



Effect of Maternally-Derived Antibodies on The Performance and Immunity of Broilers Induced by *in Ovo* or Post-Hatching Immunizations with a Live Vaccine Against Infectious Bursal Disease

■ Author(s)

Michell BC¹
Gomes AD²
Baião NC¹
Resende M²
Lara LJC¹
Martins NRS^{1*}

¹ Escola de Veterinária - UFMG

² Instituto de Ciências Biológicas - UFMG

■ Mail Address

Nelson Rodrigo S. Martins

E-mail: rodrigo@vet.ufmg.br

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ABSTRACT

The interference of low or high maternal antibodies titers on the attenuated infectious bursal disease (IBD) virus (IBDV) vaccine infection and its effects on the performance of broilers vaccinated at the 18th day of incubation (*in ovo*), at one day of age (subcutaneously-SC), or at 15 days of age (drinking water-DW) were investigated. After a series of three live vaccinations, breeders were given or not an IBD oil emulsion vaccine (IBD-OEV) prior to sexual maturity. At day 18 of incubation (*in ovo*), a commercial vaccine containing HVT and an intermediate IBDV strain or the single HVT vaccine was given. An intermediate IBDV vaccine was given SC at one day of age, or at 15 days of age via DW. The progeny of unvaccinated breeders presented higher neutralizing IBDV-specific antibody (IBDVab) titers at 25 and 40 days of age than those of the progeny of IBD-OEV breeders ($p < 0.05$) at any broilers vaccination age and route. The lower IBDV RNA detection by RT-PCR in the bursa of Fabricius (BF) and the lower IBDV antibody titers in the serum of the groups vaccinated at one and 15 days of age derived from IBD-OEV breeders may indicate antibody-mediated IBDV neutralization. The *in-ovo* and one-day vaccinations did not interfere with performance, both in low and high antibody-titered progenies. The *in-ovo* vaccination against IBD is considered convenient and safe for industrial chickens, irrespective their maternal antibody levels.

INTRODUCTION

Infectious bursal disease (IBD) is one the most common diseases of poultry worldwide, and it is an important cause of immunodepression or immunosuppression in chickens. Biosafety measures and vaccination have been attempted to reduce the risk of infection and disease. For less than two decades, progeny protection was successfully achieved based on the transference of IgG (IgY) antibodies from the female breeder. As passive immunity in chickens is mediated by a single antibody class, it may have eventually caused the selection of progressively more virulent IBDV, as occurred in the mid-1980s, a risk which presently challenges the passive protection strategy. In addition, passive protection may prevent adequate vaccinal IBD virus (IBDV) infection, and therefore hinder effective protective immune response (Wyeth and Cullen, 1976; Van der Berg and Meulemans, 1991; Sharma, 1985; Wyeth and Chettle, 1990; Knoblich *et al.*, 2000; Kumar *et al.*, 2000; Tessari *et al.*, 2000; Alam *et al.*, 2002; Ahmed *et al.*, 2003; Ahmed e Akhter, 2003; Bolis *et al.*, 2003; and Hair-Bejo *et al.*, 2004). The challenge is to determine the exact early timeframe of susceptibility for inducing protection with the minimum risk of wild IBDV infection. This problem has been indirectly solved by monitoring breeder flocks for antibody levels and carefully establishing the best date for progeny vaccination with least vaccine



IBDV neutralization and higher resistance to field IBDV. The *in-ovo* vaccination enables early infection, and thus early immune response and protection against Marek's disease (Sharma, 1985; Whitfill *et al.*, 2002), in addition, of not interfering with chick hatching or livability; however, it does not induce immune response and protection against IBDV (Coletti *et al.*, 2001; Corley *et al.*, 2002).

The objective of the present study was to evaluate the effects of maternal antibodies on IBDV vaccinal infection and specific antibody response in broilers, using three conventional vaccination strategies.

MATERIALS AND METHODS

Two thousand and eight hundred (2,800) Avian Cobb broiler breeders (Rio Branco Alimentos, Brazil) were separated into two groups: one group (1,400) was vaccinated at 18 weeks of age with a trivalent oil-emulsion inactivated vaccine containing IBD-Newcastle disease and infectious bronchitis viruses (IBD-OEV) and the second group (1,400) was vaccinated with a bivalent vaccine only with Newcastle disease-infectious bronchitis viruses (non-IBD-OEV). A sufficient number of eggs (4,320) for incubation were collected when breeders were 32 weeks of age, 2,160 from each group. Breeders from both groups had received three live intermediate IBDV vaccines at 5, 10, and 15 weeks of age, prior to the oil-based vaccine. The progenies were distributed into the following experimental groups:

