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Infectious Bursal Disease: Pathogenicity and Immunogenicity of Vaccines

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ABSTRACT

The Infectious Bursal Disease (IBD) is a contagious viral disease that affects young chickens and may cause high morbidity and mortality. As the virus is very resistant to the environment, vaccination is required in case of high infection pressure. Due to variations in the virulence degree of the vaccines available to control IBD, this study aimed at evaluating the pathogenicity and immunogenicity of three types of vaccines. In total, 220 one-day-old specific pathogen free (SPF) chickens were immunized with recombinant, immune-complex and intermediate vaccines, or not vaccinated (55 birds per group) and challenged with IBD G11 strain on day 25. On days 25, 30, and 35, the Bursa of Fabricius (BF) were submitted to gross and histological examination, and serum samples were submitted to ELISA to determined anti-IBD antibody titers. On day 23, chickens were submitted to the test of hypersensitivity to phytohemagglutinin to evaluate the immunosuppressive effect of vaccines on the cell-mediated immunity. The results have indicated that the immune-complex vaccine induced the most severe BF lesions, whereas the recombinant vaccine preserved BF tissue and cell integrity. The three evaluated vaccines induced humoral immunity of similar intensity. The cellular reaction to phytohemagglutinin of the chickens immunized with recombinant and immune-complex vaccines was less severe compared with the unvaccinated chickens. In conclusion, these results indicate that the immune-complex vaccine was the most pathogenic and that all vaccines were effective in protecting SPF chickens against IBD.

INTRODUCTION

The Infectious Bursal Disease (IBD) is a highly contagious acute viral infection, affecting young chickens (Eterradossi & Saif, 2008). Infectious Bursal Disease Virus (IBDV), belonging to serotype 1, is an important immunosuppressive virus of chickens, hastropism for the Bursa of Fabricius (BF), and its intense viral replication may cause severe lymphocyte depletion in bursal follicles (Muller *et al*., 1979).

The infection with IBDV may exacerbate previous infections with other infectious agents, and may reduce the capacity of the bird to respond to vaccination, as the virus damages the humoral and cellular immune responses of chickens (Sharma *et al*., 2000). Previous studies have demonstrated that IBDV strains with different virulence may differ in their ability to replicate *in vivo*, to induce humoral immunity, and to cause immunosuppression. Nonetheless, the relative effectiveness of these strains to stimulate cell-mediated responses, and the relation of virulence with these responses, is not known yet (Rautenschlein *et al*., 2003).

The IBDV is highly contagious and very resistant, and tends to persist in the environment despite of the utilization of strict hygiene measures.

Therefore, vaccination is considered an important means of protecting birds during their first weeks of life (Eterradossi & Saif, 2008).

The strategy to control IBD in chicks is to hyperimmunize breeders with inactivated vaccines. Although passive immunity promotes good protection of chickens during the first weeks of life, permanent protection against IBD requires the administration of live vaccines. It is important to highlight that live vaccines have been developed and are categorized as "mild", "intermediate" and "hot" according to their degree of virulence. Mild vaccines are safe for specific pathogen free (SPF) chickens, but are not very effective in the presence of high levels of maternal antibodies or against very virulent strains of IBDV. Intermediate and hot vaccines are much more effective, but may induce moderate to severe lesions in the BF (Van Den Berg & Meulemans, 1991). Therefore, in order to overcome these problems, new vaccines, combining safety and efficacy, have been developed, such as immune-complex and recombinant vaccines.

The recombinant vaccine uses a viral vector to carry and express the immunogenic protein VP2 of IBDV, inducing, even in the presence of passive immunity, the production of specific antibodies (Goutebroze *et al*., 2003; Bublot *et al*., 2007; Le Gros *et al*., 2009). The immune-complex vaccine, on the other hand, is innovative compared with conventional live vaccines because the vaccine virus is coated with anti-IBD antibodies, and when administered to one-day-old chicks, its pathological effects are delayed up to one week, during which the level of maternal antibodies is greatly reduced (Haddad *et al*., 1997; Jeurissen *et al*., 1998).

