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PAPER

Protection conferred by a live avian metapneumovirus vaccine when co-administered with live La Sota Newcastle disease vaccine in chicks

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Abstract

This paper examines the effects on specific pathogen-free (SPF) chicks when avian metapneumovirus (aMPV) and Newcastle disease virus (NDV) La Sota strain vaccines are co-administered. Day-old SPF chicks were divided into five groups. The first group was inoculated with sterile water (SW) and the rest of the groups were inoculated with live NDV vaccine VG/GA by the oculo-oral route. At 21 days-old, the unvaccinated chicks were again inoculated with SW. The four VG/GA-vaccinated groups were further inoculated with (i) SW, (ii) live aMPV vaccine, (iii) live NDV La Sota, or (iv) combined live NDV La Sota and live aMPV, respectively. Chicks were monitored for post-vaccination reactions and oropharyngeal swabs were collected for vaccines detection. Blood samples were collected to detect aMPV ELISA and NDV haemagglutination-inhibition antibodies. Twenty-one days following the second vaccination, six chicks from each group were challenged with virulent NDV or aMPV respectively. Chicks were monitored for clinical signs and mortality and oropharyngeal swabs collected for aMPV detection. Results showed that, when challenged with a virulent aMPV, both chicks previously vaccinated with VG/GA and subsequently given aMPV vaccine singly or in combination with La Sota were equally protected against clinical signs. Chicks that were vaccinated against NDV either once with VG/GA or followed by La Sota (singly or in combination with aMPV) were fully protected when challenged with velogenic NDV. We concluded that simultaneous administration of live aMPV and NDV La Sota vaccines have no adverse effects on protection conferred by either live vaccine.

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Introduction

In the last two decades, avian metapneumovirus (aMPV) infection has become an important component of respiratory disease in chickens. It is associated with swollen head syndrome which also causes drop in egg quality and production (Cook, 2000; Cook and Cavanagh, 2002; Sugiyama *et al.*, 2006; Alexander and Jones, 2008; Cecchinato *et al.*, 2011). Newcastle disease (ND), which is caused by paramyxovirus serotype 1 (APMV-1), remains as one of the most economically important poultry diseases worldwide (Alexander and Jones, 2008). For control and prevention of ND, live and inactivated vaccines are available for more than half a century (Alexander and Jones, 2008). In contrast, live and inactivated vaccines for aMPV control and prevention were only available in the last two decades and restricted to certain countries (Cook, 2000; Cook and Cavanagh, 2002; Alexander and Jones, 2008). It was reported that when live aMPV and ND virus (NDV) vaccines were given in combination in day-old chicks, both singly and dually vaccinated chicks were equally protected against aMPV challenge (Ganapathy *et al.*, 2005, 2007). In addition, protection against virulent NDV was not affected in single and dual vaccination. It has also been demonstrated that live aMPV and NDV can be safely administered to broiler chicks with NDV maternal antibodies (Ganapathy *et al.*, 2006). Working with another important respiratory pathogen, Cook *et al.* (2001) found that when aMPV and infectious bronchitis virus (IBV) vaccines were co-administered, the latter virus inhibited the replication of aMPV vaccine virus, resulting in reduced humoral antibody response to aMPV vaccine, though protection against aMPV or IBV challenges were not affected. There have not been reports on co-administration of aMPV and NDV La Sota vaccines, where the latter vaccine is widely used in ND endemic countries, mostly after priming with another lentogenic ND vaccine. This paper reports on the protection conferred by live aMPV or NDV La Sota vaccines applied singly or dually in chicks already primed with a live NDV VG/GA vaccine at day-old.

Materials and methods

Chickens

Eighty white Leghorn day-old specific pathogen-free (SPF) chicks (Lohmann Animal Health Gmbh & Co., Cuxhaven, Germany) were randomly allocated into five groups of sixteen birds each and were placed in separate isolators in an experimental house. Food and water were provided *ad libitum*.