- A) Progeny of IBD-OEV breeders; day-old chicks given the IBDV intermediate attenuated vaccine strain subcutaneously.
- B) Progeny of non-IBD-OEV breeders given the IBDV intermediate attenuated vaccine strain via drinking water at 15 days of age.
- C) Progeny of IBD-OEV breeders given IBDV intermediate attenuated vaccine strain via drinking water at 15 days of age.
- D) Progeny of non-IBD-OEV breeders; day-old chicks given IBDV intermediate attenuated vaccine strain subcutaneously.
- E) Progeny of non-IBD-OEV breeders vaccinated *in ovo* with IBD-HVT bivalent intermediate attenuated vaccine.
- F) Progeny of IBD-OEV breeders vaccinated *in ovo* with IBD-HVT bivalent intermediate attenuated vaccine.

The experiment was designed to evaluate the

progenies of breeders with low or high antibody titers, vaccinated at the most common ages and through the most frequent routes of IBD vaccination. As to *in-ovo* vaccination, eggs from groups A, B, C and D were vaccinated at 18 days of incubation with a commercially available HVT FC-126 strain (at least 1,000 PFU/dose) and eggs from groups E and F with a commercial combination of HVT FC-126 (9,600 PFU/dose) and intermediate IBDV attenuated vaccine ($10^{3.50}$ TCID₅₀/dose) using *Inovoject*[®] equipment (Embrex, The Netherlands). Chicks from treatments A and D were subcutaneously vaccinated at hatch with an intermediate IBDV attenuated vaccine ($10^{4.1}$ TCID₅₀/dose), and those from treatments B and C received the same vaccine via drinking water at 15 days of age. Chicks vaccinated for IBDV or not were housed in separate houses at the experimental farm "Prof. Hélio Barbosa" of Universidade Federal de Minas Gerais (Igarapé, Minas Gerais, Brazil). Feed and water were provided *ad libitum*. Two different feeds were formulated: a starter feed was fed from 1 to 21 days, and a grower feed, from 22 to 40 days of age.

Serology

Passive Maternal Humoral Immunity

In order to evaluate IBDV-specific serum neutralizing (SN) antibody levels in the sera of breeders given or not the IBDV-oil based vaccine at the time of egg collection (IBD-OEV), at 32 weeks of age, blood samples were collected from the ulnar superficial vein. Thirty hatchlings from each breeder experimental group (vaccinated or not the oil-emulsion vaccine) were sampled 24 hours post-hatching for antibody levels.

Active Humoral Immunity

Active IBDV-specific SN antibody response was evaluated for each experimental group by sampling 30 chicks on days 10, 25, and 40 post-hatching. The SN assay was performed in 96-well microtest plates with 100 TCID₅₀ of an IBDV Moulthorp chicken embryo fibroblast (CEF)-adapted strain with monolayers evaluated at 72h post-infection.

Reverse-Transcriptase PCR

The vaccinal infection was verified in the cloacal bursa of embryos at day 21 of incubation or in chicks 96 hours post-vaccination using reverse-transcription polymerase chain reaction (RT-PCR), with specific primers for the VP1 gene, as previously described (Gomes *et al.*, 2005).



Live Performance

Cumulative live performance parameters (mean weight, feed conversion and livability) were determined at 7, 21 and 40 days of age for a total of 1,080 chickens from all experimental groups.

Experimental Design and Analyses

In order to evaluate performance, a completely randomized experimental design with a 2x3 factorial arrangement of 6 treatments with six replicates of 30 birds each was applied. Differences were compared using the Student-Newman-Keuls (SNK) test or Kruskal-Wallis (livability). Serological and PCR results were statistically analyzed using the same experimental design, using one bird per replicate, and means were compared using Kruskal-Wallis, Mann-Whitney or χ^2 tests.

RESULTS AND DISCUSSION

Chickens

The chicks used in this experiment were passively protected against Newcastle disease and infectious bronchitis viruses and derived from breeders free from *Mycoplasma gallisepticum*, *M. synoviae*, *Salmonella gallinarum* and *S. pullorum*. The experimental birds were raised in insect- and bird-proof houses, and did not show any sign of disease, being sent for normal processing at the end of the experiment.

