Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Moraes, HLS; Salle, CTP; Padilha, AP; Nascimento, VP; Souza, GF; Pereira, RA; Artencio, JO; Salle, FO
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Revista Brasileira de Ciência Avícola, vol. 6, núm. 4, octubre-diciembre, 2004, pp. 243-247
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2004 / v.6 / n.4 / 243 - 247

Infectious Bursal Disease: Evaluation of Pathogenicity of Commercial Vaccines from Brazil in Specific Pathogen Free Chichens

■ Author(s)

Moraes HLS[1,2]
Salle CTP[1]
Padilha AP[1]
Nascimento VP[1]
Souza GF[1]
Pereira RA[3]
Artencio JO[1]
Salle FO[1]

[1] Centro de Diagnóstico e Pesquisa em Patologia Aviária - CDPA/UFRGS
[2] Faculdade de Medicina Veterinária da Universidade Luterana do Brasil - ULBRA
[3] Faculdade de Medicina Veterinária da Universidade de Passo Fundo - UPF

■ Mail Address

Hamilton Luiz de Souza Moraes
Centro de Diagnóstico e Pesquisa em Patologia Aviária - CDPA/UFRGS
Av. Bento Gonçalves, 8824
Bairro Agronomia
91.540 000 - Porto Alegre, RS, Brasil

E-mail: hamilton.souza.moraes@ufrgs.br

■ Keywords

bursa of Fabricius, infectious bursal disease, pathogenicity, vaccines.

■ Acknowledgements

Grants to this study were given by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Arrived: january / 2004
Approved: october / 2004

ABSTRACT

Infectious Bursal Disease (IBD) is a chicken disease economically important for the poultry industry in function of the immune depression that it causes. Disease control is made with different vaccines and vaccination programs. In present work, the pathogenicity of 3 intermediate vaccines (I1, I2 and I3), 2 intermediate more pathogenic (IP1 and IP2) and 3 vaccines containing strong virus (F1, F2 and F3) was evaluated. Birds vaccinated with IP1, IP2, F1, F2 and F3 showed significantly lower bursa size in relation to control animals and animals vaccinated with I1, I2 and I3. On the other hand, vaccines I1 and I3 induced antibody titers higher than the control and lower than I2, IP1, IP2, F1, F2 and F3. Histological scores showed that vaccines I1, I2 and I3 induced similar injury degree, although I2 and I3 were not different from the control, whereas I1 was slightly different. Strong vaccines induced more pronounced lesions than the other tested vaccines. These findings suggest that strong vaccines are able to cause severe bursal injuries.

However, bursometry and relative weight of the bursa of Fabricius wereconsidered inadequate to evaluate vaccine pathogenicity. Moreover, strong vaccines induced higher antibody titers than the other vaccines, although some intermediate vaccines induced similar titers.

INTRODUCTION

Infectious Bursal Disease (IBD) is a viral disease that affects mainly young chickens and is economically important to the poultry industry (Van den Berg, 2000). Consequently, several types of vaccines and vaccination programs have been developed in order to prevent this disease. Solano *et al.* (1986) evaluated the effect of the levels of maternal antibody in chicks on the prime vaccination against IBDV and reported better antibody response when birds were vaccinated at 28 days of age. On the other hand, Kumar *et al.* (2000) considered 21 days old as the ideal age for vaccination, since maternal antibodies were not detectable anymore and could not interfere with the replication of the vaccine virus. The immunosuppressive effect of IBD on the vaccination against infectious bronchitis was assessed and it was observed that younger birds inoculated with IBDV were generally more susceptible to infectious bronchitis virus than animals inoculated with IBDV at older ages (Pejkovski *et al.*, 1979). Although there is variability in the persistence of maternal antibodies in the progeny, antibody levels at the first day of age can be known according to the breeder immunity, and it is thus possible to estimate antibody half life and establish the most appropriate period for prime vaccination (Alam *et al.*, 2002).