Viruses

Live NDV VG/GA (AVINEW®), NDV La Sota (BIO LA SOTA) and aMPV subtype B (NEMOVAC®) vaccines were provided by Merial SAS (Lyon, France). On the other hand, as for aMPV challenge, a virulent subtype B aMPV (Ganapathy *et al.*, 2007) was carried out as previously described. This virus was propagated and titrated in tracheal organ cultures (TOCs) (Cook *et al.*, 1976). For NDV challenge, the velogenic APMV-1 chicken/Italy/3015/00 (intra cerebral pathogenicity index of 1.8) was used. This virus was kindly provided by OIE:FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza, Legnaro, Italy. The virus was grown and titrated in SPF embryonated eggs.

Vaccine preparation

One vial each of VG/GA, La Sota or aMPV vaccines was thoroughly mixed with 100 mL of

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sterile water (SW). For dual-vaccination, aMPV followed by NDV La Sota were both mixed in 100 mL of SW. Immediately after preparation, SW and the vaccines were placed in a cold box containing crushed ice. Each chick received 50 µL of SW or the appropriate vaccine ocularly and 50 µL orally.

Experimental design

Day-old chicks were randomly divided into five groups as shown in Table 1. One group was inoculated with 0.1 mL of SW and the other groups were inoculated with VG/GA. At 21 days of age, the group which received SW was inoculated with 0.1 mL of SW (Group: SW:SW). The four VG/GA-inoculated groups were further inoculated as follows: i) the first was sham-inoculated with SW (Group: VG/GA:SW); ii) the second group received aMPV (Group: VG/GA:aMPV); iii) the third group received NDV La Sota (Group: VG/GA:LASOTA); and iv) the forth group (Group: VG/GA:aMPV+LASOTA) was inoculated with both aMPV and NDV La Sota vaccines simultaneously. Dosages received by each bird were in accordance with the manufacturer's recommendations (Table 1). Three weeks after the second vaccination, six birds from each group were transferred into separate isolators and challenged with virulent aMPV. Each bird received 0.1 mL of 4.0 log_{10} CD_{50} of a virulent aMPV subtype B challenge virus via the ocular route. At the same time and in a similar manner, another six birds from each group were transferred into new isolators. These chicks were challenged by intraocular inoculation of 0.2 mL of 5.0 log_{10} EID_{50} velogenic Italian NDV isolate. Four birds per group remained unchallenged and were used to determine aMPV and NDV post-vaccination antibody titres and NDV vaccine shedding.

Clinical signs

All chicks were observed every day and after aMPV challenge were examined daily for clinical signs and the severity scored as described before (Jones *et al.*, 1992; Catelli *et al.*, 2010). Briefly, a score of 0 (=no signs), 1 (=clear nasal exudates), 2 (=turbid nasal exudates), 3 (=frothy eyes and/or swollen infraorbital sinuses in conjunction with nasal exudates). Following NDV challenge, all chicks were examined daily for clinical signs and mortality. Those birds showing signs of illness such as paralysis were humanely killed.

Sampling

For aMPV and NDV vaccine detection wet and dry swabs were collected from the oropharynx of chicks at 0, 2, 7, 14 and 21 days post-VG/GA vaccination and 7, 14, 21 and 28 days following the second vaccination, oropharyngeal (OP) swabs were collected from vaccinated-unchallenged chicks. After virulent aMPV challenge, OP swabs were collected at 5 and 7 days for the detection by real time-polymerase chain reaction (RT-PCR) and virus isolation (VI) of aMPV. Wet swabs previously moistened in TOC medium [Eagles serum-free MEM with glutamine, streptomycin (50 µg:mL) and penicillin (50 U:mL)] were used. The dry and wet swabs were processed for RT-PCR and VI respectively. Swabs from the challenged birds were processed individually whilst the other swabs were pooled. As for serology, blood was collected at 0, 14 and 21 days post-NDV VG/GA vaccination and at 7, 14, 21 and 28 days post-second vaccination from vaccinated-unchallenged chicks. Prior to termination of the experiment at 7 days post-challenge (aMPV or NDV), blood was collected from 6 chicks per challenge group for detection of aMPV ELISA or NDV haemagglutination-inhibition (HI) antibodies.