Thereby, this study aimed at characterizing the pathogenicity and immunogenicity of IBD vaccines currently utilized to control IBD in Brazil in SPF chickens, as well as to determine the protection degree of chickens challenged with a highly-virulent strain of IBDV provided by those vaccines by analyzing their effect on the cell-mediated immunity of SPF birds.

MATERIAL AND METHODS

The experiment was conducted in the Veterinary Research Institute Desidério Finamor (IPVDF), located in Eldorado do Sul, RS, Brazil. The laboratory analyses were carried out at the Center for Diagnosis and Research in Avian Pathology (CDPA) of the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil.

Vaccines and vaccination

Three commercial vaccines, commonly utilized by the Brazilian poultry industry for the immunization of chickens against IBD, were tested. The first vaccine consisted of an intermediate vaccine developed with the Lukert strain, and was administered by the ocular route to one-day-old SPF chicks (0.03 mL/chick). The second was a complexed live vaccine, with Winterfield 2512 strain and anti-IBDV antibodies, and administered at a volume of 0.1 mL by injection under the skin of the neck of each one-day-old SPF chick. The third vaccine was a vectored recombinant vaccine, with the gene coding for the VP2 protein of IBD Vinserted into the genome of the HVT virus, was administered by subcutaneous route to one-day-old SPF chicks (0.1 mL/chick). One-day-old SPF chicks of the negative control group were inoculated with 0.03 mL of PBS (Phosphate Buffered Saline) by ocular route, for standardization.

Experimental groups

In total, 220 SPF White Leghorn female one-day-old chicks were divided into four groups. Each group of 55 chicks was allotted to specific isolation units (Table 1), and supplied feed and water *ad libitum* during the entire experimental period (35 days).

Table 1 – Experimental groups and their characteristics.

Group	Characteristics
G1	negative control (unvaccinated chickens)
G2	recombinant vaccine (HVT + VP2)
G3	immune-complex vaccine (Winterfield 2512 + anti-IBDV antibodies)
G4	intermediate vaccine (Lukert)

Challenge

The very virulent strain of IBDV used to challenge the chicks belonged to genomic group eleven (G11) and it is called GAR-1 by RT/PCR-RFLP assay, according to Ikuta *et al*. (2001). The viral inoculum was titrated in embryonated SPF eggs, and the viral titer was estimated by Reed & Muench's method (1938) as $10^{4.7}$ 50% embryo infective dose /mL (EID 50/mL). All SPF chickens were challenged at25 days of age with 100mL of the previously prepared viral inoculum by ocular route (50mL per eye).

Data collection before and after the challenge

On day 25 post-vaccination (dpv), 15 chickens from each experimental group were examined, euthanized, bled by cardiac puncture, weighed and necropsied for the collection and analysis of BF. The same procedures were carried out 5 and 10 days post-challenge (dpc) in 20 chickens/group.

Serology

Serum samples obtained 25 dpv (15 chickens/group), 5 dpc (20 chickens/group) and 10 dpc (20 chickens/group) were analyzed for the presence of anti-IBD antibodies by ELISA using a commercial kit (IDEXX flockchek® IBD, IDEXX Laboratories).

Cellular immunity

The cellular immunity of SPF chickens was evaluated by the cutaneous basophil hypersensitivity test to phytohemagglutinin (PHA - *Phaseolus vulgaris*, Sigma Aldrich Company). A volume of 0.1mL phytohemagglutinin solution was injected in the skin of the inter-digital space (Stadecker*et al*., 1977) on 23 dpv. Skin thickness was individually measured using a digital caliper (DIGIMESS®, 0.01 mm precision), before and 12 and 24 hours after injection.

Gross and microscopic examination of the Bursa of Fabricius

The diameter of BF was measured in the region of the greatest diameter using a caliper. The relative weight of BF (RWBF) was calculated according to the formula: (BF weight x 100) / bodyweight. All BF were submitted to histopathological examination to determine the presence of lesions. The lymphoid depletion score was obtained through mathematical modeling by utilizing artificial neural networks, in accordance to the method proposed by Moraes *et al*. (2010).