Serology

Average antibody titers of breeders at time of egg collection were 11,434 for the IBD-OEV and 810 for the non-IBD-OEV and chicks 24 hours post-hatching were 16,816 for IBD-OEV and 547 for the non-IBD-OEV. At 32 weeks of age, mean IBDV-specific SN titer of breeders vaccinated with the combined oil-emulsion IBDV vaccine at the 18-week of age was much higher than the unvaccinated group ($p < 0.05$), suggesting that the progeny of the vaccinated breeders would have high titers of passive antibody at hatching. These results are consistent with previous reports Wood *et al.* (1983) and Van der Berg and Meulemans (1991). At hatching, passive IBDV-specific SN antibody titers were higher in the progeny of breeders given the IBDV-containing oil-emulsion vaccine, as shown in literature (Naqi *et al.* (1982); Knoblich *et al.* (2000). Progeny mean IBDV-specific SN antibody titers at 10, 25, and 40 days of age are shown in Table 1. At 10 days post-hatching, chicks from IBD-OEV breeders presented the highest ($p < 0.05$) IBDV-specific antibody titers. At 25 days post-

hatching, the chicks from unvaccinated breeders and vaccinated *in ovo* had higher antibody titers as compared to chicks from IBD-OEV vaccinated breeders ($p < 0.05$). These findings are in agreement with previous reports evaluating the interference of maternal antibodies on vaccinal IBDV infection (Van der Berg and Meulemans, 1991; Knoblich *et al.*, 2000; Alan *et al.*, 2002; Rautenschlein *et al.*, 2005). Indeed, Sharma (1985) found similar *in ovo* responses, with low passive antibody-titered progenies at hatching presenting low response to day-old subcutaneous vaccination and progenies with no IBDV-specific antibody titers presenting better protection to vvIBDV challenge. At 40 days of age, chicks from unvaccinated breeders responded with higher titers ($p < 0.05$), irrespective of vaccination route (*in ovo*, subcutaneous, or drinking water). The results of the progeny derived from oil-emulsion vaccine vaccinated breeders and vaccinated at hatching are consistent with those of Naqi *et al.* (1982), Knoblich *et al.* (2000), Kumar *et al.* (2000), Bolis *et al.* (2003), and Ahmed *et al.* (2003).

Table 1 - Mean IBDV-specific SN* antibody titers of broilers at 10, 25 and, 40 days of age.

Age (days)	Chick vaccination	Breeder IBD-OEV**	
		Yes	No
10	<i>In ovo</i>	784 A a	206 A b
	1st day	841 A a	111 A b
	15th day***	1,313 A a	19 A b
25	<i>In ovo</i>	60 A b	2,366 A a
	1st day	107A a	2,159 AB a
	15th day	35 A b	1,003 B a
40	<i>In ovo</i>	201 A b	10,223 A a
	1st day	432 A b	4,799 A a
	15th day	5.7 A b	6,189 A a

*Serum neutralization; **IBD-OEV: Infectious bursal disease inactivated oil-emulsion vaccine; ***Group sampled for serology prior to the vaccination at 15 days of age; Means followed by different capital letters in the same column or lower-case letter in the same row are different by the Kruskal-Wallis test ($p < 0.05$).

In-ovo vaccination titers were similar to those previously described (Coletti *et al.*, 2001). *In-ovo* vaccinated IBD-OEV breeders progenies had lower responses ($p < 0.05$) at 25 and 40 days of age as compared to unvaccinated breeders progenies, which agrees with previous findings (Coletti *et al.*, 2001), and suggests that high titers of IBDV-specific passive antibodies negatively interfere with intermediately-attenuated IBDV vaccine strain replication.

RT/PCR

The IBDV RT-PCR results determined in the progeny cloacal bursa 96 hours post-vaccination are presented



in Table 2. Unvaccinated breeders produced progenies that were more susceptible to vaccinal IBDV infection, as detected by PCR of the reversely-transcribed bursal RNA as compared to the chicks derived from breeders with high IBDV-specific antibodies. Vaccinal IBDV may have been inactivated by maternal antibodies in IBD-OEV breeder progenies vaccinated at hatching or at 15 days of age. However, in progenies with high IBDV-specific antibody titers, the *in-ovo* vaccination resulted in a higher rate of bursal infection (58%) as compared to the other vaccination routes and ages (36.6% subcutaneous at hatching and 22% via drinking water at day 15). The higher susceptibility of chicks vaccinated at 18 days of incubation day embryos to vaccine infection may be due to the fact that the passively acquired antibodies are not yet available in the embryonic circulation and are still retained in the yolk. Similar findings of vaccinal IBDV post-vaccination with live attenuated IBDV were previously reported (Coletti *et al.*, 2001; Corley *et al.*, 2002), although these authors tested SPF-breeder progenies for the absence of passive antibodies.

