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Protective Efficacy of Different Live Attenuated Infectious Bronchitis Virus Vaccine Combination against Challenge with GI-11 (BR-Type) Strain

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Research Article

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Abstract

The infectious bronchitis virus (IBV) is an avian coronavirus that causes a highly contagious disease that results in substantial economic losses to the poultry industry worldwide. The prevention is mostly based on biosecurity measures and vaccination. The vaccination programs are defined by epidemiological status and cross-immunity provided against different serotypes. Programs using vaccine combinations can be designed to increase protection. We conducted an experimental study to compare two vaccine programs on protection against a virulent strain belonging to the GI-11 genotype (BR-type) isolated in Brazil using two different Mass-type vaccines combined with a BR-type live vaccine. Two groups of commercial chicks were vaccinated at day-1 using two commercially available Mass-type vaccines + BR-type vaccine. They were challenged intranasally at 28-day using 10^4EID50/0.1 microliter/chick of wild-type G11 IBV (IBV/24W). Ciliostasis, macroscopic and microscopic lesion scores were evaluated at 4 and 11-days post vaccination (dpv) and 5 days post infection (dpi) and not showed differences between vaccinated groups. MLV+BR-Type group presented highest amount of BR strain vaccine and lowest amount of Mass strain vaccine in tracheal and cloacal swabs at 4 and 11 dpv. In the other hand, the Ma5+BR-type group showed fewer BR-type positive in both samples tested. After challenge, MLV+BR group showed higher titer of antibody measured by ELISA. These results suggest that the selection of a Mass-type vaccine strain can impact directly in the BR vaccine replication, when used associated, and consequently the induction of humoral immune response. Despite that, both protocols provided protection against the challenge with G11 strain.

Keywords: Infectious Bronchitis; Vaccine; Massachusetts Strain

Introduction

Infectious bronchitis (IB) is a rapidly spreading, highly contagious and characterized by respiratory and urogenital disease mainly. IB is caused by infectious bronchitis virus (IBV), a coronavirus belonging to *Nidovirales* order, *Coronavidae* family and *Gammacoronavirus genus* [1].

Infectious bronchitis is widely distributed in poultry flocks worldwide. Brazil is one of the major poultry-producing countries in the world, and IB has been a serious problem in the last few years. The IBV is divided into 6

genotypes (GI-GVI) and 32 lineages, with other potential groups present as unique variants (UVs) based in the S1 phylogeny [2]. Two main IBV genotypes are predominant in Brazilian poultry flocks: GI-1 (Mass-type strains) and GI-11 (BR-type strains). Although IBV can be considered as a major cause of respiratory infection, other clinical manifestations including renal and reproductive signs is found in outbreaks of Brazilian infectious bronchitis [3,4].

Since the 90s, after the characterization of different serotypes circulating in Brazilian flocks (Di Fabio et al., 2000), this genotype/serotype has become to be the predominant in Brazil [5-8]. Additionally, two of these new Brazilian variants were clustered together with few Argentinean isolates recovered from broilers and layers during different outbreaks in commercial poultry flocks in different geographic regions of Argentina between 2001 and 2008 [9].

Brazilian IBV viruses have been isolated mostly from broiler flocks, broiler breeders and layers associated with drop in egg production, increased feed conversion and condemnation at slaughterhouse [10]. It is known that homologous vaccines against field viruses induce expected clinical protection, however the possibility of having two genotypes circulating in the field at the same time or at different periods could be a problem. This justifies the need for a broad vaccination program through combination.

The use of one serotype for vaccination does not ensure high or complete protection score from heterologous strains [11]. Otherwise, many studies have been shown that the use of different combinations of live IBV vaccines is able to induce high and broad protection against challenges with heterologous virulent strains [11-14]. It was demonstrated that the combined use of homologous and heterologous vaccines increase the protection score [15].

However, few studies have compared the forms of combination by choosing the origin of the Mass-like vaccine strain. Therefore, the objective of this study was to evaluate different vaccine protocols using distinct Massachusetts vaccines with a BR type vaccine, by evaluating the replication profile of each strain after vaccination and the pre- and post-challenge immune response.

Material and Methods

Virus

The IBV/24W strain, previously characterized as G11 genotype - GenBank access number KY565553 [16], was propagated in SPF eggs for virus scaled up production. The suspension of allantoic fluid (AF) was harvested from the inoculated SPF eggs and stored at -70ºC. The infectivity of this strain in AF suspension was determined as 50% of embryo infectivity doses (EID_{50}) by titration in embrocated SPF eggs, according to the method recommended by Gelb, et al. [17].

RNA Extraction and IBV Detection by RT-qPCR

RNA was extracted with commercial reagents (New-Gene, city, country), according to the supplier protocol (Simbios Biotecnologia, Cachoeirinha, RS, Brazil). Reactions were performed in a total volume of 30 microliters including 2 microliters of viral RNA. The RT-qPCR was performed for 40 cycles with the commercial kit MASSAmp and BRAmp (New-Gene), according to the supplier protocol (Simbios Biotecnologia) to detect the Massachusetts and GI-11 genotypes (BR-type) vaccine strain, respectively. All reactions were performed in a Rotor-Gene Q (Qiagen, Hilden, Germany).