Statistical analysis

Antibody titers did not present normal distribution and were transformed to logarithm to the base 10 (log10). Treatments were distributed according to a completely randomized experimental design, and the obtained data were submitted to analysis of variance (ANOVA), using the general linear procedure model (PROC GLM) of SAS statistical software (SAS Institute, Inc., Cary, NC). Means were compared by Tukey's test. All analyses were performed at 0.05 significance level.

RESULTS

No clinical signs or mortality were observed in any of the four groups of birds during 25 days of observation after vaccination. Following the challenge with the G11 strain, the immunized birds, independently of the administered vaccine, seemed to be normal. On the other hand, the SPF chickens of the negative control group showed severe clinical signs (100%) and high mortality rate (62%).

The parameters BF diameter and relative weight are described in Table 2.

Table 2 – Diameter and relative weight of Bursa of Fabricius evaluated on 25 days post-vaccination and 5 and10 days post-challenge with the highly virulent G11 strain. Data expressed as mean ± standard deviation.

Variable	Group	25 dpv	5 dpc	10 dpc
Diameter (mM)	Negative control	12.77±1.20 ^{A}	12.84±1.88 ^{A}	07.75±2.42 ^{A}
	Recombinant	12.91±1.44 ^{A}	15.28±1.27 ^{B}	16.94±1.53 ^{B}
	Immune-complex	07.83±1.03 ^{B}	10.26±1.53 ^{C}	09.95±1.86 ^{C}
	Intermediate	11.18±3.04 ^{A}	13.87±1.88 ^{AB}	14.94±1.68 ^{D}
RWBF (%)	Negative control	0.606±0.109 ^{AB}	0.624±0.193 ^{A}	0.163±0.069 ^{A}
	Recombinant	0.717±0.147 ^{A}	0.732±0.140 ^{A}	0.778±0.198 ^{B}
	Immune-complex	0.213±0.116 ^{C}	0.268±0.105 ^{B}	0.218±0.104 ^{A}
	Intermediate	0.505±0.283 ^{B}	0.587±0.156 ^{C}	0.640±0.187 ^{C}

dpv= days post-vaccination; dpc= days post-challenge; RWBF= relative weight of Bursa of Fabricius. Means followed by different capital letters (A, B or C) in the same column are significantly different ($p<0.05$, Tukey's test) among experimental groups.

The BF of the chickens immunized with recombinant and intermediate vaccines showed no lesions in the *post-mortem* exam, 25 dpv, whereas those immunized with the immune-complex vaccine exhibited severe BF atrophy (Figure 1).

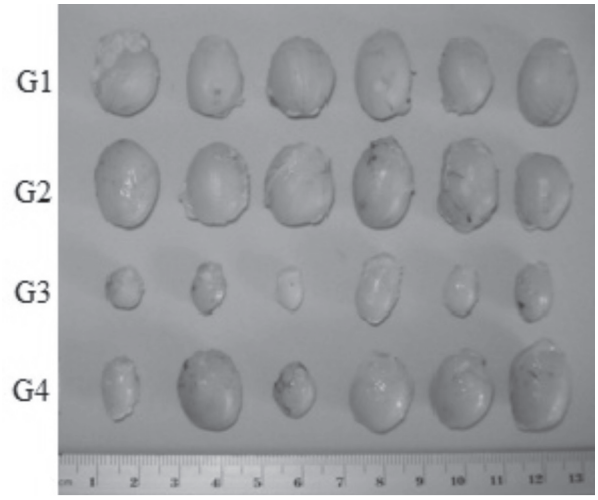

Figure 1 – Bursas of Fabricius examined on 25 days post-vaccination. G1, negative control. G2, recombinant vaccine. G3, immune-complex vaccine. G4, intermediate vaccine.

In addition, the BF of the negative control group presented severe lesions when examined after the challenge. It should be noted that the BF of birds that died at the peak of infection peak was enlarged and presented pale-yellow discoloration, hemorrhages, and peribursal edema. Bursal atrophy was only observed 10 dpc, and the lesions observed were compatible with the virulence of the G11 strain.

