EVALUATION OF ANATOMOPATHOLOGICAL, SEROLOGICAL, IMMUNOLOGICAL RESPONSES AND PROTECTION IN BROILERS VACCINATED WITH LIVE INFECTIOUS BURSAL DISEASE VACCINES

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ABSTRACT

The study was divided into three experiments. In the first one, broilers were distributed into six groups and vaccinated against infectious bursal disease at 14 days of age: T1-not vaccinated, T2-Lukert[1] (intermediate), T3-Lukert[2] (intermediate plus), T4-228E, T5-V877 and T6-Winterfield 2512 ("hot" strains). Bursas of Fabricius (BF) were collected at 17, 21, 28 and 35 days to measure BF relative weight (BFRW), diameter, histological examination and image processing analysis (IPA). At one, 14, 21, 28 and 35 days of age, samples of blood taken from eight birds from each group for serology analysis by ELISA test. Hot strains vaccines induced reduction of BFRW and BF diameter, higher histological score lesion degree, more lymphocyte depletion on the BF follicles and higher IBD antibody titer. In the second experiment, 16 birds from groups T1 to T6 were isolated and challenged with a very virulent strain of IBDV (vvIBDV) at 25 days of age. Only groups T4, T5 and T6 were totally protected against vvIBDV challenge. In the third experiment, the immunosuppressive potential of each vaccine was determined by examining the ability of IBDV-vaccinated birds to respond to Newcastle disease (ND) vaccination and challenge. None of the vaccines was found to be immunodepressive.

KEY WORDS: Infectious Bursal Disease, very virulent infectious bursal disease virus, live vaccines, bursa of Fabricius, broilers, immunosuppression.

RESUMO

AVALIAÇÃO DAS RESPOSTAS ANATOMOPATOLÓGICA, SOROLÓGICA, IMUNONOLÓGICA E PROTETORA EM FRANGOS DE CORTE VACINADOS COM VACINAS VIVAS CONTRA A DOENÇA INFECCIOSA DA BURSA. O estudo foi dividido em três experimentos. No primeiro, frangos foram distribuídos em seis grupos e vacinados contra a Doença Infecciosa da Bursa (IBD) aos 14 dias de idade: T1-não vacinados, T2-Lukert[1] (intermediária), T3-Lukert[2] (intermediária plus), T4-228E, T5-V877 e T6-Winterfield 2512 (cepa forte). As bursas de Fabrícius (BF) foram colhidas aos 17, 21, 28 e 35 dias para mensurar o seus pesos relativos da BF (BFRW), diâmetros, exames histológicos e processamento de imagem imagens(IPA). Ao primeiro, 14, 21, 28 e 35 dias de idade, foram colhidas amostras de sangue de oito aves de cada grupo para a realização de sorologia através do método ELISA indireto. As vacinas cepa forte induziram redução do BFRW peso e do diâmetro da BF, maior grau de lesão histológica e depleção linfocitária e títulos mais elevados de anticorpos. No segundo experimento, 16 aves dos grupos T1 ao T6 foram isoladas e desafiadas com o vírus muito virulento da IBD (vvIBDV) aos 25 dias de idade. Somente os grupos T4, T5 e T6 foram completamente protegidos do desafio com vvIBDV. No terceiro experimento, o potencial imunossupressor de cada vacina foi determinado através da capacidade das aves vacinadas responderem à vacinação contra a Doença de Newcastle (ND) e posterior desafio. Nenhuma vacina apresentou-se imunossupressora.

PALAVRAS-CHAVE: Doença Infecciosa da Bursa, vírus muito virulento, vacinas vivas atenuadas, bursa de Fabrícius, frangos de corte, imunossupressão.

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INTRODUCTION

Infectious bursal disease virus (IBDV) is a member of Birnaviridae family, non-enveloped with icsosahedral symmetry and a diameter varying from 55 to 65 nm. The virus has genomes consisting of two segments of double-stranded RNA (Lobardo et al., 1999; Schröder et al., 2000).