Detection of viruses

Oropharyngeal swabs were examined for aMPV by isolation in TOC (Catelli *et al.*, 1998; Ganapathy *et al.*, 2005) and for aMPV genome by RT-PCR as previously described (Cavanagh *et al.*, 1999; Ganapathy *et al.*, 2005). Newcastle disease vaccine VI was attempted following VG/GA and after the LaSota vaccinations from OP swabs by inoculation of 9 to 11 days old embrionated chicken eggs.

Detection of vaccinal antibodies

Newcastle disease antibodies were detected by HI (Allan and Gough, 1974). For detection of aMPV antibodies, a commercial ELISA kit (BioChek B.V., Gouda, The Netherlands) was used and the assay was carried out as recommended by the manufacturers.

Statistics

The antibody titres within the groups were log-transformed and then examined by analysis of variance (ANOVA) with MINITAB® for WINDOWS® 14 (MINITAB Ltd., Coventry, UK). A P value of <0.05 was considered statistically significant.

Results

Clinical signs

No clinical signs were observed after the first or the second vaccinations. After aMPV challenge, no clinical signs were seen in the groups that received aMPV vaccine either alone (VG/GA:aMPV) or in combination with NDV La Sota (VG/GA:aMPV+LASOTA). In contrast, nasal exudate and watery eyes were observed in the groups not given aMPV vaccine [the following mean daily scores resulted:

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Table 1. Experimental design for protection against virulent avian metapneumovirus or Newcastle disease virus challenge in specific pathogen-free chicks vaccinated singly or in combination.

	Vaccination			Challenge (21 days post-vaccination)	
Groups	Birds, n	Vaccination age, days		Birds, n	Challenge virus
		Day-old	21		
SW:SW	16	SW	SW	6	aMPV
				6	NDV
				4	Unchallenged
VG/GA:SW	16	NDV VG/GA°	SW	6	aMPV
				6	NDV
				4	Unchallenged
VG/GA:aMPV	16	NDV VG/GA	aMPV#	6	aMPV
				6	NDV
				4	Unchallenged
VG/GA:LASOTA	16	NDV VG/GA	NDV La Sota$	6	aMPV
				6	NDV
				4	Unchallenged
VG/GA:aMPV+ LASOTA	16	NDV VG/GA	aMPV+ NDV La Sota	6	aMPV
				6	NDV
				4	Unchallenged

SW, sterile water; aMPV, avian metapneumovirus; NDV, Newcastle disease virus. °6.52 log_{10} EID_{50}, #2.4 log_{10} CCID50, $7.54 log_{10} EID_{50}.

SW:SW (0.5, day 5 post challenge), VG/GA:SW (0.83, day 5 post challenge; 0.5, day 6 post challenge); VG/GA: LASOTA (0.5, day 6 post challenge)]. Also, no clinical signs were seen in any of the NDV-vaccinated groups. However, within five days post-NDV challenge, five out of six chicks in the NDV-unvaccinated control group (SW:SW) died and one was humanely killed.

Detection of avian metapneumovirus

Groups that did not receive aMPV vaccine remained negative for this virus (Table 2). In aMPV-vaccinated chickens, the combined (VG/GA:aMPV+LASOTA) vaccinated group was positive for aMPV by RT-PCR at 7, 14 and 21 days post-vaccination, but the group that received aMPV vaccine (VG/GA:aMPV) alone was positive at only 7 days post-vaccination. aMPV was only isolated from both aMPV-vaccinated groups at day 7 post-vaccination.

Five days following challenge with a virulent aMPV, four out of six birds in the aMPV-unvaccinated groups were aMPV positive by VI, but all six were positive by RT-PCR (Table 2). In contrast, there was no detection of aMPV in the aMPV-vaccinated groups except 1 of 6 in the single aMPV-vaccinated group (VG/GA :aMPV) which was positive by RT-PCR only. Seven days after challenge, OP samples from all groups were negative for aMPV by VI and RT-PCR except for the VG/GA:LASOTA group where one of six birds was positive for aMPV by both methods.

