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Bilateral Effects of Vaccination Against Infectious Bursal Disease and Newcastle Disease in Specific-Pathogen-Free Layers and Commercial Broiler Chickens

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SUMMARY. Different infectious bursal disease virus (IBDV) live vaccines (intermediate, intermediate plus) were compared for their immunosuppressive abilities in specific-pathogen-free (SPF) layer-type chickens or commercial broilers. The Newcastle disease virus (NDV) vaccination model was applied to determine not only IBDV-induced immunosuppression but also bilateral effects between IBDV and NDV. None of the IBDV vaccines abrogated NDV vaccine–induced protection. All NDV-vaccinated SPF layers and broilers were protected against NDV challenge independent of circulating NDV antibody levels. Sustained suppression of NDV antibody development was observed in SPF layers, which had received the intermediate plus IBDV vaccine. We observed a temporary suppression of NDV antibody development in broilers vaccinated with one of the intermediate, as well as the intermediate plus, IBDV vaccines. Different genetic backgrounds, ages, and residual maternal antibodies might have influenced the pathogenesis of IBDV in the different types of chickens. Temporary suppression of NDV antibody response in broilers was only seen if the NDV vaccine was administered before and not, as it was speculated previously, at the time the peak of IBDV-induced bursa lesions was detected. For the first time, we have demonstrated that the NDV vaccine had an interfering effect with the pathogenesis of the intermediate as well as the intermediate plus IBDV vaccine. NDV vaccination enhanced the incidence of IBDV bursa lesions and IBDV antibody development. This observation indicates that this bilateral effect of an IBDV and NDV vaccination should be considered in the field and could have consequences for the performance of broiler flocks.

RESUMEN. Efectos bilaterales de la vacunación contra la enfermedad infecciosa de la bolsa y la enfermedad de Newcastle en ponedoras libres de patógenos específicos y en pollos de engorde comerciales.

Diversas vacunas vivas (intermedias, intermedias plus) del virus de la enfermedad infecciosa de la bolsa (Gumboro) fueron comparadas por sus capacidades inmunosupresoras en ponedoras libres de patógenos específicos y pollos de engorde comerciales. El modelo de vacunación del virus de la enfermedad de Newcastle fue aplicado para determinar no solo la inmunosupresión inducida por el virus de la enfermedad infecciosa de la bolsa, sino también los efectos bilaterales entre este virus y el virus de Newcastle. Ninguna de las vacunas contra Gumboro deterioró la protección inducida por el virus vacunal de Newcastle. Todas las ponedoras comerciales libres de patógenos y los pollos de engorde vacunados contra Newcastle fueron protegidos contra el desafío de Newcastle, independientemente de los niveles de anticuerpos circulantes contra Newcastle. Se observó una supresión sostenida del desarrollo de anticuerpos para Newcastle en las ponedoras libres de patógenos específicos que habían recibido la vacuna intermedia plus contra Gumboro. Observamos una supresión temporal del desarrollo de anticuerpos contra Newcastle en pollos de engorde vacunados con una vacuna intermedia, al igual que en los inmunizados con una vacuna contra Gumboro intermedia plus. Diversos antecedentes genéticos, así como la edad, y los anticuerpos maternales residuales, podrían haber influido sobre la patogenesis del virus de Gumboro en los diferentes tipos de aves. La supresión temporal de la respuesta de anticuerpos contra Newcastle en pollos de engorde fue observada únicamente cuando la vacuna contra Newcastle fue administrada antes, y no simultáneamente con la detección del máximo de producción de lesiones inducidas en la bolsa, como se había especulado anteriormente. Se demostró por primera vez, que la vacuna contra Newcastle tuvo un efecto de interferencia en la patogenesis de las vacunas intermedias e intermedia plus contra Gumboro. La vacunación contra Newcastle aumentó la incidencia de lesiones en la bolsa y el desarrollo de anticuerpos contra Gumboro. Estas observaciones indican que el efecto bilateral de una vacunación contra Gumboro y Newcastle debe ser tenido en cuenta a nivel de campo y puede tener consecuencias sobre el desempeþo productivo de lotes de pollo de engorde.