Immune suppression is inversely related to the degree of histological integrity of the Bursa of Fabricius (BF) and also to the age at which it was induced (Iván *et al.*, 2001). Some vaccines are capable of inducing

similar or more severe bursal lesions than those caused by field virus strains. Luengo *et al.* (2001) evaluated the severity of macroscopic and microscopic lesions caused by IBDV infection in three groups of animals vaccinated at 4 weeks of age with a commercial live vaccine (intermediary strain). Bursa atrophy and reduced bursa weight:body weight ratio was observed after seven weeks, which could be responsible for immunodeficiency. In a second experiment, vaccinated birds were challenged with a classical IBDV strain; follicular necrosis and lymphocyte depletion were observed, associated to acute inflammation, edema, hemorrhage, and heterophil infiltration. Kim *et al.* (1999) studied the long-term effects of IBD infection, evaluating the restoration of BF lesions after chicks were inoculated in the first day of life with two types of vaccine (intermediate and virulent strains). At the beginning of the infection, an extensive bursal B lymphocyte necrosis was observed together with T lymphocyte infiltration. Bursal necrosis was not observed later, and follicular B lymphocyte population was partially restored. Lymphoid repopulation occurred faster in chicks vaccinated with the intermediate vaccine than in the chicks vaccinated with virulent virus sample; repopulation at seven weeks was 80% and 40%, respectively.

The present study evaluated the pathogenicity of commercial vaccines, and contributes to vaccine selection to be used in vaccination programs for chicks.

MATERIALS AND METHODS

Vaccines

Eight vaccines were used according to the specification of the manufacturers: three intermediate (I1, I2, I3), two intermediate plus (IP1, IP2), and finally three virulent ("hot") vaccine strains (F1, F2, F3).

Birds

Ninety SPF birds were distributed in 9 groups with 10 animals each. The groups were vaccinated with one vaccine sample each and one last group was the control group. Birds were raised in batteries located in separate rooms with positive pressure and filtered airflow, and feed was given *ad libitum*.

Vaccination

The birds of the eight experimental groups were submitted to ocular vaccination (one eye drop) at 21 days old and sacrificed at 28 days of age. Vaccine titers were determined in SPF eggs (Sadia SA, Uberlândia, MG, Brazil), and a standard titer of $10^{3.0}$ DIE50 was used for all vaccinations. The control group was not vaccinated.

Bursometry

At 28 days of age, birds were weighed, slaughtered and BF diameters were measured with a ruler called bursometer (Fort Dodge Animal Health). In the bursometer, each orifice from 1 to 8 represents a diameter, as follows:

1=3.17 mm	2=6.35mm	3=9.52mm	4=12.70
5=15.87m	6=19.05	7=22.22mm	8=25.40mm.

Relative Weight of the Bursa of Fabricius

Birds were weighed and the BF was immediately weighed after being removed. BF relative weight was calculated as BF weight (BW) divided by bird body weight (BBW) multiplied by 1000.

Histopathology

The collected BF were fixed in 10% buffered formalin, and transversally cut at the median region. Both sections were dehydrated, clarified and embedded in paraffin. Cuts were performed and stained with hematoxylin and eosin, and the intensity of lesions was scored according to Muskett *et al.* (1979). Each score, numbered from 0 to 5, represents the degree of lymphocitary depletion corresponding to normal, lower than 24%, from 25 to 49%, from 50 to 69%, from 70 to 89% and higher than 90% respectively.

ELISA

Serum samples were collected from the 90 birds before slaughter at 28 days of age and IBDV antibody levels were evaluated using a commercial ELISA kit (Civites™ AVI IBD®, Laboratórios Hipra SA. Avda. la selva, 135 – 17170 Amer, Girona, Spain.

