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Research Article

Influence of Maternally Derived Antibodies on Vaccination using a IBV H120 Vaccine Virus

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Keywords

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Abstract

Infectious bronchitis (IB) is a highly contagious viral disease of chickens, resulting in animal discomfort and economic damage caused by production loss and instalment of interventions. Protection against IB is normally provided in young chicks by passive maternally acquired immunity and active immunity induced by early vaccination. The most widely used IBV vaccine strain is H120, which is administered to chicks in the first week of their life. At this age, the potential interference of maternally derived antibodies (MDA) with vaccines may hamper the development of active immunity. In this study, the onset and duration of immunity and the interference of MDAs on the immunogenicity of a live attenuated IB H120 vaccine was assessed. Broilers with and without MDA to IB virus were vaccinated by spray, eye-nose-drop, orally (mimicking drinking water vaccination) or were left non-vaccinated. At three different time points after vaccination, antibody levels to IB virus, and ciliary activity of tracheal explants were measured to assess the efficacy of the vaccine against challenge. No correlation between serum antibody titers and protection could be established, suggesting that local and cell-mediated immunity plays an important role in preventing IBV infections in young birds. There was no influence of MDAs on the vaccination by any of administration methods tested, as judged by level of protection against challenge using ciliostasis scores.

ABBREVIATIONS

IBV: Infectious Bronchitis Virus; MDA: Maternally Derived Antibodies; ELISA: Enzyme Linked Immunosorbent Assay; CMI: Cell-Mediated Immunity

INTRODUCTION

Infectious bronchitis virus is a gammacoronavirus in the subfamily Coronavirinae and family Coronaviridae that causes respiratory disease in domestic fowls. The disease is highly contagious with morbidity typically up to 100%. Mortality is low but can be >50% with some strains that cause nephritis or when opportunistic pathogens such as *Escherichia coli* complicate the disease [1,2]. Disease signs include respiratory symptoms, reduced weight, and egg production, increased frequency of abnormal eggs and increased rates of mortality [3]. Eight decades after its first discovery, the disease continues to cause significant losses in poultry production [4].

Although strict bio security and working with one-age systems are essential control measures, vaccination is normally required to protect chickens against challenge with virus strains of IBV. Since there are many variants of IBV, this is a challenging task [3,5,6].

For vaccination of chickens against IBV, both live attenuated and inactivated vaccines are used.

The most widely used IBV vaccine strain is H120, which has been used successfully as a primary vaccine in broilers and also for initial vaccination of breeders and future layers all over the world for more than 50 years [7]. Field strains of Massachusetts serotype are still causing disease in many areas of the world [1,3] and this together with the evidence for broad protection with the H120 strain vaccines accounts for its continuing success [7]. To stimulate protective immunity, the H120 vaccine virus must replicate in the respiratory tract [6,7]. IBV H120 virus is ideally suited for use in young, susceptible chicks [7].

Besides careful application which cannot be overstated, another point of concern is influence of maternally derived antibodies on vaccine efficacy.

MDAs are passively transmitted from the hen to the offspring to provide protection of young birds against pathogens during the first week of life [8].

Since vaccination of 1-day-old commercial broilers against IBV is widely performed, the vaccine virus may be partially or fully neutralized by circulating MDAs and prevent the development of active immunity. Furthermore, some have reported that vaccine

virus may increase the rate of depletion of MDAs resulting in an undesired effect [9].

Several groups have reported a negative effect of high levels of MDAs against the vaccine strain when it is applied on day of hatch [9,10], whereas others did not detect a lower efficacy of the day-old vaccination in presence of maternal antibodies [11,12].

In the present study we assessed the efficacy of Avishield IB H120 in young birds with and without MDA. The vaccine was applied on 1-day-old broiler chicks by spray and oculonasal route and on 7-days-old broiler chicks via drinking water. At different time points after vaccination, antibody levels and protection against homologous IBV challenge were tested to assess the onset and duration of the immunity achieved by the vaccine.

MATERIALS AND METHODS

Viruses

The infectious bronchitis virus H120 vaccine used in this study (Avishield IB H120, Genera Inc., part of Dechra Veterinary Products) is produced in requirement with European Pharmacopoeia and Directive 2004/28/EEC concerning quality, safety and efficacy of live poultry vaccines.

The vaccine virus was reconstituted in an appropriate volume of sterile water, depending on the inoculation route (see below) to provide 3.5 log10 EID_{50} per bird, which is the minimal dose recommended for vaccination as required by Ph. Eur. monograph 04/2013:0442 and the specifications of the manufacturer.

Challenge of chickens in vaccination challenge studies was conducted using the virulent IBV strain M41.

Chickens

Chickens without MDA were SPF broilers hatched from the in-house SPF broiler flock (GD Animal Health). MDA positive chickens were commercial broilers (Ross308) originating from breeders that had been vaccinated using live IBV vaccines of serotypes Mass, D274, 793B and QX) and had been boosted using an inactivated multivalent vaccine containing Mass and D274 antigens. Each group was further divided into four experimental subgroups (Refer "Experimental design") to be vaccinated by eye-nose-drop, spray or drinking water while fourth group consisted of non-vaccinated control chickens.

All animal experiments were approved by the Animal Experimental Committee of GD. GD is licensed to conduct animal trials according Dutch law (licence number: TVWA/07/22279).