Detection of Newcastle disease virus

Newcastle disease vaccine VI was attempted following VG/GA and after the La Sota vaccinations. No NDV vaccine virus was detected in the control group (SW:SW). In the NDV-vaccinated groups, vaccine was isolated at two and seven days post-VG/GA vaccination (Table 3), but not at all following the NDV La Sota vaccination.

Serology

No aMPV antibodies were detected in groups that did not receive either aMPV vaccine or the challenge virus. Following aMPV vaccination, there were no significant differences in the levels of aMPV antibodies between the groups given aMPV alone (VG/GA:aMPV) and those given aMPV in combination with La Sota vaccine (VG/GA:aMPV+LASOTA), except at 7 days, where the titre in the latter group was significantly higher than the singly vaccinated group (Figure 1). Following the challenge, levels of aMPV antibodies in the challenged groups increased significantly than those of the unchallenged chickens (Table 4). No NDV HI antibodies were detected in the NDV-unvaccinated (SW:SW) group. In all other groups, antibodies were detected either in chicks that were vaccinated only with NDV VG/GA or those followed by NDV La Sota. However, between the groups, there were no significant differences in the HI titres (Figure 2). As regards velogenic NDV challenge, there were no significant differences in the levels of antibody titres between the vaccinated-challenged and corresponding vaccinated-unchallenged groups, except that the titres of the dually vaccinated-challenge group (VG/GA:aMPV+LASOTA) were significantly lower than the corresponding vaccinated-unchallenged group (Table 4).

Discussion

In our previous work (Ganapathy *et al.*, 2005, 2007), it was demonstrated that when live aMPV and VG/GA vaccines were given simultaneously to day-old SPF chicks, the efficacy of the vaccines was not affected. In addition, local and humoral immune responses in SPF (Cook *et al.*, 2001; Ganapathy *et al.*, 2005, 2007; Ganapathy and Jones, 2007; Tarpey *et al.*, 2007) and broiler chicks (Ganapathy *et al.*, 2006) have been reported before. Those reports concentrated on single or dual vaccination of young chicks. In some Asian, African and Latin American countries where ND is endemic, the live aMPV vaccines are often administered simultaneously with live La Sota vaccines, which are normally given to boost the protection against ND field challenge. This study was undertaken to examine the potential effect of live aMPV and La Sota vaccines in such situations when simultaneously administered to chicks already vaccinated with another lentogenic NDV vaccine. Chicks that had already been primed with VG/GA were used to

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Table 2. Detection of vaccine or challenge aMPV virus in the swabs by reverse transcriptase-polymerase chain reaction or passage in tracheal organ cultures.

Groups	Methods of detection	Days post-aMPV vaccination					Days post-virulent aMPV challenge[§]	
		0	7	14	21	28°	5	7
SW:SW	RT-PCR	-	-	-	-	-	6	0
	VI[#]	-	-	-	-	-	4	0
VG/GA:SW	RT-PCR	-	-	-	-	-	6	0
	VI	-	-	-	-	-	4	0
VG/GA:aMPV	RT-PCR	-	+	-	-	-	1	0
	VI	-	+	-	-	-	0	0
VG/GA:LASOTA	RT-PCR	-	-	-	-	-	6	1
	VI	-	-	-	-	-	4	1
VG/GA:aMPV+ LASOTA	RT-PCR	-	+	+	+	-	0	0
	VI	-	+	-	-	-	0	0

aMPV, avian metapneumovirus; SW, sterile water; RT-PCR, reverse transcriptase-polymerase chain reaction; VI, virus isolation. °From unchallenged groups; [#]attempted by TOC; [§]by oropharyngeal swabs.

Table 3. Detection of Newcastle disease virus in the swabs by passage in embryonated chicken eggs.