Statistical analysis

Data were analyzed using the statistical softwares Sigmastat Statistical™ (Version 2.0, SPSS Inc., Chicago, Illinois, USA) and Minitab™ Statistical (Version 13.2, Minitab Inc., Chicago, Illinois, USA)

RESULTS

Bursa of Fabricius diameter

The diameter of the bursa of Fabricius was compared among vaccine groups. No differences ($p>0.05$) in the size of the bursa were seen between

animals from the control group and those vaccinated with I1, I2 and I3. However, animals that were vaccinated with IP1, IP2, F1, F2 and F3 had smaller bursas ($p<0.05$) when compared to the control group. Bursa sizes in animals vaccinated with I1, I2, I3 were greater ($p<0.05$) than in the birds vaccinated with IP1, IP2, F1, F2 and F3. Table 1 shows bursa diameter averages and the standard deviation for the different vaccine groups.

Table 1 – Mean diameter of bursa of Fabricius (mm) and standard deviation (SD) for each vaccine group.

Vaccines	n	Mean diameter	SD
Control	10	4.8[a]	0.63
I1	10	4.4[a]	0.51
I2	10	4.3[a]	0.48
I3	10	4.8[a]	0.63
IP1	10	3.5[b]	0.52
IP2	10	3.2[b]	0.42
F1	10	2.9[b]	0.31
F2	10	3.0[b]	0.00
F3	10	2.9[b]	0.31

Different letters in the same column indicate different means ($p<0.05$).

Relative weight of the bursa of Fabricius

Intermediate vaccines (I1, I2 and I3) were not able to reduce bursa size significantly compared to the control group ($p>0.05$). However, the more pathogenic intermediate (IP1 and IP2) and the very virulent vaccines (F1, F2 and F3) were able to cause enough reduction ($p<0.05$) in bursa size, and were different from the control group as well as from the group of birds that were vaccinated with the intermediate vaccines (I1, I2 and I3). Table 2 presents mean relative BF weight and standard deviation according to the different vaccines.

Table 2 – Means of bursa relative weight and standard deviation (SD) for each vaccine group.

Vaccine	n	Relative weight	SD
Control	10	5.88[a]	0.90
I1	10	5.88[a]	1.38
I2	10	6.06[a]	1.64
I3	10	7.22[a]	1.20
IP1	10	3.05[b]	1.15
IP2	10	2.62[b]	0.81
F1	10	1.74[b]	0.41
F2	10	2.00[b]	0.40
F3	10	1.75[b]	0.46

Different letters in the same column indicate different means ($p<0.05$).

Antibody titers

IBD antibody titers induced by the vaccines were measured using ELISA. Antibody levels in I3-vaccinated birds were not different ($p>0.05$) from the levels in the control group. On the other hand, levels were different ($p<0.05$) from all other vaccine groups. I1 and IP1 developed a similar antibody response, which was different ($p<0.05$) compared to the control group and similar ($p>0.05$) to IP2 and very virulent vaccines (VV). Although I2 induced antibody titers similar to I1 and IP1 vaccines ($p>0.05$), these values were different ($p<0.05$) from IP2 and VV vaccines. The more pathogenic intermediate vaccine IP2 and the VV vaccines (F1, F2 and F3) showed similar antibody titers ($p>0.05$).

Table 3 presents the medians of antibody titers induced by the different vaccines.

Table 3 – Medians of antibody titers induced by different vaccines.

Vaccine	n	Median ($\log_{10}$)
Control	9	2.10[a]
I1	10	3.28[bc]
I2	9	3.00[b]
I3	10	2.23[a]
IP1	10	3.36[bc]
IP2	10	3.69[c]
F1	10	3.77[c]
F2	10	3.67[c]
F3	10	3.77[c]

Different letters in the same column indicate significant differences by the Kruskal-Wallis Test ($p<0.05$).

Histological lesion scores in the Bursa of Fabricius

Histological lesion scores in the bursas were defined for each evaluated vaccine. Scores were similar ($p>0.05$) among the intermediate vaccines I1, I2 and I3. However, whereas I2 and I3 scores were similar ($p< 0.05$) to the control group, I1 score was different ($p< 0.05$).