Experimental Design

One hundred-forty 1-day old commercial broiler chickens were equally divided and housed into four isolators with air control by positive pressure. At 1-day old, one group received a dose of a commercial attenuated Massachusetts vaccine strain by oculo-nasal route (MLV strain - Volvac® IB Fit, Boehringer Ingelheim) associated with a dose of a commercial attenuated GI-11 (BR strain) vaccine strain (Cevac® Ibras, Ceva), while the second group received a dose of a commercial attenuated Massachusetts vaccine strain by oculo-nasal route (Ma5 strain - Nobilis® IB Ma5, MSD) with the same attenuated BR-type vaccine. The third and fourth group (mock vaccinated) received only the ocular vaccine diluent. After 28 days, vaccinated and one of mock group were challenged with $10^{4.0}$ EID50%/bird of IBV/24W by intranasal and ocular routes, while the fourth group was not challenged and received only the ocular vaccine diluent. The clinical signs were observed throughout the experimental period. Three birds of each group were euthanized at 4 and 11-days post vaccination (dpv) and 5 days post-infection (dpi). Trachea and cloacal swabs and blood samples were collected from five birds.

Ciliostasis

The tracheal samples were divided in proximal, medial, and distal portions. Each portion was submitted to ciliary kinetic analysis and scored ranged from 0 to 4 according to the percentage of ciliary activity: 0 =100% of ciliary movement, 1 = 75–100% of ciliary movement, 2 = 50–75% of ciliary movement, 3 = 25–50% of ciliary movement, and 4 = 0–25% of ciliary movement. Individual chickens were recorded as protected against challenge if the ciliostasis score for each group according to Cook, et al. [11].

Histopathology

Trachea samples were taken and fixed in 10% buffered formaldehyde solution. The samples were embedded in paraffin wax, sectioned (5 µm) and stained with haematoxylin and eosin (HE). Histopathological analysis of tracheas was performed according to the parameters described by Nakamura, et al. [18] and Chen, et al. [19]. Trachea lesions were evaluated according to the loss of epithelial cells, the depletion of mucus secreting cells, the lymphoid cell infiltration of the lamina propria and the hyperplasia of epithelial cells.

The scores were assigned according to the lesion's intensity, ranging from no pathological change (-), slight (+), moderate (++) or severe changes (+++).

Measurement of Systemic Antibodies

Serum samples collected were tested for the presence of anti-IBV antibodies by a commercial IBV ELISA kit (BioChek, Ascot, England), according to the manufacturer's instructions.

Results

Clinical Findings, Ciliostasis and Histopathology

All birds were monitored at random intervals throughout the experiment (total of 33 days) for observation of clinical sings. No clinical signs, neither macroscopic nor microscopic lesions were observed in the birds of the mock-vaccinated group. The vaccinated groups did not show respiratory symptoms and macroscopic changes. Chicks from non-vaccinated group and challenged with IBV/24W showed typical clinical signs.

Inhibition of tracheal ciliary activity was measured at 4 and 11 dpv and 5 dpi. Vaccinated groups showed lower ciliostasis score at 4 dpv and increased at 11 dpv. After challenge, the non-vaccinated group had intense damage in the trachea at 5 dpi with the highest score (score 4), while the MLV+BR-type vaccinates showed a score of 0.84 and the Ma5+BR-type vaccinates a score of 0.86.

The most marked microscopic changes in both vaccinated groups at 4 and 11 dpv were mild ciliary degeneration and desquamation of epithelial cells of the mucosa. After challenge both vaccinated groups showed mild heterophil infiltration in the lamina propria, characterized as tracheitis, congestion and edema of the submucosa. Further, Ma5+BR-type vaccinate group had 2 birds with moderate tracheitis and degeneration of mucus-secreting glands. The non-vaccinated group presented severe inflammatory infiltration of the lamina propria and desquamation of epithelial cells with congestion.

Viral Detection by RT-qPCR

A summary of the viral detection in trachea and cloacal swabs are showed in Table 1. At 4 and 11 dpv, the BR strain vaccine was detected in 50% and 70% of tracheal and cloacal swab samples, respectively, from MLV+BR group, while the Ma5+BR group had 10% and 40% in tracheal and cloacal swab samples. For Mass-like vaccine strains, the MLV group had a detection of 40% in tracheal and 20% in cloacal swab samples, while the Ma5 was of 60% and 40% in trachea and swab samples, respectively (Table 2).