Live performance

Feed intake, weight gain and livability from 1- to 40-day-old chicks are presented in Tables 3, 4, and 5, respectively.

No significant differences were observed in feed, feed conversion ratio or intakelivability among the treatments. Gagig *et al.* (1999) found similar results in SPF chickens vaccinated with an intermediately-attenuated IBDV vaccine strain at 18 days of incubation, which did not affect livability at 7 days post hatching.

At 7 days post-vaccination, the weight gain of IBD-OEV breeder chickens was not higher ($p < 0.05$) than that of the other groups (Table 3). A possible explanation is that the detrimental effects of vaccinal infection may have been prevented by the higher titers of IBDV-specific passive antibodies in the OEV breeder progenies.

Vaccination at 15 days of age resulted in lower weight gain, lower feed intake, but better feed conversion ratio ($p < 0.05$), as evaluated in the period of 1 to 21 days of age (Table 4). The lower intake/feed intake may have resulted from vaccinal virus replication

Table 2 - IBDV reverse transcription PCR of bursal RNA extracts 96 hours post-vaccination *in ovo*, from 1 to 15 days of age.

Progeny Vaccination	Breeder*			
	IBD-OEV**		non-IBD-OEV***	
	Positive (expected)****	Negative	Positive (expected)****	Negative
<i>in ovo</i>	17 (22.0) A a	12	27 (22.0) A a	3
Day 1	11 (18.5) AB b	19	26 (18.5) A a	4
Day 15	6 (14.5) B b	21	23 (14.5) A a	5

*Live intermediate-IBDV vaccinations, at 5, 10 and 15 weeks of age; **IBD-OEV breeders: given IBD oil emulsion vaccine at 18 weeks of age; ***non-IBD-OEV: not given the oil emulsion vaccine; ****Number of positive birds (expected frequency); Means followed by different lower-case letters (row) or capital letters (column) are different by the χ^2 test ($p < 0.05$).

Table 3 - Live performance of broiler chickens from 1 to 7 days of age.

Variable	Chicks	Breeder*		Mean	VC**
		IBD-OEV***	non-IBD-OEV****		
Feed intake (g)	Vac <i>in ovo</i>	143	129	136	9.40
	Vac 1 st day	132	124	128	
	Vac 15 th day	130	125	127	
	Mean	135	126		
Body weight (g)	Vac <i>in ovo</i>	128	115	121 A	7.06
	Vac 1 st day	118	112	115 A	
	Vac 15 th day	117	112	117 A	
	Mean	121 a	114 b		
Feed conversion (g/g)	Vac <i>in ovo</i>	1,123	1,119	1,121	4.10
	Vac 1 st day	1,112	1,103	1,108	
	Vac 15 th day	1,103	1,074	1,088	
	Mean	1,116	1,099		
Livability (%)	Vac <i>in ovo</i>	99.33	98.88	99.11	
	Vac 1 st day	100.00	98.33	99.17	
	Vac 15 th day	99.33	98.64	98.99	
	Mean	99.55	98.62		

*Live intermediate IBDV vaccinations, at 5, 10 and 15 weeks of age; **Coefficient of variation; ***IBD-OEV breeders: given the IBD oil emulsion vaccine at 18 weeks of age; ****non-IBD-OEV: not given the oil emulsion vaccine; Means followed by different lower-case letters (row) or capital letters (column) are different ($p < 0.05$) by the SNK test or Kruskal-Wallis test (livability).



Table 4 - Live performance of broiler chickens from 1 to 21 days of age.

Variable	Chicks	Breeder*		Mean	VC**
		IBD-OEV***	non-IBD-OEV****		
Feed intake (g)	Vac <i>in ovo</i>	1,107	1,093	1,100 A	3.84
	Vac 1 st day	1,116	1,093	1,104 A	
	Vac 15 th day	1,065	1,031	1,048 B	
	Mean	1,095 a	1,072 a		
Body weight (g)	Vac <i>in ovo</i>	803	770	787 A	3.32
	Vac 1 st day	787	761	774 AB	
	Vac 15 th day	766	749	757 B	
	Mean	785 a	760 b		
Feed conversion (g/g)	Vac <i>in ovo</i>	1,379 A a	1,418 B b	1,399	1.96
	Vac 1 st day	1,418 A a	1,435 B a	1,427	
	Vac 15 th day	1,390 A a	1,364 A a	1,377	
	Mean	1,396	1,406		
Livability (%)	Vac <i>in ovo</i>	99.33	96.99	98.16	
	Vac 1 st day	100.00	98.33	99.17	
	Vac 15 th day	97.33	97.98	97.66	
	Mean	98.89	97.77		

