120, 4/91, 1/96, and locally serial propagated virus strain MH5365/95 P88 showed antibody response at 7 to 28 dpi (Table 1). The mean antibody titre for pre-vaccination is at 78.88 ± 43.41. The cut-off antibody titre is at 396.

Table 1. Antibody profiles following inoculation of different IBV strains in SPF chickens

Virus strain	Antibody titre at different dpi, mean ± SD			
	7	14	21	28
Mass H120	121.76 ± 87.40^{a}	162.10 ± 112.91	213.94 ± 95.80^{a}	491.28 ± 383.36^{a}
4/91	109.29 ± 68.57^{a}	51.59 ± 35.11	250.18 ± 246.03^{b}	267.67 ± 167.68^{a,b}
1/96	86.23 ± 56.04^{A,a}	43.91 ± 36.05^{B}	307.79 ± 148.98^{a,b,c}	429.28 ± 318.28^{A,B,a,b,c}
MH5365/95 P88	95.64 ± 73.42^{a}	787.75 ± 33.53	1,266.61 ± 1,221.10	1,161.65 ± 883.05^{a,b,c}
Control	119.24 ± 96.45^{a}	68.63 ± 53.04	62.89 ± 31.05^{c}	28.27 ± 20.74^{a}

^{A,B} Capital letters represent significant differences of antibody titres between dpi time points within the group at $p<0.05$

^{a,b,c} Small letters represent significant differences of antibody titres amongst the group at $p<0.05$

Chickens in Mass H120 group were negative for antibody titre at 7 until 21 dpi but the level increased at 28 dpi (491.28 ± 383.36). The antibody titres increased significantly between 21 and 28 dpi ($p<0.05$), but the increased in the antibody titre were not significant between 7 and 21 dpi (ρ=0.263). Chickens in 4/91 group were negative for antibody titre at 7 until 28 dpi. Even though the antibody titre decreased between 7 and 14 dpi, but the decreased in the antibody titre were not significant (ρ=0.938). The antibody titres increased significantly between 14 and 21 dpi (ρ<0.05), but the increased in the antibody titre were not significant between 21 and 28 dpi (ρ=0.998). Chickens in 1/96 group were negative for antibody titre at 7 until 21 dpi, but the level increased at 28 dpi (429.28 ± 318.28). Even though the antibody titre decreased between 7 and 14 dpi, but the decreased in the antibody titre were not significant (ρ=0.981). The antibody titres increased significantly between 14 and 28 dpi (ρ<0.05). On the other hand, a significant (ρ<0.05) increment of antibody response was observed in chickens inoculated with MH5365/95 P88 with the highest level antibody titre detected at 21 dpi (1266.61 ± 1221.10). Antibody titres of chickens in control group remained negative throughout the experimental period.

At 7 dpi, significant difference of antibody levels was observed between group H120 and group 4/91 (ρ=0.002), group 1/96 (ρ=0.001), group MH5365/95 P88 (ρ=0.011). Significant difference was observed between group 4/91 and group 1/96 (ρ=0.001), group MH5365/95 P88 (ρ=0.011). Significant difference was also observed between group 1/96 and group MH5365/95 P88 (ρ=0.011).

At 14 dpi, no significant difference of antibody levels was observed between group H120 and group 4/91 (ρ=0.084), group 1/96

(ρ=0.125), group MH5365/95 P88 (ρ=0.350). No significant difference was also observed between group 4/91 and group 1/96 (ρ=0.125), group MH5365/95 P88 (ρ=0.350). In addition, no significant difference was also observed between group 1/96 and group MH5365/95 P88 (ρ=0.350).

At 21 dpi, no significant difference of antibody levels was observed between group H120 and group 4/91 (ρ=0.072), group MH5365/95 P88 (ρ=0.068), but significant difference of antibody levels was observed between group H120 and group 1/96 (ρ=0.006). Significant difference was also observed between group 4/91 and group 1/96 (ρ=0.006), but no significant difference of antibody levels was observed between group 4/91 and group MH5365/95 P88 (ρ=0.068). In addition, no significant difference was also observed between group 1/96 and group MH5365/95 P88 (ρ=0.068).

On the other hand, at 28 dpi, significant difference of antibody levels was observed between group H120 and group 4/91 (ρ=0.016), group 1/96 (ρ=0.030), group MH5365/95 P88 (ρ=0.032). Significant difference was observed between group 4/91 and group 1/96 (ρ=0.030), group MH5365/95 P88 (ρ=0.032). Significant difference was also observed between group 1/96 and group MH5365/95 P88 (ρ=0.032).

Clinical Signs Observation

No undesirable clinical signs associated with IBV infection were observed in all the inoculated and control chickens at 7 dpi to 28 dpi.

Gross and Histopathological Examination

In general, no severe lesions in the trachea, lungs and liver were observed following inoculation with the IBV virus vaccine at 7 to 28 dpi. Mild to moderate lesions in the inoculated groups were observed between 7 and 28 dpi (Table 2). No lesions were observed in the trachea, liver, and lung of the un-inoculated control group.