Massachusetts vaccine detection post vaccination (pv)					
Trachea (%)			**Cloaca (%)**		
	MLV	Ma5		MLV	Ma5
4 dpv	20	80	4 dpv	0	40
11 dpv	60	40	11 dpv	40	40
GI-11 (BR) vaccine and virus detection post vaccination (pv) and post infection (pi)					
Trachea (%)			**Cloaca (%)**		
	MLV	Ma5		MLV	Ma5
4 dpv	40	0	4 dpv	60	40
11 dpv	60	20	11 dpv	80	40
5 dpi	0	20	5 dpi	20	20

Table 1: Molecular detection of vaccine strains in trachea and cloacal swab after vaccination (4 and 11 dpv) and challenge (5 dpi) from chickens vaccinated and challenged with an GI-11 virulent strain of IBV.

Gmean IBV ELISA antibody titers		
	Groups	
	MLV+BR-type	Ma5+BR-type
28 dpv	1.094	776
5 dpi	1.599	979

Table 2: Anti-infectious bronchitis virus (IBV) geometric mean titer (Gmean) antibody from different groups, vaccinated with live vaccines at 1 day old and challenged at 28 days old with a GI-11 virulent strain.

Measurement of Antibodies against IBV

28 days after vaccination there were no significant differences in antibody titers between vaccinated groups. However, at 5 dpi, higher antibody titers were observed in groups vaccinated with MLV+BR-type.

Discussion

The IBV control in Brazil is mainly the result of the use of one single serotype (Massachusetts) live vaccine application at day-old, which has low S1 protein similarity in relation to the S1 of the the Brazilian circulating field strains [6,7]. Studies show that higher S1 gene homology results in increased chance for cross protection between strains [20,21].

Live attenuated vaccines are important components of the vaccination program against IBV, due to the mucosal immunity and its capacity to act as a primer for inactivated vaccines [22-24]. The continuous evolution of IBV in Brazil has reduced the efficacy of Massachusetts vaccines, considering that there is no evidenced of cross-protection against the IBV GI-11 lineage circulating in Brazil [3]. However, it is efficient to virulent Mass-like field strains that continue to be a problem nowadays. In this study, different protocols using live Massachusetts vaccines with BR strain vaccine in commercial broiler chickens were used to compare the respective dynamic interactions and its ability to induce protective immunity against a challenge.

The present study demonstrated high levels of protection against a BR-type when using both combinations of Mass-like and BR-like vaccines. Vaccination programs using the combination of heterologous vaccines can induce protection against challenge with various IB viruses of different serotypes. A recent study showed that the combined live H120 and CR88 vaccines simultaneously at day-old followed by CR88 vaccine at 14 days-old gave more than 80 per cent tracheal ciliary protection against Middle East IBV isolates, while the vaccination program with H120 at day-old followed by CR88 at 14 days-old showed a tracheal ciliary protection from 60 per cent to 80 per cent. Both vaccination programs used provided excellent protection against a challenge with a virulent strain.

The high amount of BR strain and low amount to Mass strain of positive samples detected post vaccination in trachea and cloacal swabs from MLV+BR-Type group suggest that MLV vaccine provided less impact to replication of BR vaccine. In the other hand, the Ma5+BR-type group presented fewer amount BR strain in both samples.

Previous study found low HI antibody response to Ma5, when this vaccine was given at the same time with a 793B serotype 4/91 strain, indicating that there was interference between both vaccine viruses, being the Ma5 virus presented low replication capacity and induce an immune response. The authors suggest that the number of susceptible cells in the respiratory tract had been reduced possibly because the 4/91 vaccine had occupied the majority of available receptors, preventing the attachment and replication of the Ma5 vaccine, given at the same time or later.

Conversely, in this study, the vaccine interactions seemed to occur differently, once our data suggest that Ma5 IBV strain replied more than the BR-type vaccine virus and possibly had occupied the available receptors of attachment, therefore reducing the replication of BR-type vaccine. ELISA serological results showing that the MLV+BR-type group had higher antibody titer before and after challenge. This may suggest that the MLV vaccine strain does not interfere with the BR strain, therefore increasing the immune response in associating with the BR-type vaccine.

Conclusion

In conclusion, we showed that the selection of the Mass-like vaccine strain used in combination with the BR-type vaccine can impact directly in the BR vaccine replication post vaccination, and consequently with the humoral immune response induced. However, it is worth mentioning that both vaccination programs provided expected protection against IBV challenge, according to the results of macroscopic and microscopic changes and ciliostasis. *In vivo* protection studies are essential besides determining the effect of vaccine strains on ciliary activity for both vaccine evaluation and proper design of vaccination program for IBV, considering the respiratory complex with other respiratory pathogens in Brazil.

Ethical Standards

The design for this study was approved by the Ethics and Animal Welfare Committee (CEUA nº. 004/2021), of Centro de Amparo a Pesquisa de Amparo, Amparo, State of São

Paulo, Brazil.

Conflict of Interest

The authors are employees of Boehringer Ingelheim witch is the manufacturer of the MLV IBV vaccine (Volvac® IB Fit).

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