Key words: infectious bursal disease, vaccination, immunosuppression, broilers

Abbreviations: EID = egg infectious dose; ELD = embryo lethal dose; ELISA = enzyme-linked immunosorbent assay; HA = hemagglutination; HI = hemagglutination inhibition; IBD = infectious bursal disease; IBDV = infectious bursal disease virus; mAbs = maternally derived antibodies; NDV = Newcastle disease virus; post-NDVvac = post–NDV vaccination; SPF = specific-pathogen-free; TCID = tissue culture infectious dose; vNDV = NDV challenge virus

Infectious bursal disease virus (IBDV) induces an immunosuppressive disease (IBD) in susceptible chickens that is often complicated by secondary infections (22). Despite widely used vaccination programs, IBD is one of the major economically important diseases, especially in broiler production (23). The main target cells for IBDV are B cells (17), but macrophages are also susceptible (18). Because of the effect of IBDV on humoral immunity and macrophage activity, immunosuppression can occur (11,18,20,33,34). Immunosuppressed flocks perform poorly and show reduced economic return. The primary antibody responses are impaired, resulting in poor response of IBDV-infected birds to vaccination such as with Newcastle disease virus (NDV). Depending on the virulence of the IBDV strain and the age of the infected bird, the destruction of the bursa of Fabricius can be permanent or temporary, and then bursa architecture and immunity may recover after IBDV infection (19).

IBDV vaccines are grouped on the basis of their residual virulence into mild, intermediate, and intermediate plus or hot strains (26). For licensing, IBDV vaccines are evaluated for their immunosuppressive

abilities using a Newcastle disease vaccination model (6,10,11,13,24). To determine immunosuppressive abilities of IBDV live vaccines, the standard protocols indicate that birds are vaccinated with the IBDV vaccine of interest and will be vaccinated with a lentogenic NDV strain at the time the most severe vaccine-related bursa lesions are expected. The timing of the most severe IBDV-induced immunosuppression is supposed to correlate with the finding of the most severe bursa lesions. NDV antibody response and protection against NDV will be evaluated at 14 days post–NDV vaccination (post-NDVvac). Specific-pathogen-free (SPF) layer-type chickens are used for these studies because they are known to be the most susceptible birds for IBD.

Field observations and some experimental studies indicate that IBDV pathogenesis can vary depending on the genetic background of the chicken (4,5,9,16,27,31,38). Furthermore, it is known that maternally derived antibodies (mAbs) can interfere with IBDV pathogenesis (2,25,31,38,39). Although IBD is of significant economic importance for the broiler industry, not many studies have been done to evaluate the efficacy and immunosuppressive abilities of IBDV live vaccines in broiler-type chickens (14,15), especially in commercial broilers with residual mAbs (2). In this study, we compared the immunosuppressive abilities of different IBDV vaccines in SPF layer-type chickens and also in following experiments in commercial broilers. We investigated the effect of IBDV vaccination on NDV vaccination, the development of NDV antibodies, and the protection against NDV challenge. In one experiment, NDV vaccination was given at different time points post–IBDV vaccination, and the effect of IBDV on NDV antibody development was correlated with the incidence of IBDV-induced bursa lesions. Furthermore, the IBDV vaccine response, such as IBDV antibody development and recovery from bursa lesions, was investigated in NDV-vaccinated and NDV-free broilers to determine eventual bilateral effects of NDV on IBDV pathogenesis.

MATERIALS AND METHODS

Chickens. SPF layer-type chickens (LSL-LITE; Lohmann Tierzucht GmbH, Cuxhaven, Germany) and commercial broilers (Ross-Type; Hatchery Weser Ems GmbH & Co., Visbek-Rechterfeld, Germany) were raised in isolation units of the Clinic for Poultry, University of Veterinary Medicine, Hannover, Germany, following animal welfare guidelines. The broiler chicks were obtained from different parent flocks of the same company, and the parents had been vaccinated once with IBDV live vaccine. Feed and water were provided *ad libitum*.

Viruses. Four commercially available vaccines were used: 3 intermediate strains (IBDV I, II, III) and 1 intermediate plus vaccine (IBDV P). Chickens were vaccinated individually by oral route following the instructions of the manufacturers. One dose was as follows: IBDV I, $10^{4.1}$ mean tissue culture infectious dose ($TCID_{50}$); IBDV II, $10^{4.8}$ $TCID_{50}$; IBDV III, $10^{5.9}$ $TCID_{50}$; and IBDV P, 2 $\log_{10}$ mean egg infectious dose (EID_{50}).