The clinical disease of IBD, first described by Cosgrove in 1962, affects chicks between 3 and 6 weeks of age, during over 8 to 10 days. It is characterized by ruffled feathers, whitish or watery diarrhea, anorexia, depression, trembling, severe prostration and finally death. The target organ of the virus is the lymphoid tissue, specially the bursa of Fabricius that has a gelatinous yellowish transudate covering the serosal surface. Longitudinal striations on the surface become prominent, and the normal white color turns to cream. The transudate disappears as the bursa returns to its normal size and becomes gray during the following period of atrophy. The infected bursa often shows necrotic foci and at times ecchymotic hemorrhages on the mucosal surface. Occasionally, extensive hemorrhage throughout the entire bursa has been observed (Lukert ; Saif, 1997; Sellers et al., 1999).

Susceptible chicks younger than 3 weeks do not exhibit clinical signs, but have subclinical infections that are economically important as a result of severe immunosuppression of the chicken (Lukert; Saif, 1997).

In the late 1980', IBD caused low mortality, with economical loss due to the immunosuppression (Skinner, 2000). At that time mild vaccines were introduced, followed by inactivated and intermediate vaccines. Since then, until the beginning of 1990 decade, samples of IBDV isolated in those countries had moderate pathogenicity (Saukas, 1978; Ito, 1981; Ito, 1990).

In the early 1990, Brazil was following the strategy of IBD prevention and control used in the USA. Mild and intermediate vaccines were produced using field IBDV with mild pathogenicity, attenuated by adaptation after several blind passages in cell culture or embryonated eggs (Ito, 2002).

The situation changed rapidly, however, in the late 1980' with the emergence of a pathotypic variant, first observed in the Benelux countries (Van den Berg; Meulemans, 1991). This variant termed very virulent, spread rapidly throughout mainland Europe (Eteradossi et al., 1992).

Since July 1997, the vvIBDV, genomic group 11 (G 11-Simbios) had been observed in some broiler farms located in Campinas, São Paulo State (Di Fabio et al., 1999). Bursas from 27-day-old diseased broilers were sent for virus characterization to the AFSSA-Ploufragan laboratory, France. In an antigen-capture ELISA base d on seven neutralizing monoclonal antibodies, homogenates derived from the submitted bursa were shown to contain IBDV particles antigenically similar to the vvIBDVs collected in Europe since 1981 (Eteradossi et al., 1997). Clinical and virological data suggested that the very virulent form of IBD was present in Brazil. One of the virus envolvedinvolved shared antigenic, genetic and pathogenic properties with European vvIBDV strains (Di Fabio et al., 1999).

The control of vvIBDV in Europe was done in part by the use of live vaccines, which primarily did not differed not primarily in their antigenicity but in their pathogenicity. Thus, instead of mild vaccines, intermediate vaccines were used routinely, with hot vaccines being used use don heavily contaminated sites. The rationale behind this successful approach was that the hotter vaccines would be able to take in the face of higher levels of maternal antibodies, allowing chicks to be vaccinated earlier, before infection with the vvIBDV. Such an approach though depends on strict hygiene and management to reduce levels of contamination with vvIBDV. Moreover, vaccination protocols have to be strictly adhered to so that vaccination is effective without causing adverse effects on growth or immune system function (Skinner, 2000).

The immunization of breeder flocks is very important to confer parental immunity to their progeny. Antibody transmitted via the yolk sac can protect chicks against early infections with IBDV, with resultant protection against immunosuppressive effect of the virus (Lukert; Saif, 1997). The half-life of maternal antibodies to IBDV is between 3 and 5 days (Skeeles et al., 1979). Such maternal antibodies protect the chick from early immunosuppressive infections, protecting them for 1 to 3 weeks, but by boosting the immunity in breeder flocks with oil-adjuvant vaccines, passive immunity may be extended to 4 or 5 weeks (Baxendale; Lutticken, 1981).

The present study was carried out to evaluate the anatomopathological, serological, immunological responses and protection in broilers vaccinated with five commercial live IBD vaccines and challenged with vvIBDV strain.

MATERIAL AND METHODS

Experimental birds and management

A total number of 560 day-old male Ross broiler chickens were distributed into seven treatments of 80 birds each. The group was divided into four repetitions with 20 broilers each, housed in boxes over litter, keeping distance between the other groups. They were watered and fed *ad libitum* a broiler corn-soyben starter diet (21.5% protein and 3.073 kcal / kg ME).