Table 3 shows the lymphoid depletion scores obtained by digital-image analysis of the BF.

Table 3 – Lymphoid depletion scores of the Bursa of Fabricius (BF) determined by digital-image analysis.

Group	Average lymphoiddepletion scores (0-5)		
	25 dpv	5 dpc	10 dpc
Negative control	1.00 ^{A}	3.30 ^{A}	3.53 ^{A}
Recombinant	1.07 ^{A}	1.15 ^{B}	1.35 ^{B}
Immune-complex	1.93 ^{B}	2.15 ^{C}	1.95 ^{C}
Intermediate	1.33 ^{AB}	1.10 ^{B}	1.05 ^{B}

dpv= days post-vaccination; dpc= days post-challenge. Means followed by different capital letters (A, B or C) in the same column are significantly different (p<0.05, Tukey's test) among experimental groups.

At histopathological examination before challenge (25 dpv), no changes were observed in the BF of SPF chickens of the negative control group and of the group immunized with the recombinant vaccine. Some alterations were found in BF of the birds immunized with the intermediate vaccine, but these were less extensive and severe compared with those immunized with the immune-complex vaccine, which BF presented moderate to intense cellular infiltration, inter-follicular fibroplasia, and epithelial folding. No BF histological differences were detected among the three vaccinated after challenge.

The serological results are presented in Table 4.

Table 4 – Antibody titers (log10) determined in the experimental groups before and after challenge by ELISA. Data expressed as mean ± standard deviation.

Group		25 dpv	5 dpc	10 dpc
1	Negative control	0.600±0.917 ^{A}	*	3.452±0.156 ^{A}
2	Recombinant	2.523±0.417 ^{B}	2.841±0.434 ^{A}	3.064±0.211 ^{B}
3	Immune-complex	2.921±0.394 ^{B}	3.167±0.412 ^{B}	3.167±0.563 ^{AB}
4	Intermediate	2.995±0.245 ^{B}	3.380±0.294 ^{B}	3.307±0.249 ^{AB}

dpv= days post-vaccination; dpc= days post-challenge. *No data as birds died as a result of the challenge or due to hemolysis of the serum samples. Means followed by different capital letters (A, B) in the same column are significantly different (p<0.05, Tukey's test) among experimental groups.

Figure 2 illustrates the cellular response to phytohemagglutinin of SPF chickens 12 and 24 hours post-injection.

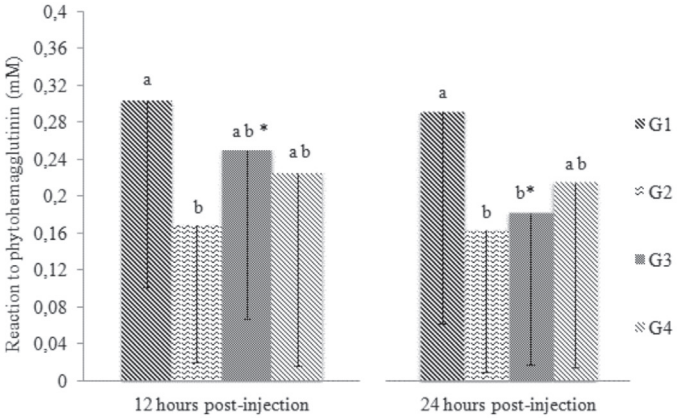

Figure 2 – Cell response to phytohemagglutinin of SPF chickens after 12 and 24 hours from injection. Hypersensitivity test performed on 23 days of age. Data expressed as mean ± standard deviation. G1, negative control; G2, recombinant vaccine; G3, immune-complex vaccine; and G4, intermediate vaccine. Different letters (A or B) have characterized significant difference (p<0,05, Tukey test) among groups in the same period. * Significant difference (p<0.05, ANOVA) between 12 and 24 hours in the same group.