A commercially available lentogenic strain (VG/GA) was used for NDV vaccination by eyedrop route following the instructions of the manufacturer. One dose referred to 5.5 $\log_{10}$ EID_{50}. The velogenic viscerotropic strain NDV Herts 33 was used as the NDV challenge virus (vNDV). vNDV had been propagated and titrated in embryonated SPF chicken eggs following standard procedures (3). Chickens received a 10^5 mean embryo lethal dose (ELD_{50}) intramusculary as indicated by standard protocols for the evaluation of IBDV vaccines for their immunosuppressive abilities.

Serology. IgG-type IBDV and NDV antibodies were detected in collected serum samples with a commercially available enzyme-linked immunosorbent assays (ELISA) kit (ProFLOK® IBD and ND ProFLOK® plus; Synbiotics Corporation, Lyon, France), as described by the manufacturer. The hemagglutination inhibition (HI) test for the detection of NDV antibodies was performed following standard procedures (37). NDV antibody titers <8 were evaluated as negative.

Histology. Bursae of Fabricius were fixed in 10% phosphate-buffered formalin, paraffin embedded, cut, and strained with hematoxylin and eosin. Bursa lesion scores were determined microscopically and compared between groups (19,33). The scoring system was as follows: 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, 4 = 76–100% of bursal follicles showing cellular depletion of more than 70%.

Immunohistochemical detection of IBDV antigen. IBDV antigen was detected in the bursa of Fabricius following previously published procedures (19,35). A polyclonal rabbit anti-IBDV serum, which was prepared against an intermediate strain of IBDV (IBDV Bursine 2) was used for the detection of IBDV antigen (36). The group means of the numbers of IBDV-infected cells per field were determined after counting 10 microscopic fields per tissue for each bird at a magnification of 400×.

Reisolation of vNDV. Tracheal swabs were taken from sick and dead animals or at 7 and 10 days post–vNDV challenge. These swabs were investigated for NDV isolation in embryonated SPF chicken eggs following standard procedures (3). The allantoic fluids of inoculated eggs were investigated for NDV in the hemagglutination (HA) test and the HI test with antisera against NDV (3).

Experimental protocol. *Experiment 1.* One-day-old SPF layer-type chickens were randomly distributed into five groups ($n = 30$). At 7 days posthatch, birds of four groups were inoculated orally with different IBDV live vaccines (IBDV I, IBDV II, IBDV III, IBDV P). One group of birds was kept as a virus-free control group. At 14 days posthatch, birds of each group were further distributed into three subgroups ($n = 10$), and two of these subgroups received the NDV live vaccine by eyedrop route. At 10, 14, and 21 days post-NDVvac, serum samples from four to eight birds per group were tested for NDV antibodies, and at 21 days post–IBDV vaccination, they were tested for IBDV antibodies. Fourteen days post-NDVvac, one NDV-vaccinated and one NDV-free subgroup were challenged intramusculary with NDV Herts 33. Morbidity and mortality rates were determined up to 14 days postchallenge. The experiment was finished at 14 days post–NDV challenge, and final serum NDV antibody levels were measured.

Experiment 2. One-day-old broilers were randomly distributed into three groups ($n = 45$–60). Fifteen serum samples were collected at different days before (data not shown) and the day of IBDV vaccination (day 18 posthatch) to determine the decline of IBDV mAbs. At 18 days posthatch, birds were inoculated orally with the different IBDV live vaccines (IBDV I, IBDV P). One group of birds remained noninoculated as a virus-free control. At 23 days posthatch, each vaccinated group and the virus-free control group were further divided into three or four subgroups ($n = 10$–15), respectively. Two subgroups of each group were inoculated with the NDV live vaccine by eyedrop route. At 23, 30, and 37 days posthatch, serum samples of from eight to 15 birds per group were tested for IBDV and NDV antibodies. Fourteen days post-NDVvac, one NDV-vaccinated and one NDV-free subgroup were challenged with NDV Herts 33. Morbidity and mortality rates were determined. At 7 and 10 days post–NDV challenge, tracheal swabs were examined for reisolation of NDV. At 10 days post–NDV challenge, birds were necropsied, and bursa samples were collected for histologic examination.