* Live intermediate IBDV vaccinations, at 5, 10 and 15 weeks of age; **Coefficient of variation; ***IBD-OEV breeders: given the IBD oil emulsion vaccine at 18 weeks of age; ****non-IBD-OEV: not given the oil emulsion vaccine; Means followed by different lower-case letters (row) or capital letters (column) are different ($p < 0,05$) by the SNK test or Kruskal-Wallis test (livability).

in the chickens, as observed in other vaccinal infections, such as those with infectious bronchitis virus vaccine strains (Talebi *et al.*, 2005).

The *in-ovo* vaccination of the progeny from unvaccinated breeders may have impaired feed conversion ratio ($p < 0.05$). Their lower body weight as compared to the vaccinated breeder progenies may be related to a lower protection against vaccinal infection.

No significant differences in livability were observed up to 21 days of age among the experimental groups (Table 4). These results are different from those of Giambone *et al.* (2001), who observed lower livability at 21 days of age in SPF chickens as compared to commercial ones, when using *in-ovo* vaccination. SPF chickens are different from commercial chickens in

several performance aspects and the type of chicken may account for the observed differences. The experimental breeders and progenies used in the present experiment were managed and fed according to the high standards of a chicken-produce exporting company; no deviation from the regular management was observed, and the breeders continued to produce until culling at the end of the production cycle.

The results shown in Table 5 do not indicate any significant differences in body weight and livability up to day 40. However, chickens vaccinated at 15 days of age presented higher feed intake and better feed conversion ($p < 0.05$) as compared to the other treatments. At day 15, the IBD-OEV progeny has a lower rate of vaccinal infection, as detected by RT-PCR.

Table 5 - Live performance of broiler chickens from 1 to 40 days of age.

Variable	Chicks	Breeder*		Mean	VC**
		IBD-OEV***	non-IBD-OEV****		
Feed intake (g)	Vac <i>in ovo</i>	3,867	3,785	3,827 A	2.47
	Vac 1 st day	3,812	3,795	3,804 A	
	Vac 15 th day	3,706	3,690	3,698 B	
	Mean	3,795 a	3,757 a		
Body weight (g)	Vac <i>in ovo</i>	2,306	2,259	2,283	2.42
	Vac 1 st day	2,280	2,247	2,264	
	Vac 15 th day	2,244	2,247	2,246	
	Mean	2,276	2,251		
Feed conversion (g/g)	Vac <i>in ovo</i>	1,678	1,676	1,677 A	1.60
	Vac 1 st day	1,672	1,689	1,681 A	
	Vac 15 th day	1,651	1,642	1,647 B	
	Mean	1,667 a	1,669 a		
Livability (%)	Vac <i>in ovo</i>	98.00	96.66	97.33	
	Vac 1 st day	99.44	97.50	98.47	
	Vac 15 th day	97.33	97.97	97.65	
	Mean	98.26	97.98		

* Live intermediate IBDV vaccinations, at 5, 10 and 15 weeks of age; **Coefficient of variation; ***IBD-OEV breeders: given the IBD oil emulsion vaccine at 18 weeks of age; ****non-IBD-OEV: not given the oil emulsion vaccine; Means followed by different lower-case letters (row) or capital letters (column) are different ($p < 0,05$) by the SNK test or Kruskal-Wallis test (livability).



CONCLUSIONS

The vaccination of broiler breeders at puberty (18 weeks of age) with an IBD inactivated oil-emulsion vaccine, inducing high titers of circulating antibodies, may negatively affect the infection of the progeny by an intermediate attenuated vaccinal IBDV strain due to the presence of high titers of passive antibodies. Paradoxically, in more susceptible birds, such as the progeny of breeders that did not receive the oil-based IBD vaccine, the vaccinal infection by an intermediate attenuated strain may result in a significant performance loss. The vaccination with an intermediate vaccine strain *in ovo* or at hatching did not affect feed intake, feed conversion ratio or weight gain up to the 40 days of age of the progenies derived from breeders given or not the oil-emulsion vaccine. Although the induction of high levels of circulating antibodies in breeders has been a gold standard for passively protecting progenies, the results of the present experiment show that there is a negative effect on subsequent vaccine uptake by the progeny. Hence, paradoxically, when 15-day-old broilers are vaccinated with an intermediately attenuated IBDV strain, the protective titers produced by vaccinal IBDV infections in the progeny may be necessary to prevent performance losses.

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