Experimental Design

The study was divided into three experiments. In the first, there were six groups of 80 birds each. Each group represented four replicates of 20 birds each. The first group (T1) was unvaccinated (IBD control group). The Group T2 group received Lukert intermediate strain, T3 Lukert intermediate plus strain, T4 228E strain, T5 V877 strain and T6 Winterfield 2512 strain. At 17, 21, 28 and 35 days of age, eight randomly selected broilers of each group were necropsied. They were individually weighted and had their BF measured, weighted and taken for histological and IPA observation. At one, 14, 21, 28 and 35 days of age, eight birds from each group had their blood samples collected for serology analysis by ELISA test.

In the second experiment, at 25 days of age, 16 birds from each group (T1 to T6) were kept in isolators and were challenged. Each bird received, oral via, 0.5 mL of vvIBDV suspension ($10^{4.0}$ DIE_{50} / 0.1 mL) by, oral via. The protection against vvIBDV challenge was measured by the mortality, clinical signs and macroscopic lesions observation observed during 10 days of challenge. Those birds that did not showed mortality or clinical signs of the disease were killed and necropsied at the end of the challenge period.

In the last experiment, the same six vaccine groups consisting of four replicates per group, as in the first experiment were used. In addition, a seventh group consisting of four replicates of 20 birds each, were was not IBDV nor ND vaccinated. Birds from T1 to T6 groups were vaccinated at 21 days of age against ND. At 35 days of age, 16 birds from each group (T1 to T7) were kept into isolators and challenged with viscerotropic NDV. Serum Sera were collected at 21, 28 and 35 days of age to perform hemagglutination inhibition test (HI) to check antibody concentration. They were maintained under observation for 10 days post challenge. The protection against ND challenge was measured by the mortality, clinical signs and macroscopic lesions observation observed during the challenge period. At 45 days of age, all remainigremaining challenged birds were killed and necropsied (Table 1).

IBD vaccines and vaccination

The following five live commercial vaccines were given by intra-conjunctiva instillation at 14 days of age: T2-Lukert[1] (intermediate strain), T3-Lukert[2] (intermediate plus strain), T4-228E, T5-V877 and T6-Winterfield 2512 ("hot" strains). Groups T1 and T7 were not vaccinated against IBD.

Bursa of Fabricius relative weight (BFRW)

At 17, 21, 28 and 35 days of age, eight birds of each group were individually weighted, sacrificed and necropsied. The bursas were sampled, weighted and had their diameter measured. The ratio between the bursa of Fabricius and body weight was calculated by dividing the bursa weight for the body weight multiplied by 1000.

Diameter of the Bursa of Fabricius

A total of 32 bursas of Fabricius per group were submitted to diameter qualitative measurement and given scores from 1 to 8, based on the following parameters: score 1 (diameter up to 3.17 mm); score 2 (from 3.18 to 6.35 mm); score 3 (from 6.36 to 9.52 mm); score 4 (from 9.53 to 12.7 mm); score 5 (from 12.8 to 15.87 mm); score 6 (from 15.88 to 19.05 mm); score 7 (from 19.06 to 22.22 mm); and score 8 (from 22.23 to 25.4 mm). For this, it was used a ruler that contains holes with different diameters to each score (Bursometer suppiedsupplied by Fort Dodge Animal Health, Brazil).

Table 1 - Experimental groups.

Groups	Number of birds	IBD vaccination (14 days)	ND vaccination (21 days)	IBD challenge (25 days)	ND challenge (35 days)
T1	80	-	LaSota	vvIBDV	NDV
T2	80	Lukert[1]	LaSota	vvIBDV	NDV
T3	80	Lukert[2]	LaSota	vvIBDV	NDV
T4	80	228E	LaSota	vvIBDV	NDV
T5	80	V87	LaSota	vvIBDV	NDV
T6	80	Winterfield 2512	LaSota	vvIBDV	NDV
T7	80	-	-		NDV