Experiment 3. One-day-old broilers were distributed randomly into three groups ($n = 39$–54). Fifteen serum samples were collected at different days before (data not shown) and on the day of IBDV vaccination (day 17 posthatch) to determine the decrease of maternally derived IBDV antibodies. At 17 days posthatch, two groups received IBDV vaccines orally (IBDV I, IBDV P). One group remained virus-free. At 5, 7, 14, and 20 days post–IBDV vaccination, three to five birds per group were necropsied, and bursa samples were investigated for histologic lesions and IBDV antigen detection. At 5, 7, and 14 days post–IBDV vaccination, five to seven birds per group were separated from the main groups, placed in different isolators, and inoculated with the NDV live vaccine by eyedrop route. Five to seven serum samples per group were collected at 5, 7, 14, 20, and 28 days post–IBDV vaccination and investigated for NDV and IBDV antibodies by ELISA. On day

Table 1. Induction of NDV ELISA antibodies post-NDVvac of IBDV-inoculated SPF layer-type chickens (Experiment 1).

Group	Median $\log_{10}$ NDV ELISA antibody titers on days post-NDVvac (no. of NDV antibody–positive birds/group)[A]			
	10	14	21	28
IBDV-free	n.d.	3.02 ab (4/5)	3.04 a (4/5)	n.d.
IBDV I	2.56 (3/5)	0.00 ab (2/5)	2.56 a (4/5)	2.60 a (4/7)
IBDV II	2.69 (3/5)	2.79 a (5/5)	3.08 b (8/8)	3.47 b (8/8)
IBDV III	2.80 (4/5)	2.81 ab (3/5)	3.29 ab (5/7)	3.52 b (6/7)
IBDV P	2.69 (3/5)	0.00 b (0/5)	0.00 c (0/5)	0.00 c (0/4)

[A]Non–NDV-vaccinated chickens did not have detectable NDV ELISA antibody levels. Values in a column followed by different lowercase letters differ significantly by the Kruskal–Wallis test ($P < 0.05$.). n.d. = no data available.

28 post–IBDV vaccination, the remaining birds were necropsied, pathologic and histopathologic lesions were determined, and intrabursal IBDV antigen was detected.

Statistical analysis. Group responses within experiments were analyzed by Kruskal–Wallis one-way analysis of variance on ranks and pairwise multiple comparison procedure by the Dunn method, Student's *t*-test, or median test as indicated in the table and figure legends.

RESULTS

Induction of NDV antibodies. In Experiment 1, IBDV-vaccinated SPF chickens were inoculated with the NDV live vaccine at 7 days post–IBDV inoculation. SPF chickens vaccinated with IBDV I and IBDV P showed a temporary and permanent suppression, respectively, of NDV ELISA antibody production, whereas the inoculation with IBDV II and IBDV III did not affect NDV antibody production (Table 1). IBDV P–induced NDV antibody suppression was confirmed in the HI test. None of the IBDV P–inoculated birds developed HI NDV antibodies during the experiment, whereas all the other IBDV-vaccinated and IBDV-free birds developed NDV antibodies of similar HI titers, varying between $\log_2$ 5 and $\log_2$ 6 at 28 days post-NDVvac. In Experiment 2, all broilers had received NDV vaccination at 5 days post–IBDV inoculation. No significant differences in NDV antibody titers were observed between IBDV-vaccinated and IBDV-free birds at 7, 14, and 24 days post-NDVvac, either with the ELISA or with the HI test (data not shown).

In Experiment 3, significant differences in NDV antibody production were observed between IBDV-vaccinated and IBDV-free broilers depending on the day of NDV vaccination in relation to the IBDV inoculation ($P < 0.05$; Table 2). Birds inoculated with IBDV P showed a temporary suppression in HI (data not shown) and ELISA NDV antibody production (Table 2) at 9 days post-NDVvac when the NDV vaccine was given at 5 days post–IBDV P inoculation. When IBDV I–inoculated broilers were given NDV vaccine at 7 days post–IBDV inoculation, the HI (data not shown) and ELISA NDV antibody levels were significantly suppressed (Table 2) at 13 days post-NDVvac ($P < 0.05$). For both groups, the suppression was transient. Seven days later, antibodies did not differ between the groups that had been inoculated with the NDV vaccine the same day with or without IBDV vaccination.