Birds vaccinated with the more pathogenic intermediate vaccines (IP1 and IP2) and the VV vaccines (F1, F2 and F3) had similar scores ($p> 0.05$), which were different from the control group and the intermediate vaccines I1, I2 and I3. The medians obtained for histopathological lesion scores of the different vaccines are shown in Table 4.

Table 4 – Medians from the scores of histopathological lesions induced by the different vaccines.

Vaccine	n	Medians
Control	10	1.00[a]
I1	10	2.00[b]
I2	10	1.50[ab]
I3	10	1.00[ab]
IP1	10	4.00[c]
IP2	10	4.00[c]
F1	10	4.00[c]
F2	10	3.00[c]
F3	10	4.00[c]

Different letters in the same column indicate significant differences by the Kruskal-Wallis Test ($p<0.05$).

DISCUSSION AND CONCLUSIONS

There was no correlation between bursa diameter and bursal histopathological lesion scores. This fact corroborates the hypothesis that bursa measurement is a less sensitive method to evaluate vaccination effect when compared to histopathological score. Similarly, no correlation was found between BF diameter and disease or vaccination when birds vaccinated in the first day of life were challenged with very virulent IBDV samples (Moraes *et al.*, unpublished results). The histopathological test was compared with the results of bursa measurement in a previous study of Gumboro disease monitoring that was carried out in our institution. It was observed that, although 155 animals had histological scores that were considered compatible with disease, 137 of these were considered well immunized by bursa measurement. This result might reflect the fact that bursa measurement does not consider bird weight. Therefore, even though the Bursa of Fabricius in sick birds is atrophied, its diameter in sick birds with high body weight might be similar to the bursa diameter in lighter birds that have been vaccinated (Pereira *et al.*, personal communication).

Serological evaluation showed that some intermediate vaccines were able to induce antibody titers similar to VV vaccines. Rautenschlein *et al.* (2002) also showed that a intermediate strain and a very virulent strain induced similar antibody titers ($p>0.05$) during a 29-day period of observation. In the present study, the more pathogenic intermediate strains (IP1 and IP2) induced levels of antibodies against IBDV similar ($p> 0.05$) to the VV vaccines. However, it should be noted that it is not known whether the vaccines that were used in the paper above are from to the same IBDV virus sample.

It was observed that the vaccine I1 induced antibody titers similar ($p>0.05$) to the very virulent vaccines, although the histopathological lesion scores were significantly lower than the scores observed for F1, F2 and F3. However, we should consider the antigenic relationship between the studied vaccine virus and the virus used in the ELISA kit.

Ezeokoli *et al.* (1990) evaluated histological modifications of BF associated with poultry vaccination against IBDV, describing severe lesions in the bursa between three and seven days after vaccination. The authors suggested a vaccination program that should be made in the first days of life, based on the observation that the bursa tissue was completely recovered 15 days after vaccination and that there was no difference between vaccinated and control birds. However, the type of vaccines used in that experiment was not described. These findings disagree from the observations made by Kim *et al.* (1999), who reported only partial disappearance of bursal necrosis and recovery of B lymphocyte population in the follicles after a long term observation of the infection caused by the IBDV with vaccines containing intermediate and virulent strains. They have also observed seven weeks after inoculation about 80% of repopulation in the bursal follicles of animals exposed to the vaccine prepared with intermediate strain, and 40% in birds that have received a virulent strain vaccine. Thus, the use of vaccines that are proved to induce accentuated lesion degrees in the lymphoid system must be carefully considered, since follicle recovery might not be total. Besides, longer periods of time might be needed for follicular repopulation, during which the animal would be susceptible to several diseases.

The present study reveals that the more pathogenic intermediate vaccines as well as very virulent vaccines caused severe BF injury in vaccinated animals, resulting in lymphocyte depletion of approximately 90%. It must be noted that such histological lesions are compatible with the signs of disease induced by the pathogenic field virus strains. Consequently, the induced degree of pathogenicity must always be considered before determining which vaccine samples should be used in a vaccination program.

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O arquivo disponível sofreu correções conforme ERRATA publicada no Volume 7 Número 3 da revista.