[1]=intermediate strain

[2]=intermediate plus strain

vvIBDV =very virulent infectious bursal disease virus

NDV= Newcastle Disease Virus

Histological observation

At 17, 21, 28 and 35 days of age, the bursas of four birds of each group had theirwere bursa fixed in 10% phosphate-buffered formalin. They were included in paraffin and fine cuts of 5µ were prepared, mounted between glass slides and coverslips and stained with hematoxilin and eosin. All cuts were examined in a light microscope and scored according to severity of damage on a scale of 0 (none), 1 (minimal), 2 (mild), 3 (moderate), 4 (marked) and 5 (severe), by the following criteria: hemorrhage, lymphoid necrosis, lymphoid depletion, heterophilic inflammation, fibrosis, cyst formation and reticuloendothelial hiperplasia. A mean score for each lesion was then determined from the collected bursas.

Image processing analysis

The histological samples were used to measure the percentage of lymphocytes present in the bursa. A microscope connected to a computer reads the percentage of lymphocytes containing in the bursa by a computer program called E-bursa, developed by Fort Dodge Animal Health.

Serological analysis

Eight serum samples per group were collected aseptically at one, 14, 17, 21, 28 and 35 days of age. Samples were heated at 56^0 C for 30 minutes and kept frozen at -20^0 C until used. They were submitted to ELISA commercial test (IBD-Idexx®).

IBD challenge inoculum

A strain of IBDV was isolated from bursa of Fabricius of natural infected birds. The strain was characterized as a very virulent strain (vvIBDV) and identified by RT-PCR as genomic pattern 11 (G11), as described by IKUTA et al. (2001).

IBD challenge

Sixteen birds from groups T1 to T6 groups were challenged by oral via at 25 days of age. They were given a suspension of 0.5 mL per broiler of vvIBDV. Fifty percent of the birds of each group were chosen at random to be sacrificed four days post challenge and the rest of them were sacrificed ten days after challenge. All of these broilers were observed to identify clinical signs suggesting IBD and were necropsied to observe macroscopic lesions, such as edema, hemorrhage and gelatin-like exsudate on the BF.

Newcastle disease (ND) vaccination

A LaSota strain of Newcastle disease virus (NDV) was used in this trial. Birds from T1 to T6 groups were vaccinated at 21 days of age, each with one drop (0.03 mL) of LaSota vaccine by intra-conjuntictiva instillation. Birds from T7 group (Table 1) were not vaccinated neither against IBD nor ND, constituting the control group of ND.

Table 2 - Mean bursa weight in grams divided by body weight in grams x 1000 (BFRW) of the experimental groups at different ages.

Groups	Vaccines	Mean bursa weight body weight (BFRW) Age (days)			
		17	21	28	35
T1**	-	3.11[a]*	3.04[a]	3.00[a]	3.02[a]
T2	Lukert[1]	2.84[a]	2.86[a]	2.84[ab]	2.54[b]
T3	Lukert[2]	2.98[a]	3.45[a]	2.72[ab]	2.14[b]
T4	228E	3.22[a]	3.37[a]	1.69[bc]	0.95[c]
T5	V877	2.77[a]	2.41[a]	1.06[c]	0.85[c]
T6	Winterfield 2512	3.33[a]	2.53[a]	1.36[c]	0.92[c]

[1]= intermediate strain

[2]= intermediate plus strain

*Means with different subscripts with the same column differ significantly ($p<0.05$)

**IBD control group: non vaccinated against IBD

ND challenge

At 35 days of age, 16 birds per group (T1 to T6 groups) were challenged with viscerotropic ND virus strain. The virus has intra-cerebral pathogenic index of 1.78 and embryonic death time of 48 hours, with a 50% embryo infecting dose titer of 8.15 $\log_{10}$ / 0.1 mL. Distilled water was used as diluent for the inoculum that was instilled by oculo-nasal route, according to the U.S. CODE OF FEDERAL REGULATIONS (1993).

Statistical analysis

Data were submitted to statistical analysis by using GLM of SAS, and treatment means were compared at 5% probability level ($p < 0.05$).