Induction of protection against NDV challenge. NDV vaccine protection was not affected by any of the IBDV vaccines in either SPF layer-type birds or broilers. All NDV-vaccinated birds independent of NDV antibody levels were protected against NDV challenge. None of the NDV-vaccinated birds showed clinical signs. As tested in Experiment 2, no NDV was detected in tracheal swabs taken at 7 or 10 days post–NDV challenge of NDV-vaccinated broilers (data not shown). SPF layer-type chickens that were not vaccinated against NDV died 48–72 hr after NDV challenge. They showed pathologic lesions such as mottled spleens, swollen livers, and hemorrhages in muscle, pancreas, and proventriculus and necrotic foci (boutons) in the intestines. Non–NDV-vaccinated broilers died or were sacrificed because of severe clinical disease within 96 hr post–NDV challenge with similar pathologic lesions as seen in NDV-challenged SPF layer-type birds. NDV was isolated from tracheal swabs of all challenged nonvaccinated broilers (data not shown).

Induction of IBDV antibodies in SPF layer-type birds and broilers. In Experiment 2 at 18 days posthatch, which was the day of IBDV vaccination, one of 15 tested broilers had

Table 2. Induction of NDV ELISA antibodies in IBDV-vaccinated broilers that received NDV vaccinations at different time points post–IBDV inoculation (Experiment 3).

Group	No. of days post–IBDV inoculation of NDV vaccination	Average $\log_{10}$ NDV ELISA antibody level on days post-NDVvac[A]		
		9	13 or 15[B]	14, 21, or 23[B]
IBDV-free	5	3.11 ± 0.24 a	2.70 ± 1.35 a	3.53 ± 0.38
IBDV I	5	2.87 ± 0.22 a	3.11 ± 0.34 a	3.46 ± 0.28
IBDV P	5	2.46 ± 1.10 b	2.64 ± 1.20 a	3.42 ± 0.37
IBDV-free	7	NA	3.21 ± 0.32 a	3.33 ± 0.16
IBDV I	7	NA	2.16 ± 1.49 b	3.42 ± 0.24
IBDV P	7	NA	3.18 ± 0.13 a	3.39 ± 0.20
IBDV-free	14	NA	NA	3.49 ± 0.25
IBDV I	14	NA	NA	3.29 ± 0.42
IBDV P	14	NA	NA	3.27 ± 0.36

[A]Non–NDV-vaccinated chickens did not have detectable NDV antibody levels. IBDV-free birds and IBDV-vaccinated groups in the same column followed by different lowercase letters differ significantly by the Student's *t*-test ($P < 0.05$.). NA = not applicable; $n = 5$–7.

[B]The number of days post-NDVvac differs depending on the time of NDV vaccination, which varied between 5, 7, and 14 days post–IBDV inoculation.

Table 3. Induction of IBDV ELISA antibodies in IBDV- and NDV-vaccinated broilers (Experiment 3).

Group	No. of days post–IBDV inoculation of NDV vaccination	IBDV ELISA antibody level (log_{10}) at 28 days post–IBDV vaccination (group average ± SD)[A]
IBDV I	NDV-negative	2.39 ± 1.64 a
	5	3.46 ± 0.17 b
	7	3.59 ± 0.15 b
	14	3.56 ± 0.18 b
IBDV P	NDV-negative	3.68 ± 0.09 a
	5	3.67 ± 0.14 a
	7	3.76 ± 0.07 b
	14	3.74 ± 0.12 a

[A]No IBDV antibodies were detectable in birds that did not receive an IBDV vaccination. Different lowercase letters within a column indicate significant differences between IBDV-vaccinated birds with and without the NDV vaccination by the Student's *t*-test ($P < 0.05$).

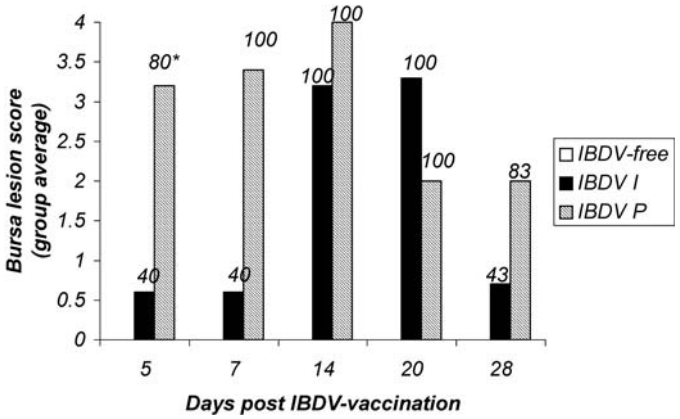

Fig. 1. At 5, 7, 14, 20, and 28 days post–IBDV inoculation, histologic bursa lesion scores were determined ($n = 3$–7). *Percentage of birds per group with bursa lesion scores ≥1.