DISCUSSION

The intermediate vaccine showed good results in terms of pathogenicity and protection of SPF chickens. After vaccination, the immunized birds showed no clinical signs, and their BF diameter, relative weight, and lymphoid depletion score was similar to those of the negative control group, characterizing low vaccine pathogenicity. The reduced number of BF microscopic lesions demonstrated only mild atrophy. The intermediate vaccine provided complete protection to SPF chickens against infection with challenge virus (at 25 days of age), as there were no clinical signs of the disease or any BF changes relative to the period previous to the challenge. The serological analyses also demonstrated that the intermediate vaccine induced a good production of anti-IBDV antibodies, which titers were similar to those obtained with the immune-complex vaccine, which contains a "hotter" viral strain (2.995 and 2.921 log10, respectively). Studies have consistently reported the low pathogenicity of the intermediate vaccines in SPF chickens, and induction of antibody levels similar to those obtained with hot vaccines (Rautenschlein *et al*., 2003; Moraes *et al*., 2004; Padilha *et al*., 2005). However, it must be emphasized that commercially available intermediate vaccines may have different virulence degrees.

The immune-complex vaccine, as it contains a hot IBDV strain, caused greater pathogenicity compared with the other evaluated vaccines, as shown by the lower BF diameter and relative weight, and higher severity of BF lesions. Due to the higher virulence of the virus strain in the immune-complex vaccine, it was expected that it would promote higher antibody titers compared with the other groups; however, that was not the case. The resistance to the challenge showed that the immune-complex vaccine conferred complete protection to birds.

The results of the present study are consistent with the reports of Link *et al*. (2003) on the pathogenicity and immunogenicity of the immune-complex vaccine administered *in ovo* to SPF birds challenged Delaware E and Mississippi IBD strains 21 days post-vaccination. Avakian *et al*. (2001) reported two experiments in which SPF birds were vaccinated *in ovo* with an

immune-complex vaccine and observed BF atrophy after vaccination and good protection of the birds against challenges with very virulent DV 86 strain and the classic STC strain at 28 days of age.

Despite the BF lesions caused by immune-complex vaccines, some researchers stated that the vaccine antibodies may reduce the pathogenicity of the virus, resulting in less severe depletion of B lymphocytes when compared with live vaccines with the same virus strain (Haddad *et al*., 1997; Jeurissen *et al*., 1998). On the other hand, Moraes *et al*. (2004) recommends caution when using live vaccines that may cause BF lesions, as the recovery of bursal follicles may be only partial. Moreover, according to Iván *et al*. (2001), the pathological effects caused by immune-complex vaccines are different on SPF birds compared with commercial birds with maternal immunity. In their study, commercial birds exhibited bursal depletion later (starting on day 33), was less severe, and for a shorter time than SPF birds, in which depletion started 7 days post-vaccination. In addition, several studies have shown that the immune-complex vaccine is an excellent alternative for the vaccination of broiler chickens, promoting high levels of maternal antibodies, providing total protection against heterologous strains, and do not affect live performance (Haddad *et al*., 1997; Avakian *et al*., 2001; Chansiripornchai & Sasipreeyajan, 2009; Santos, 2009).

In the present study, the comparison of the pathogenicity and protection results of the three tested vaccines showed that the recombinant vaccine exhibited the best performance, as the lymphoid tissue of the BF was completely preserved (before the challenge). As expected, these results are in agreement with other investigations (Darteil *et al*., 1995; Perozo *et al*., 2009), because the viral vector of the vaccine, by loading only the gene of the immunogenic protein of IBDV, is not able to synthesize virulent viral particles, and therefore, it does not cause infection and lysis of B lymphocytes (Bublot *et al*., 2007). The recombinant vaccine demonstrated to be capable of inducing humoral immunity, presenting similar antibody titers on 25 dpv statistically similar to those of the other groups. This suggests that the VP2 gene is efficiently expressed and that the translated transgenic protein maintained conformational native VP2 characteristics. Similar protection levels of SPF birds induced by another recombinant vaccine, also with the HVT vector, were previously reported (Tsukamoto *et al*., 2002).

The present results are in agreement with Goutebroze *et al*. (2003), Perozo *et al*. (2009), and Le Gros *et al*. (2009), who previously reported the good performance of recombinant vaccines in terms of immunogenicity, pathogenicity, and protection of both broilers with maternal immunity and SPF chickens, as well as good safety and security. Therefore, recombinant vaccines may be considered an excellent alternative for the immunization of birds against IBD.