RESULTS

Experiment 1: "Hot" strain vaccines (T4, T5 and T6) were more invasive and pathogenic than the other groups, as evidenced by lower BFRW ratio, smaller

diameter of the BF, higher lymphocyte depletion degree, lower lymphocyte percentage in the BF and higher antibody titers. As expected, the non-IBD vaccinated group (T1) did not show any disorder in the BF and did not presented elevation in the IBD antibody titer.

Bursa of Fabricius relative weight (BFRW)

The analysis of Table 2 revealed that birds from T4, T5 and T6 groups had significant reduction ($p<0.05$) in BFRW ratio from 28 to 35 days of age. A little reduction could be observed in T2 and T3 groups at 35 days of age.

Diameter of the bursa of Fabricius

The Bursa of Fabricius from T4, T5 and T6 groups, representing the "hot" IBD strains, was smaller than the other groups from 28 to 35 days of age (Table 3).

Table 3 - MeasurMesurement of the Bursa of Fabricius by the bursometer at 17, 21, 28 and 35 days of age in broiler chickens vaccinated at 14 days of age.

Groups	Vaccine	Mean of bursa diameter			
		Age (days)			
		17	21	28	35
T1**	-	4.63[a*]	5.50[a]	6.00[a]	6.88[a]
T2	Lukert[1]	4.38[a]	5.38[a]	5.63[ab]	6.00[a]
T3	Lukert[2]	4.50[a]	5.38[a]	5.63[ab]	5.88[a]
T4	228E	4.63[a]	5.25[a]	4.63[bc]	4.50[b]
T5	V877	4.50[a]	4.75[a]	4.25[c]	4.75[b]
T6	Winterfield 2512	4.88[a]	4.88[a]	4.38[c]	4.50[b]

[1]= intermediate strain.
[2]= intermediate plus strain.
* Means with different subscripts with the same column differ significantly ($p<0.05$).
**IBD control group: non vaccinated against IBD.

Histological observation

Based on lesions distribution and scores (Table 4) at 17 and 21 days of age, it was not found significant differences in the BF of birds from T1, T2 and T3 and T4 groups. After 28 days of age, birds from T4, T5 and T6 groups had scores equal or higher than 3.

Image processing analysis

According to the results shown in Table 5, there was more lymphocyte depletion in T4, T5 and T6 groups in comparison to the other ones ($p < 0.05$) due to the more evident pathogenic ability in these kindkinds of vaccines ("hot" strains) over the BF. There was a little recovery and elevation in the percentage of B-lymphocytes in the BF at 35 days of age in these groups.

Table 4 - Quantitative histological analysis of Bursa of Fabricius expressed in lesion scores of the experimental groups at different ages. Quantitative lesion scores from histological analysis of the Bursa of Fabricius expressed of the experimental groups at different ages.

Groups	Vaccines	Mean of histological analysis of Bursa of Fabricius			
		Age (days)			
		17	21	28	35
T1**	-	0[a]	0[a]	0[a]	0[a]
T2	Lukert[1]	0[a]	0.25[a]	0[a]	0[a]
T3	Lukert[2]	0[a]	0.25[a]	0.25[a]	0.25[a]
T4	228E	0.25[a]	0.5[a]	2.5[b]	3[b]
T5	V877	0.5[b]	1.5[b]	2.5[b]	3.25[b]
T6	Winterfield 2512	0.5[b]	1.5[b]	3[b]	3.25[b]

[1]= intermediate strain
[2]= intermediate plus strain
*Means with different subscripts with the same column differ significantly ($p<0.05$)
**IBD control group: non vaccinated against IBD

Table 5 - Mean of lymphocyte percentage in the bursa.

Groups	Vaccines	Mean of lymphocyte percentage in the bursa			
		Age (days)			
		17	21	28	35
T1**	-	43.62[a*]	42.50[a]	40.92[a]	44.06[a]
T2	Lukert[1]	41.99[ab]	38.43[ab]	38.34[a]	43.44[a]
T3	Lukert[2]	39.34[ab]	36.49[ab]	34.44[a]	37.19[a]
T4	228E	36.64[c]	36.01[ab]	25.71[b]	28.05[b]
T5	V877	32.13[c]	30.30[c]	23.45[b]	26.58[b]
T6	Winterfield 2512	33.00[c]	28.06[c]	16.29[b]	28.05[b]

[1]= intermediate strain
[2]= intermediate plus strain
*Means with different subscripts with the same column differ significantly ($p<0.05$)
**IBD control group: non vaccinated against IBD

Serological analysis

Geometric mean of antibody titers in T1, T2 and T3 groups presented declination along the time. Birds

from T4, T5 and T6 groups had higher levels of antibody titers at 35 days of age, indicating the competence of producing neutralizing antibodies against IBD after vaccination (Table 6).