a maternally derived IBDV antibody titer of 788 in the ELISA. This antibody titer was below the estimated cutoff level in this ELISA system for both vaccines. In Experiment 3, 27% of the tested broilers had remaining average mAb titers of 850, which was below the estimated cutoff titer in this ELISA system for the vaccines. All IBDV-vaccinated groups showed seroconversion, whereas non–IBDV-vaccinated birds did not have detectable IBDV ELISA antibody levels. IBDV-vaccinated SPF layer-type birds developed IBDV ELISA antibodies ranging from an average log_{10} 2.73 to log 3.64 at 21 days post–IBDV vaccination. When IBDV II–vaccinated SPF layer-type birds were vaccinated against NDV, the anti-IBDV antibodies were significantly higher, at log_{10} 3.64, than in the non–NDV-vaccinated birds at log_{10} 3.54 ($P < 0.02$; data not shown). Similar observations were made in Experiments 2 and 3. In Experiment 2, 80% and 87% of IBDV P– and IBDV I–vaccinated birds had developed detectable IBDV ELISA antibodies at 12 days or 19 days post–IBDV inoculation, respectively, post-NDVvac. At these same time points, only 40% and 7% of the IBDV P– and IBDV I–vaccinated birds, respectively, that did not receive the NDV vaccine had developed IBDV antibody titers ($P < 0.05$; data not shown). In Experiment 3, birds vaccinated with NDV at 7 days post–IBDV P inoculation and at 5, 7, and 14 days post–IBDV I inoculation developed significantly higher IBDV antibody levels at 28 days post–IBDV inoculation than birds without NDV vaccination (Table 3; $P < 0.05$).

Induction of bursa lesions and intrabursal IBDV antigen detection. In Experiment 3, the time of bursa lesion induction was compared in broilers only vaccinated with IBDV I or IBDV P (Fig. 1). The severest lesions were seen after vaccination with IBDV I at 20 days and with IBDV P at 14 days post–IBDV inoculation. These bursa lesions might be exacerbated by NDV vaccination, as seen in Experiments 2 and 3. In Experiment 2, birds vaccinated with IBDV I did not show any bursa lesions at 33 days post–IBDV inoculation (last day of the experiment), whereas 100% of the birds that were IBDV I–and NDV-vaccinated showed bursa lesion scores of 3–4 (data not shown). In Experiment 2, no differences were seen between NDV-vaccinated and non–NDV-vaccinated IBDV P–inoculated birds. Remaining bursa lesions were seen in 40%–60% of these birds, with an average score of 3 (data not shown). In Experiment 3, when IBDV I–and IBDV P–inoculated birds had received the NDV vaccine at 7 or 14 days or 7 and 14 days post–IBDV inoculation, bursa lesion scores were significantly higher than in non–NDV-inoculated birds ($P < 0.05$; Fig. 2).

In Experiment 3, IBDV antigen was detected in bursa sections by immunohistochemistry (Table 4). In IBDV P–vaccinated birds, IBDV antigen was detected in 100% of the investigated birds between 7 and 14 days post–IBDV inoculation. IBDV I–vaccinated birds showed IBDV antigen in their bursa in two of five and three of five birds at 7 and 14 days postinoculation, respectively. At 20 days postvaccination, only one of five vaccinated birds in each group showed IBDV antigen in the bursa, and at 28 days, no IBDV antigen was detectable in bursae of IBDV-vaccinated NDV-free birds by this method. Six of 18 IBDV I–vaccinated + NDV-vaccinated birds and one of 21 IBDV P–vaccinated + NDV-vaccinated birds still had detectable IBDV antigen in their bursae at 28 days post–IBDV vaccination.