In addition of antibody protection provided the vaccines and of their pathogenicity , this study also evaluated the level of cellular immunity of SPF birds after vaccination. Contrary to the expectations, the results demonstrated that the birds immunized with the recombinant vaccine presented lower cellular reaction to phytohemagglutinin in comparison with the control group. And, although the cellular reaction to immune-complex vaccine was similar to that of the control group in 12 hours, the intensity of the response was significantly reduced 24 hours post-injection.

To date, no scientific explanation for the weaker cell-mediated response to phytohemagglutinin in chickens immunized with recombinant IBD vaccines has been published in literature. Corley & Giambrone (2002), testing a different immune-complex vaccine *in ovo* also reported that the immunized SPF birds presented a weaker cellular response compared with unvaccinated birds, utilizing the *in-vitro* assay of mitogenic proliferation of spleen T cells with concanavalin A. However, the mechanisms through which IBDV may induce partial reduction of the T lymphocyte response to mitogens are not known yet.

Despite of the reduced cellular response determined when chickens were 23 days of age, their immuno competence in face of the challenge was not affected. Independently of the experimental group, the birds presented a highly variable cellular response to phytohemagglutinin, which suggests that other *in-vivo* or *in-vitro* methods should be applied to evaluate cell-mediated immunity, not only at cellular level, but also at molecular level.

Therefore, under experimental conditions, this study clearly demonstrated that all SPF chickens immunized on the day 1, regardless of the type of vaccine administered, resisted to the challenge with the G11 strain, and, although the immunogenicity of the vaccines were similar, their pathogenicity was considerably different. In addition, it is important to highlight that the achieved results were analyzed under experimental conditions, in which birds were vaccinated with one day of age and challenged 25 days later.

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REFERENCES

Avakian AP, Whitfill CE, Haddad EE, Van Den Wijngaard JK, Chettle NJ. The characteristics of infectious bursal disease virus-antibody complex vacines and their application on broilers with maternal immunity. Proceedings of the 3° Meeting of Working Group 3, COST Action 839. Passive protection and vaccination (current and future possibilities) in the presence of maternally derived antibody; 2001; Pulawy. Poland.

Bublot M, Pritchard N, Le Gros FX, Goutebroze S. Use of a vectored vaccine against infectious bursal disease of chickens in the face of high-titred maternally derived antibody. Journal of Comparative Pathology 2007;137:S81-S84.

Chansiripornchai N, Sasipreeyajan J. Comparison of the Efficacy of the Immune Complex and Conventionally Live Vaccine in Broilers against Infectious Bursal Disease Infection. Journal of Veterinary Medicine 2009;39:115-120.

Corley MM, Giambrone JJ. Immunosuppression in specific pathogen free broilers administered infectious bursal disease virus vaccines by in ovo route. Avian Diseases 2002;46:2002.

Darteil R, Bublot M, Laplace E, Bouquet JF, Audonnet JC, Riviere M. Herpesvirus of turkey recombinant viruses expressing infectious bursal disease vírus (IBDV) VP2 immunogen induce protection against an IBDV virulent challenge in chickens. Virology 1995;211(2):481-490.

Eterradossi N, Saif YM. Infectious Bursal Disease. In: Saif YM, Fadly AM, Glisson JR, Mcdougald LR, Nolan LK, Swayne DE. Diseases of poultry. 12th ed. Ames: Iowa State University Press; 2008. p.185-208.

Goutebroze S, Curet M, Jay ML, Roux C, Le Gros FX. Efficacy of a recombinant vaccine HVT-VP2 against Gumboro disease in the presence of maternal antibodies. British Poultry Science 2003;44(5):824-825.

Haddad EE, Whitfill CE, Avakian AP, Ricks CA, Andrews PD, Thoma JA, Wakenell PS. Efficacy of a novel infectious bursal disease virus immune complex vaccine in broiler chickens. Avian Diseases 1997;41(4):882-889.

