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IN VIVO ASSAY OF VACCINE PROTECTION TO BRAZILIAN VARIANT STRAINS OF INFECTIOUS BRONCHITIS VIRUS

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ABSTRACT

Infectious bronchitis (IB) is an important poultry disease caused by a coronavirus named Infectious Bronchitis Virus (IBV). Failure of protection as well as variation on clinical manifestations associated with the emergence of many different antigenic types of the IBV have been reported in the last years. Although vaccines may protect against heterologous strains, it has been speculated that the vaccine serotype Massachusetts has not always been able to confer full protection against some field strains of IBV. In this study, multiple tests were performed in order to evaluate the protective potential of a commercial vaccine against four strains IBV previously classified as variants by sequencing, phylogenetic and pathogenic analyses. Seven day-old chicks were vaccinated with H120 vaccine strain by eyedrop and challenged with field IBV strains (BIBR116, 438, 1496 and 212). Degrees of ciliary activity were measured at 5 days after challenge to assess the ability of the vaccine to protect the chicks. In two vaccinated groups and challenged with variants strains (BIBR116 and BIBR212), a dose of $10^{3.5}EID_{50}$/bird H120 vaccine (serotype Massachusetts) was able to induce protection to the respiratory tract, demonstrating good cross-protection of the vaccine against the field strains. However, in other two groups (strains BIBR438 and BIBR1496), the challenge failed, despite the accurate titration and previous pathotyping assay that had proven that the challenge virus was pathogenic. These results suggest that the infectivity of IBV was reduced from the time it was prepared until the moment of inoculating the chickens. This reinforces the knowledge of the high liability of IBV and thus the importance of care on handling the vaccine during vaccination to ensure a good immunity.

KEYWORDS: IBV, sample variants, protectotypes.

INTRODUCTION

Infectious bronchitis (IB) remains one of the main diseases causing economic losses in commercial poultry due to respiratory, renal, reproductive disease, and more recently also associated with enteric problems (Villarreal *et al.*, 2007, Cavanagh and Gelb, 2008). Infectious bronchitis is often controlled with vaccines. However, due to the different clinical manifestations and the high frequency of mutation in the viral genome (RNA single-stranded non-segmented), this virus has been associated with failure of vaccine cross protection (Picault *et al.*, 2003, Wang *et al.*, 1994). The concept of protectotypes has been suggested to be a valuable one to consider in terms of developing strategies to control IBV infection, since it provides direct information about the efficacy of a vaccine (Cook *at al.*, 1999). Strains that induce protection to each other belong to the same protectotype. Within this concept, this study evaluated four field strains of IB genotypically classified as variants of the Massachusetts serotype vaccine strain.

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MATERIALS AND METHODS

The field strains of IBV were recovered from birds with respiratory signs, and drop in egg production. Samples were insolated in SPF fertile eggs (OIE, 2008). After viral isolation, the extraction of viral RNA was obtained using standard methodology (Trizol, Gibco/BRL) according to manufacturer's instructions. The synthesis of complementary DNA (cDNA) was performed using the enzyme reverse transcriptase (Super Script II, Invitrogen) and the primer reverse S1 3'. After the production of cDNA, the amplification of the target region was performed as described in Kwon *et al*., 1993. The samples confirmed as positive in RT-PCR were subjected to DNA sequencing and analysis to distinguish between classical strain like Massachusetts serotype and variant strains using the system "BigDye^{TM} terminator - cycle sequencing ready reaction - Applied Biosystems' (Perkin Elmer) v. 3.0 in automated DNA sequencer ABI 3130. Sequences were aligned by using the Clustal W programs (Thompson *et al*., 1991) compared to other sequences available in Genbank. Phylogenetic analysis was performed using the software MEGA version 5 (Kumar *et al*., 2004). The obtained sequence data were used for the construction of a phylogenetic tree. The alignment of the nucleic acid sequences was used to calculate a distance of Massachusetts serotype to the field strains. Four strains that were farthest from the Massachusetts serotype and the H120 vaccine strain were titrated and diluted to $10^{3.5}EID_{50}$/bird for the *in vivo* protection studies (assay to define protectotypes). The study was approved by the Ethics Committee on Use of Animals in Experiments (ECUAE) of Embrapa Swine and Poultry. . Sixty-eight one day-old chicks, derived from a specific pathogen free (SPF) flock of White Leghorn parents, were randomly divided into 4 groups of 14 and 4 groups of 3 chicks, respectively. All birds were maintained in poultry isolator units with filtered air and positive pressure. The groups of 14 chicks received a dose of H120 vaccine by eyedrop at 1-week-old (vaccinated groups). The groups of 3 chicks were not vaccinated. After 4 weeks, one bird of each group of vaccinated birds, was euthanized to perform the ciliary beat evaluation test. After confirming the ciliary activity, each IBV field strain (BIBR116, 438, 1496 and 212) was inoculated by eyedrop in the two previously groups: vaccinated and non-vaccinated.Five days post-challenge, tracheas of all groups were carefully removed and examined for ciliostasis test. Briefly, the parameter for the ciliostasis test are described as follow: **0)** all cilia are beating vigorously; **1)** all cilia are beating slowly; **2)** some cilia are beating very slowly; and **3)** no cilia are beating (Trevisol *et al.,* 2011). The response of individual birds on the tracheal ciliostasis test was qualified in: **a.** protected bird (when the median of the results was ≤ 1) or **b.** not protected bird (when the median of the results was ≥ 2). To validate the *in vivo* vaccine protection study, at least 90% of the challenged vaccinated birds should have no evidence of IBV in their trachea (degrees zero to one), while 90% or more of the control birds (only challenged) should have evidence of the presence of the virus (degrees 2 to 3).