DISCUSSION

On the basis of official guidelines for the licensing of IBDV live vaccines, most IBDV vaccines are tested for their immunosuppressive effects in SPF layer-type chickens, and many experimental studies are also done in SPF layer-type chickens (12,13). Previous studies and field observations indicated that IBDV pathogenesis can differ between chicken lines with different genetic backgrounds and the influence of residual mAbs (1,5,25,29,31). Because economic losses from IBD play an important role for broiler production, broiler breeders are vaccinated with live and inactivated IBD vaccines to assure the transfer of mAbs to their progeny, and broiler flocks in many countries are vaccinated against IBD at least once during their growing period. However, not much is known so far about the immunosuppressive abilities of IBD vaccines in commercial broilers (7,28,32). In this study, we compared the immunosuppressive abilities of different commercially available IBDV vaccines in commercial broilers and in SPF white Leghorn chickens. Our study demonstrated that the immunosuppressive abilities of different IBDV vaccines differed in SPF layer-type chickens and broilers. Although the intermediate plus IBDV vaccine induced a permanent suppression of NDV antibody development post-NDVvac of SPF layer-type chickens, only a temporary suppression was observed in broilers.

Previously, it was assumed that the peak of IBDV-induced immunosuppression correlated with the incidence of the severest bursa lesions. In this study, it was demonstrated for the first time that the suppression of NDV antibody development observed in broilers did not correlate in time with the most severe bursa lesions. The NDV vaccine had to be inoculated before the most severe

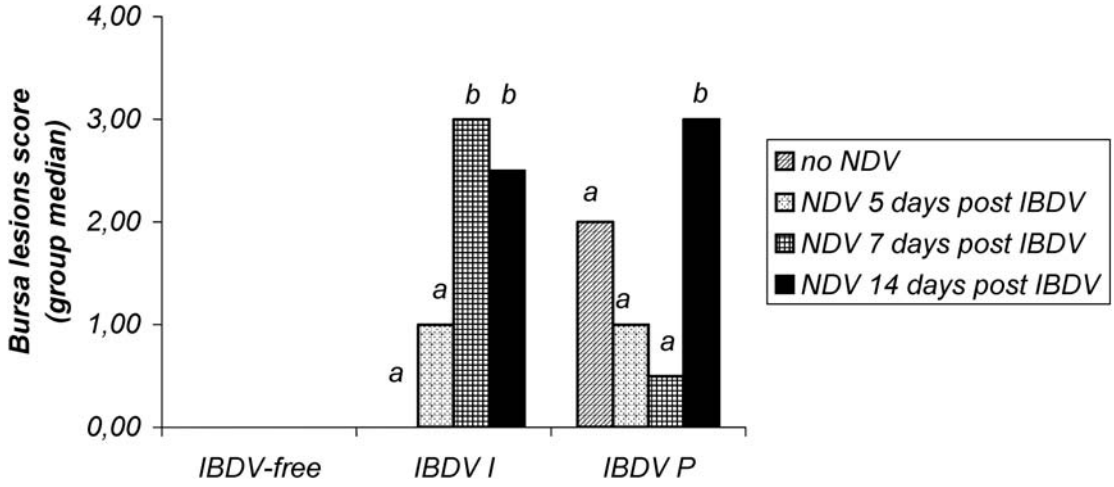

Fig. 2. Influence of NDV vaccination on IBDV lesion development in the bursa of Fabricius. At 5, 7, or 14 days post–IBDV vaccination, broilers were vaccinated with a lentogenic NDV vaccine. Bursa lesions were determined at 28 days post–IBDV vaccination. Different superscript letters indicate significant differences between NDV-free and NDV-vaccinated groups inoculated with the same IBDV vaccine (median test $P < 0.05$).

bursa lesions occurred to observe suppression in NDV antibody development compared with non–IBDV-inoculated birds. This new observation leads to the speculation that the destruction of the bursal B cells might not be the only factor interfering with NDV antibody development. Other studies indicated that IBDV can also affect other branches of the immune system, such as systemic T cell activity (20). During the early phase of IBDV infection, macrophage activation can induce the release of cytokines and nitric oxide (18), which can suppress T cell activity (20). Suppression of T helper cell activity might contribute to the reduced NDV antibody response in IBDV-inoculated chickens.