Table 6 - Geometric means of titers of neutralizing antibodies against IBDV in broilers at one, 14, 21, 28 and 35 days of age by ELISA test (log 10), in the different treatments.

Groups	Vaccines	Geometric means of IBDV titers by ELISA test (log 10)			
		Age (days)			
		17	21	28	35
T1**	-	3.73[a*]	3.11[a]	2.67[a]	2.66[a]
T2	Lukert[1]	3.73[a]	3.03[a]	2.75[a]	2.82[a]
T3	Lukert[2]	3.73[a]	3.18[a]	2.81[a]	2.69[a]
T4	228E	3.73[a]	3.19[a]	2.75[a]	3.05[ab]
T5	V877	3.73[a]	3.30[a]	2.76[a]	3.38[b]
T6	Winterfield 2512	3.73[a]	3.05[a]	2.94[a]	3.32[b]

[1]= intermediate strain

[2]= intermediate plus strain

*Means with different subscripts with the same column differ significantly ($p<0.05$)

** IBD control group: non vaccinated against IBD

Experiments 2: During the challenge period, 15/16 birds from T1 group presented signs and macroscopic lesions of IBD, such as ruffled feathers, whitish diarrhea, depression and severe prostration. The BF presented gelatinous yellowish transudate covering the serosal surface. Birds from T2 (10/16) and T3 (4/16) groups presented clinical signs of the disease and macroscopic lesion in the bursa and kidney, such as T1 group. None of the birds from T4, T5 and T6 groups showed any alteration or IBD clinical signs.

Experiment 3: Data in Table 7 revealed that none of the five IBD vaccines was immunosuppressive. Birds receiving any of the five IBD vaccines were able to produce as high NDV antibody titer and were as resistant to ND clinical challenge infection as birds from T1 group that were vaccinated against ND but not against IBD. Birds from T7 group that were not vaccinated against ND were totally susceptible to NDV challenge (16/16).

DISCUSSION

Body weight ratio is one of the most important parameters to evaluate the immunosuppression caused by IBD virus and by IBD vaccines (Bolis et al., 2003). The first experiment demonstrated that only chicks from T4, T5 and T6 groups presented considerable reduction in the BFRW (Table 2) and in the diameter of BF parameters. This can be explained by the lower degree of attenuation of this kind of vaccine, who was capable to destroy B-lymphocytes present on the BF, reducing their size. It is relevant to show that there were not an important reduction of BFRW and diameter of BF in T2 and T3 groups. More severe histological lesions and lower percentage of lymphocytes in the BF were observed in broilers from

Table 7 - Newcastle Disease (ND) serology and challenge.

Groups	IBD vaccines (14 days)	ND vaccine (21 days)	Means of ND titers by HI test (log 2)			NDV challenge
			Age (days)			
			21	28	35	10dpi
T1**	-	LaSota	5.13[a*]	4.87[a]	7.47[a]	0/16
T2	Lukert[1]	LaSota	4.50[a]	5.97[a]	8.06[a]	0/16
T3	Lukert[2]	LaSota	4.53[a]	6.08[a]	7.10[a]	0/16
T4	228E	LaSota	4.20[a]	5.74[a]	7.99[a]	0/16
T5	V877	LaSota	4.68[a]	5.34[a]	7.58[a]	0/16
T6	Winterfield 2512	LaSota	4.74[a]	5.82[a]	7.59[a]	0/16
T7***	-	-	5.12[a]	4.46[a]	3.86[b]	16/16

dpi=days post infection

[1]=intermediate strain

[2]=intermediate plus strain

*Means with different subscripts with the same column differ significantly ($p<0.05$)