Groups	Days post-NDV VG/GA vaccination					Days post-NDV La Sota vaccination			
	0	2	7	14	21	7 (28)°	14 (35)	21 (42)	28 (49)
SW:SW	-	-	-	-	-	-	-	-	-
VG/GA:SW	-	+	+	-	-	-	-	-	-
VG/GA:aMPV	-	+	+	-	-	-	-	-	-
VG/GA:LASOTA	-	+	+	-	-	-	-	-	-
VG/GA:aMPV+ LASOTA	-	+	+	-	-	-	-	-	-

NDV, Newcastle disease virus; SW, sterile water; aMPV, avian metapneumovirus. °Numbers in parenthesis represent days of age; detection was attempted from unchallenged birds.

mimic the common field practices in ND endemic countries. In some countries, a milder live lentogenic vaccine (*e.g.* B1, VG/GA) are used within first few days of hatching and 14-21 later flocks are re-vaccinated with a live La Sota vaccine to boost the protection levels against field ND challenge.

Based on findings from this experiment, chicks that were vaccinated either singly with aMPV or dually with NDV La Sota vaccines were protected against virulent aMPV challenge. In addition, no aMPV was isolated from the aMPV-vaccinated groups even though one of six chicks in the VG/GA:aMPV was positive for aMPV by RT-PCR. However, the detection of aMPV genome in the absence of viable virus isolation has been reported before (Hess *et al*., 2004; Ganapathy *et al.*, 2007). Thus, it appears that simultaneous application of live aMPV and NDV La Sota vaccines in chicks already primed with NDV VG/GA showed no adverse effects in conferring protection against aMPV challenge.

In this experiment, it was demonstrated that both chicks that received either NDV VG/GA alone at day-old or followed by NDV La Sota at 21 days old were protected against a velogenic Italian NDV. No clinical signs or mortality were recorded in these groups but all the chicks in the NDV-unvaccinated group became ill, died or were humanely killed. Although no attempts were made to detect the NDV challenge virus in the vaccinated-challenged chicks, based on protection conferred against clinical signs and mortality, it appears that aMPV vaccine does not interfere with the protection conferred by the NDV La Sota vaccine. Previously, it has been shown that chicks simultaneously vaccinated with aMPV and NDV VG/GA vaccines gave full protection against velogenic NDV Texas GB strain (Ganapathy *et al.*, 2007). This appears to be the first study to demonstrate protection conferred against velogenic NDV and field aMPV by simultaneous vaccination whit aMPV and La Sota in chickens.

To evaluate the immune responses to live aMPV and NDV vaccines, humoral antibodies were monitored following the vaccination. The antibody levels were similar to previous report

Table 4. Avian metapneumovirus and Newcastle disease virus post challenge mean antibody titres in groups of chicks vaccinated at day old with AVINEW and at 21 days old vaccinated singly with NEMOVAC or NDV BIO LA SOTA or with a combination of both vaccines.

Groups	Antibody assay	Challenge virus			
		aMPV		NDV	
		Unchallenged	Challenged°	Unchallenged	Challenged°
SW:SW	NDV HI	-	-	<2	All died
	aMPV ELISA	213^{a} (50)	1459^{b} (335)	-	-
VG/GA:SW	NDV HI	-	-	4.67 (0.52)	5.67 (0.61)
	aMPV ELISA	142^{a} (41)	2461^{b} (792)	-	-
VG/GA:aMPV	NDV HI	-	-	4.83 (0.38)	5.17 (0.49)
	aMPV ELISA	2779^{a} (566)	5899^{b} (1219)	-	-
VG/GA:LASOTA	NDV HI	-	-	5.17 (0.38)	4.50 (0.42)
	aMPV ELISA	98^{a} (29)	1496^{b} (286)	-	-
VG/GA:aMPV+LASOTA	NDV HI	-	-	5.83^{a} (0.4)	4.67^{b} (0.52)
	aMPV ELISA	2818^{a} (610)	5940^{b} (1780)	-	-

aMPV, avian metapneumovirus; NDV, Newcastle disease virus; SW, sterile water; HI, haemagglutination-inhibition. °Blood collected seven days after aMPV or NDV challenge. Numbers in parentheses indicate standard deviations. ^{a,b}Different upper case superscripts between unchallenged and challenged groups indicate that the values differ significantly (P<0.05).