It is not clear whether differences in immunosuppression regarding NDV antibody development between SPF layers and broilers were due to differences in genetic backgrounds, age of the chicken at the time of IBDV infection, or the presence of residual mAbs in broilers (5,24,31,38), but certainly, IBDV vaccination was applied when broiler chicks had residual maternal IBDV antibody levels below the breakthrough titer of the IBDV vaccines (8). More than 73% of the birds had no detectable IBDV ELISA antibodies at the time of IBDV vaccination. The remaining 27% of broilers had antibodies below the breakthrough titer.

Although, IBDV P and IBDV I had affected the NDV antibody production permanently and temporarily in SPF layer-type birds and broilers, respectively, all vaccinated birds were protected against NDV challenge by the intramuscular route. The protection rate might have been different if a more natural route, such as eyedrop administration, would have been used. In this study, all non–NDV-vaccinated birds showed high morbidity and mortality rates after NDV challenge. No NDV was detected in tracheal swabs of NDV-vaccinated birds at 7 and 10 days post–NDV challenge. This observation demonstrates that cell-mediated immunity could contribute to NDV protection. Lambrecht *et al.* (21) demonstrated that cell-mediated immunity against NDV might be activated by vaccination. Possibly, at the time of challenge, the T cell–mediated immunity was sufficiently active while the B cell–mediated immunity was below detectable levels in the case of the IBDV P–vaccinated SPF layer-type birds.

Interestingly, our study also demonstrated that not only IBDV vaccination can influence the outcome of the NDV vaccination, but NDV also influenced IBDV pathogenesis. It was demonstrated that NDV can exacerbate or prolong IBDV lesions if it is inoculated during the acute phase of IBDV infection. In some groups, NDV inoculation increased IBDV-induced bursa lesions and enhanced IBDV antibody production. The time between IBDV and the subsequent NDV vaccination and the virulence of the IBDV strain could be critical for the detection of the possible interference of the two vaccines. Our data from Experiments 2 and 3 indicate that the effect of NDV on IBDV might be more pronounced in the IBDV I–inoculated group. Exacerbation of the bursa lesions in the IBDV I–vaccinated group was observed in all the NDV-vaccinated birds compared to only with IBDV I–inoculated birds, whereas enhancement of bursa lesions in the IBDV P–inoculated birds was only observed if the NDV vaccine was given at 14 days post–IBDV inoculation. The mechanisms of NDV-induced exacerbation of IBDV lesions are not known. We can speculate that NDV might affect the immune system in a critical phase of IBDV pathogenesis, which interferes with IBDV clearance and restoration of the bursa of Fabricius. Similar observations have been made with other infectious agents in which viral infections interfered with the immune responses generated against coinfections, which contributed to the delayed clearance of the organism (30). The higher incidence of IBDV antigen in IBDV I–inoculated birds at 27 days post–IBDV vaccination might support this hypothesis. These findings are important for the evaluation of field situations. In a chicken flock,

Table 4. IBDV antigen detection after IBDV and NDV vaccination of broilers (Experiment 3).

		No. of birds/group with detectable IBDV antigen in the bursa of Fabricius at days post–IBDV vaccination[A]				
Group	NDV-vaccinated	5	7	14	20	28
Virus-free	−	0/5	0/3	0/5	0/3	0/7
	+	ND	ND	ND	ND	0/16
IBDV I	−	2/5	2/5	3/5	1/5	0/7
	+	ND	ND	ND	ND	6/18
IBDV P	−	3/5	5/5	5/5	1/5	0/6
	+	ND	ND	ND	ND	1/21

[A]IBDV antigen was detected in bursa sections of IBDV-vaccinated and IBDV- + NDV-vaccinated birds by immunohistochemistry. ND = not done.

severe bursa lesions might be observed for a longer time period than would be expected after an IBDV vaccination. This study indicates that other vaccine viruses, such as NDV, can affect the outcome of an IBDV vaccination and enhance or prolong bursa lesions. Overall, this study shows that IBDV-induced immunosuppression is difficult to determine in broilers. Its effect on NDV antibody production might only be transient, and the chance of detecting immunosuppression could depend on the timing of NDV vaccination in relation to IBDV inoculation. The ''right'' timing for NDV vaccination seems not to correlate with the severity of bursa lesions. NDV vaccination might have to take place before the peak of IBDV lesions occur to detect IBDV-induced immunosuppression. The bilateral effect between two vaccines such as NDV and IBDV should always be considered, even if the vaccines are administered more than 7 days apart.

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