**IBD control group: non vaccinated against IBD

***ND control group: non vaccinated neither against IBD nor ND

T4; T5 and T6 groups at 28 and 35 days of age (Tables 4 and 5). This can be explained by the attenuation degree of the IBD vaccine strains used in this trial. Less attenuated strains have the ability to overcome high levels of maternally derived antibodies (MDA), conferring active immunity to the vaccinated chicks (VAN DEN BERG, 2000). As nearly all of the chickens will have some residual maternal antibody at one day old of age, a more invasive, yet not nonimmunodepressive vaccine would be needed to overcome maternal antibody (GIAMBRONE; CLAY, 1986). The passive immunity, although protective, interferes with the vaccination. There is a strong competition between field and vaccines strains to break through MDA, and the optimal timing has become the crucial problem in the establishment of the vaccination schedule. Attenuated live vaccines achieve lifelong and broad protection, but possess a residual pathogenicity (VAN DEN BERG, 2000). This residual pathogenicity is able to induce more damage in the BF, causing destruction of B-lymphocytes in this organ. "Hot" strain vaccines were able to stimulate the active immunity of chicks vaccinated at 14 days of age, inducing higher levels of neutralizing antibodies observed at 35 days of age in these groups.

Only birds from groups T4, T5 and T6 groups were completely protected against vvIBDV challenge. Birds from T1 (15/16), T2 (10/16) and T3 (4/16) groups succumbed to vvIBD infection, demonstrating that birds from these groups were not enough immunized, what was already expected for T1 group, whose chicks were not vaccinated against IBDV. what was already expected for T1 group, whose chicks were not vaccinated against IBDV As intermediate vaccine is more attenuated than intermediate plus vaccine, there was more incidence of the disease on T2 group because this strain of vaccine is not capable to overcome high levels of MDA, so that most part of the vaccine was probably neutralized by the MDA. So, birds from T2 and T3 groups were only partially protected against the IBD challenge virus. Broilers vaccinated with "hot" strain IBD vaccines (groups T4, T5 and T6) were completed protected against vvIBDV challenge because they produced neutralizing antibodies titers capable to recognize and neutralize IBD virus. "Hot" strain vaccines are able to break into high levels of MDA and to stimulate the chicks active immunity, without being neutralized and afterwards destroyed by IBDV antibodies passively acquired. The antibodies were able to neutralize the vvIBDV antigenic epitopes. This mechanism reduced the invasion of the susceptible cells by the virus, protecting the bird against IBDV infection. The intensity of microscopic alterations in the bursa may be quantified to evaluate the level of immunity protection (ABDEL-ALIM; SAIF, 2000; MAAS et al., 2001) or immune modulation of the infection (POONIA ; CHARAN, 2000).

Another explanation for the 100% protection of birds from T4, T5 and T6 groups may lay in the homology of the IBD "hot" strain vaccines and the vvIBDV used in this trial. Of the two serotypes, only serotype 1 strains are pathogenic for poultry (JACKWOOD et al., 1984; ISMAIL et al., 1988) and are therefore used in vaccines. A considerable antigenic diversity exists within serotype1 IBDV strains. In cross-neutralization tests, at least six subtypes can be distinguished with serotype 1 (JACKWOOD; SAIF, 1987). Such subtypes differences could be relevant for the protection against vvIBDV used in this trial. The influence of the vvIBDV challenge strain on the correlation between serology and protection is either caused by a difference in homology between the individual challenge strains and the vaccine strains, or by the difference in virulence of the challenged strains (MAAS et al., 2001).

Birds from T1 to T6 groups presented high levels of protective neutralizing antibody, except T7 group that was not vaccinated against ND. At challenged, only group T7 succumbed to the inoculation of the viscerotropic ND virus, demonstrating that, "hot" strains IBD vaccines did not present immunosuppressive ability in vaccinated broilers.

Although the three "hot" strain IBD vaccines tested in this study (228E, V877 and Winterfield 2512) produced slightly atrophic bursa with moderate microscopic lesions, they conferred 100% protection against vvIBDV challenge and were not immunosuppressive. This study provides data about the use of hot strain IBDV vaccine to control vvIBDV infection in broilers.

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