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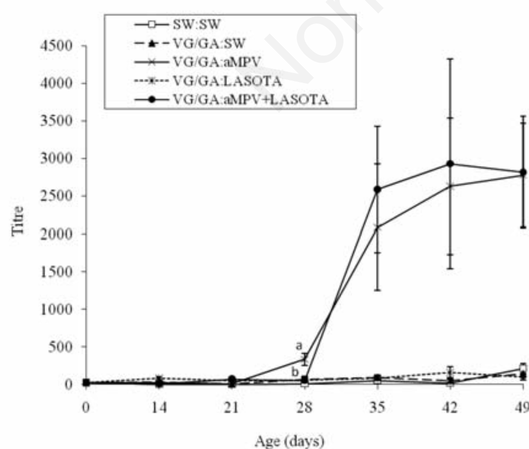

Figure 1. Mean avian metapneumovirus ELISA antibody titres in the unvaccinated and vaccinated-unchallenged groups. Different superscripts between groups indicate that the values differ significantly (P<0.05).

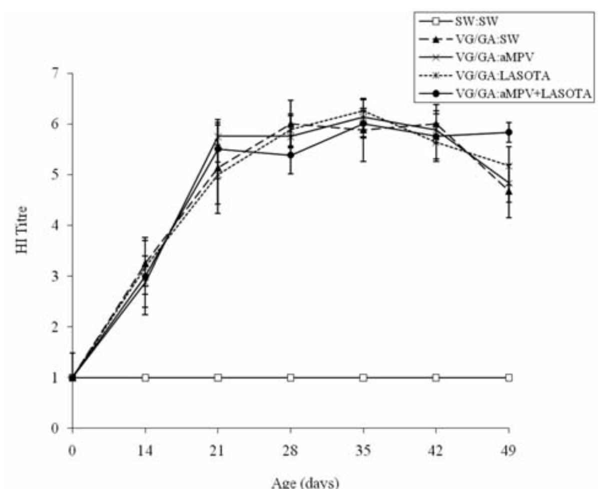

Figure 2. Mean Newcastle disease virus haemagglutination-inhibition antibody titres in the unvaccinated and vaccinated-unchallenged groups. There was no significant difference in the levels of antibodies between the groups.

(Ganapathy *et al.*, 2007) where there were no significant differences between the aMPV singly or dually vaccinated groups, except on 7 days post-vaccination. In contrast, Tarpey *et al.* (2007) reported that following administration of live aMPV, IBV H120 and NDV C2 vaccines, the humoral antibody response to aMPV were significantly lower than the singly vaccinated aMPV group. These findings reaffirm previous reports that the protection was due to the local and cell mediated immunity, which provided protection against clinical signs and for clearance of the challenge virus (Cook *et al.*, 1989; Jones *et al.*, 1992; Khehra and Jones, 1999; Ganapathy *et al.*, 2005; Liman and Rautenschlein, 2007).

For NDV HI titres, there were no significant differences in the levels of humoral antibodies between the groups that received single VG/GA and those that also received La Sota vaccine. Furthermore, the La Sota vaccine may have induced the production of local antibodies and/or cell-mediated immunity rather than solely boosting the humoral antibody responses. However, at 49 days post-vaccination, the levels of antibodies in the groups that received NDV La Sota were higher than the other groups, thought not significantly. Roy *et al.* (1997) vaccinated chicks with live lentogenic followed by mesogenic NDV vaccines and found no significant increase in the humoral antibody titres. It has been reported that for protection against NDV, local immunity on the mucosal surface of respiratory tract plays an important role in protection (Takada and Kida, 1996). At the time of challenge, each of the NDV-vaccinated groups had HI titres above $\log_2$ 5, which means that such titres were sufficient or above the protective titre. Levels of humoral antibody titres have been associated with protection against NDV (Beard and Brugh, 1975; Kapczynski and King, 2005; van Boven *et al.*, 2008).

Conclusions

In conclusion, using NDV VG/GA-primed SPF chicks, it was demonstrated that subsequent simultaneous vaccination with live NDV La Sota and aMPV vaccines does not adversely affect the protection of the chicks against virulent NDV or aMPV challenges.

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