Table 2. Histopathological scores in trachea, lung, and liver of SPF chicken inoculated with different IBV vaccines at different time points

Time points	Groups	Histopathological lesion scores in different types of tissue*		
		Trachea	Lung	Liver
7 dpi	Mass H120	3	3	3
	4/91	3	3	3
	1/96	3	3	3
	MH5365/95	3	3	3
	Control	1	1	1
28 dpi	Mass H120	2	2	2
	4/91	1	2	2
	1/96	2	2	2
	MH5365/95	2	2	2
	Control	1	1	1

*Scores: 1 = indicating no lesions; 2 = indicating mild lesions; 3 = indicating moderate lesions; 4 = indicating extensive or severe lesion.

Ciliostasis and Protection Score Estimation

Estimation of the ciliostasis was performed to evaluate the protection against ciliary damage following IBV vaccine inoculation. In brief, a chicken is considered protected if ≥ 50% ciliary movement is retained. Chickens in the control group showed 90 to 100% cilia beating. While, chickens of group Mass H120, 4/91, 1/96, and MH5365/95 P88 showed a mean ciliary movement between 70 and 80%, thus, indicating that the inoculated chickens were protected from ciliary damage following IBV vaccine inoculation.

DISCUSSION

According to the scientific literature data gained, IBV is usually inducing poor cross-protective immunity, mostly due to the differences in their antigenic properties. However, some IBV strains can provide cross-protection against other different serotypes, which are identified as protectotypic strains (Cook *et al.,* 1999; Karimi *et al.,* 2018). Therefore, evaluating the serologic antigenic differences may provide useful information regarding the protective immunity in vaccinated chickens.

In this study, by administrating a single specific IB vaccination, the detected antibody showed inconsistent antibody titres at different days post-inoculation. Based on the antibody profile findings in this study, it is observed that the MH5365/95 isolates are able to provide better antibody stimulation as early as 14 dpi compared to Mass H120, 4/91 and 1/96 isolates. Hence, the highest increments of antibody levels were observed at 28 dpi following inoculation of Mass H120, 1/96 and MH5365/95. The definite explanations for the dissimilar patterns in antibody titres in the inoculated groups remain unclear, but they could be influenced by several factors, such as host immune response (Chhabra *et al.,* 2015), age of the chickens (Stachowiak *et al.,* 2005), vaccine strain used (Jackwood & Lee, 2017), type of vaccination application method (de Wit *et al.,* 2010), route of administration (Al-Rasheed *et al.,* 2021), or other local variables, such as temperature and feed quality (Yunus *et al.,* 2012). The presence of antibody titres is important in determining if the vaccinated flock is protected or failed. A study by Bourogaa *et al.* (2009) demonstrated that low levels of antibodies, between 45 to 69% showed positive levels of antibodies in vaccinated chicken flocks, and only 5 to 15% of the chickens were protected. Moreover, the type of IBV strain infecting a flock determines the pathogenesis of the disease and the outcome of the infection is closely dependent on the immune status of the host (Alvarado *et al.,* 2005; Chousalkar & Roberts, 2007). A report by Bourogaa *et al.* (2014) showed that the serum antibody titres do not associate well with the presence or the lack of homologous or heterologous protection, since the protection is provided mainly by the local antibody responses and cell-mediated immunity.

Clinical signs of IBV infections are typically varied and challenging to evaluate. Furthermore, clinical signs alone are difficult to discriminate between vaccination or challenge groups (de Wit & Cook, 2014). Thus, in terms of practicality, direct observations by the naked eye, such as gross lesions examination and ciliostasis estimation, are the most important parameters for a protection evaluation (Jackwood *et al.*, 2015; de Wit & Cook, 2014). Therefore, in this study, by combining the observation of clinical signs, ciliostasis evaluation, and gross and histopathological examinations are used to determine the protection following inoculation of different IBV vaccine strains. Results of this study revealed a range of 70 to 80% ciliary protection rate, and all chickens inoculated

with IBV vaccines are healthy with complete clinical protection, and no undesirable lesions. The findings are in agreement with other studies by Bourogaa *et al.* (2014) which combine the observation of clinical signs and gross lesions examination; Bru *et al.* (2017) analyse the protection by combining the clinical signs observations with ciliostasis test, and Sultan *et al.* (2019) use a combination of clinical signs, ciliostasis, and histopathological examination to determine the protection following IBV inoculation in the chickens.

In this study, the histopathological scores are between mild to moderate lesions. These findings are in agreement with other studies that the IB vaccination leads to good protection demonstrated clinically with mild clinical and necropsy lesions (Al-Kubati *et al.,* 2022; Habibi *et al.,* 2017). Therefore, the vaccination used in this study presented relatively good protection based on the observation of antibody response, and protection against severe clinical signs and lesions. The outcome of this study is in agreement with some previous studies on IB (Al-Kubati *et al*., 2022; Awad *et al*., 2015; Bourogaa *et al.,* 2014). In addition, the vaccine and vaccination method used in this study were found to be able to induce a rise in antibody titre. Thus, this contributes to the decrease in the severity of organ lesions in addition to a decrease in mortality rates.

CONCLUSIONS

In conclusion, the IBV vaccines used in this study can stimulate the production of antibodies, although, the level of antibodies produced varies. Hence, there is no undesirable complications following vaccine inoculation in the chickens based on the clinical signs, ciliostasis, and gross and histopathological examination. Moreover, these data should help in controlling IB in chickens in a targeted area. Hence, continuous monitoring of the field circulating IBV strains in chickens and preparation of some homologous IBV vaccines for them is the main key role in protecting against IBV infections.

Conflict of interests

The authors have no conflicts of interest to declare related to this article.

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