RESULTS AND DISCUSSION

The tracheas of vaccinated groups (one bird/group) that were evaluated before challenge had 100% ciliary activity. The post-challenged results from all groups are summarized in Table 1. The challenge was satisfactory in 2 groups (BIBR116 and BIBR212 field strains), with 100% of birds evidencing the presence of the virus, confirming the pathogenicity of these strains. Vaccinated groups and post-challenged with BIBR116 and BIBR212 field strains had satisfactory results, with 100% of birds without evidence of IBV in the trachea. The field strains BIBR438 and BIBR1496 failed to induce ciliostasis. The most likely explanation for this finding is the reduction or loss of viral infectivity of these two strains due to delay in the procedures of handling and inoculation, despite the accurate titration and previous *in vivo* pathogenic studies that had proved that the challenged viruses of these groups were pathogenic. This finding confirms the fragility of IBV and careful handling of the samples

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should be followed while vaccinating birds in both research and routine activities in a hatchery or farm,. lThen, for these two samples (BIBR438 and BIBR1496 strains), the assay of vaccine protection was disregarded. The overall results of this study agree with a previous study in which birds that follow the precepts of an appropriate vaccination, respond well to challenges, even against samples considered variants (Trevisol *et al.,* 2010), promoting cross-protection. However, other studies argue that there is failure on vaccine protection using only serotype Massachusetts vaccine. Additional data on microscopic lesions and viral recovery are underway to complement this study. The *in vivo* assay to define protectotypes has shown the effectiveness of the vaccine Massachusetts H120, although they are still few Brazilian samples classified as protectotypes.

Table 1. Results of challenge assay in which chickens were vaccinated with H120 Massachusetts Holland serotype at 7 day-old and after 4 weeks were challenged with heterologous virus.

Groups	Number of birds/ ciliary activity score				% of ciliary activity	
					zero to one	2 to 3
	0	1	2	3		
NVC116	0/3	0/3	1/3	2	0	100
NVC438	0/3	3/3	0/3	0/3	100	0
NVC1496	0/3	3/3	0/3	0/3	100	0
NVC212	0/3	0/3	0/3	3	0	100
VC116	6/13	7/13	0/13	0/13	100	0
VC438	5/14	6/14	3/14	0/14	78.5	21.5
VC1496	11/14	2/14	1/14	0/14	92.8	7.2
VC212	7/13	6/13	0/13	0/13	100	0

NVC: non-vaccinated and challenged; VC: vaccinated and challenged.

CONCLUSION

Field observations have suggested that the only serotype IBV live vaccine used to control IB in Brazil (H120 Massachusetts Holland serotype) does not full protect the birds. However, this study has shown that one single dose of $10^{3.5}EID_{50}$/bird H120 vaccine was able to induce full protection against BIBR212 and BIBR116 field strains. These results reinforce the importance in classifying the new field strains in protectotypes, before changing strategies on controlling this disease. This study also demonstrated the fragility of the IBV, reinforcing careful handling of live samples, including the vaccine.

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