Ikuta N, El Attrache J, Villegas P, Garcia M, Lunge VR, Fonseca ASK, et al. Molecular caracterization of Brazilian infectious bursal disease viruses. Avian Diseases 2001;45:297-306.

Iván J, Nagy N, Magyar A, Kacskovics I, Mészáros J. Functional restoration of the bursa of Fabricius following in ovo infectious bursal disease vaccination. Veterinary Immunology and Immunopathology 2001;79:235-248.

Jeurissen SH, Janse EM, Lehrbach PR, Haddad EE, Avakian AP, Whitfill CE. The working mechanisms of an immune complex vaccine that protects chickens against infectious bursal disease. Immunology 1998;95(3):494-500.

Le Gros FX, Dancer A, Giacomini C, Pizzoni L, Bublot M, Graziani M, et al. Field efficacy trial of a novel HVT-IBD vector vaccine for 1-day-old broilers. Vaccine 2009;27:592-596.

Link D, Avakian A, Martin M, Whitfill C. Infectious Bursal Disease Virus (IBDV) variant strain delaware E and mississippi challenges in SPF chickens vaccinated with an IBDV antibody complex vaccine. Proceedings of the 52° Western Poultry Disease Conference; 2003 Mar 8-11; Sacramento. California; 2003. p.40.

Moraes HLS, Salle CTP, Padilha AP, Nascimento VP, Souza GF, Pereira RA, et al. Infectious bursal disease: evaluation ofpathogenicity of commercial vaccines from Brazil in specific pathogens free chickens. Brazilian Journal of Poultry Science 2004;6(4):243-247.

Moraes LB, Osório FS, Salle FO, Souza GF, Moraes HLS, Fallavena LCB, et al. Evaluation of follicular lymphoid depletion in the Bursa of Fabricius: an alternative methodology using digital image analysis and artificial neural networks. Pesquisa Veterinária Brasileira 2010;30: 340-344.

Müller R, Kaufer I, Reinacher M, Weiss E. Immunofluorescent studies of early virus propagation after oral infection with Infectious Bursal Disease Virus (IBDV). Zentralbl Veterinarmedizin Reihe B 1979;26(5):345-352.

Padilha AP. Doença infecciosa bursal: avaliação da patogenicidade de vacinas comercializadas no Brasil em aves livres de patógenos específicos [dissertação]. Porto Alegre (RS): Universidade Federal do Rio Grande do Sul; 2005.

Perozo F, Villegas P, Fernandez R, Cruz J, Pritchard N. Efficacy of Single Dose Recombinant Herpesvirus of Turkey Infectious Bursal Disease Virus (IBDV) Vaccination Against a Variant IBDV Strain. Avian Diseases 2009;53:624-628.

Rautenschlein S, Yeh HY, Sharma JM. Comparative immunopathogenesis of mild, intermediate, and virulent strains of classical infectious bursal disease virus. Avian Diseases 2003;47(1):66-78.

Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. American Journal of Hygiene 1938;27(3):493-497.

Santos R. Avaliação no desempenho de frangos de corte submetidos a três diferentes programas de vacinações para Doença Infecciosa da Bursa [dissertação]. Porto Alegre (RS): Universidade Federal do Rio Grande do Sul; 2009.

Sharma JM, Kim IJ, Rauntenschlein S, Yeh HY. Infectious Bursal Disease Virus of chickens: pathogenesis and immunosuppression. Developmental & Comparative Immunology 2000;4: 223-235.

Stadecker MJ, Lukic M, Dvorak A, Leskowitz S. The cutaneous basophil response to phytohemagglutinin in chickens. Journal of Immunology 1977;118:564-1568.

Tsukamoto K, Saito S, Saeki S, Sato T, Tanimura N, Isobe T, et al. Complete, long-lasting protection against lethal infectious bursal disease virus challenge by a single vaccination with an avian herpesvirus vector expressing VP2 antigens. Journal of Virology 2002;76(11):5637-5645.

Van Den Berg TP, Meulemans G. Acute infectious bursal disease in poultry; protection afforded by maternally derived antibodies and interference with live vaccination. Avian Pathology 1991;20(3):409-421.