Experimental design

In this study, 253 chickens with MDAs and 184 chickens without MDAs to IB virus were randomly divided over four treatments resulting in27 groups (15 groups of 23 vaccinated and 12 groups of 6 non-vaccinated broilers; 20 non-vaccinated broilers were euthanized on D0 to assess the level of MDAs to IB virus). Each group of 23 vaccinated broilers was housed in separate isolator, which was confounded with treatment. The non-vaccinated MDA positive and MDA negative broilers were also housed in separate isolators. The treatments given were 1) administration of IBV H120 live vaccine by eye-nose-drop, 2) administration of vaccine by spray, 3) oral administration of vaccine and 4) no administration of vaccine (also no mock). Vaccination by eye-nose-drop and spray was done on D0 (day-old chickens) and vaccination by drinking water on D7 (7 days of age). Seventy-two chickens were left non-vaccinated. Ten, 21 and 35 days after vaccination, 20 vaccinated chickens and 5 non-vaccinated chickens were challenged with IB M41, which is homologous to the vaccinal virus, in a dose of 4 $\log_{10}$ EID_{50} by eye-drop (0.05 ml per eye; 0.1 ml per chicken). Five days after challenge, ciliary activity in tracheal explants was determined. Antibodies to IB virus were determined on D0 and one day prior to challenge using ELISA. For each administration route, interference by MDAs on the immunogenicity of vaccine was assessed by comparing the efficacy between vaccinated chickens with and without MDAs. The non-vaccinated chickens without MDAs were used to validate the challenge dose.

Serology

The presence of IgG antibodies to avian IBV was assessed in the sera of the chickens by ELISA (IDEXX Flock Check IBV antibody kit; IDEXX, Maine, USA). Briefly, serum samples were incubated in ELISA wells, coated with purified avian infectious bronchitis antigen and after a wash step incubated with peroxidase-enzyme labelled anti-chicken-Ig conjugate. The binding was visualized by a hydrogen peroxide tetra-methylbenzidine substrate. A chicken was considered a responder when the ELISA titer was >396 (according to the manual of IDEXX ELISA kit).

Ciliary activity

The ciliary activity of tracheal explants from all challenged chickens was examined 5 days after challenge. Shortly after euthanizing the chickens, the trachea of each chicken was extirpated by removing the skin of the neck. Subsequently, the trachea was disconnected from the surrounding tissues, and cut just proximal of the larynx and just proximal of the thorax and put in Minimum Essential Medium with 4-2-hydroxyethyl-1-piperazineethanesulfonic acid buffer (MEM-H medium of 37°C).

Transverse sections of 0.5-2 mm of the trachea were made using a scalpel. The transverse sections of the trachea were placed in MEM-H medium (37°C) on a 20-well plate and examined under the microscope (40-100x). Ciliary activity of three sections of the upper part, four sections of the middle part and three sections of the lower part of the trachea were examined. All tracheal explants were examined within 2 hours after sampling. The activity of the cilia in each tracheal section was scored as follows:

Score 0: >50% of the tracheal section shows cilia movement.

Score 1: <50% of the tracheal section shows cilia movement.

A tracheal section was considered normal when at least 50% of the internal ring showed vigorous ciliary movement. A chicken was considered not affected if not fewer than 9 out of 10 tracheal rings showed normal ciliary activity.

Statistical analyses.

For each of the administration routes of the vaccine and time points of challenge, differences in the proportion of chickens with clinical signs after inoculation were assessed with Fishers paired exact tests, using a level of confidence of 95 percent.

Table 1: Protection of chickens vaccinated with live IBV H120 vaccine against challenge with 4 $\log_{10}$ EID_{50} of IB M41 based on ciliostasis in tracheal explants.

Administration route of vaccine	MDA[C]	*n*	Protection after challenge		
			10 days after vaccination	21 days after vaccination	35 days after vaccination
Eye-nose-drop	Positive	20	60%[A]	100%	100%
	Negative	20	84%[A]**	-	100%
Spray	Positive	20	65%[B]	100%	100%
	Negative	20	50%[B]	-	100%
Not vaccinated	Positive	5	0%	0%	0%
	Negative	5	0%	0%	0%
Oral	Positive	20	85%[C]	100%	100%
	Negative	20	70%[C]	-	100%
Not vaccinated	Positive	5	0%	0%	0%
	Negative	5	0%	0%	0%

[C] maternally derived antibodies,
n= number of chicks per group, - not tested.
*For each administration route and each time point, pairwise comparisons were made between broilers with (=positive) MDAs and broilers without (=negative) MDAs using Fisher's exact test($P \leq 0.05$). Different superscript letters designate significantly different groups.
**n=19

Table 2: Geometric mean ELISA IB antibody titers at different time points after vaccination at one day of age (eye-nose-drop and spray) and 7 days of age (oral administration).

Administration route of vaccine (day of application)	MDA	Geometric mean ELISA IB virus antibody titer # days after vaccination [Number of responders/total sampled]			
		#=0	#=9	#=20	#=34
Eye-nose-drop (D0)	Positive		183 [0/23]	9 [0/23]	56 [3/23]
	Negative		2 [0/21]	-	120 [2/21]
Spray (D0)	Positive		206 [4/23]	17 [0/23]	69 [1/23]
	Negative		1 [0/23]	-	92 [0/22]
Not vaccinated (D0)	Positive	1610[10/10]	143 [1/5]	1 [0/5]	5 [0/5]
	Negative	12 [0/10]	3 [0/5]	1 [0/5]	1 [0/5]
Oral (D7)	Positive	420 [1/6]	16 [0/23]	49 [2/23]	297 [8/23]
	Negative	22 [0/4]	1 [0/23]	-	130 [2/23]
Not vaccinated (D7)	Positive	164 [1/2]	2 [0/5]	20 [0/5]	12 [0/5]
	Negative	5 [0/2]	1 [0/5]	2 [0/5]	14 [0/5]

Abbreviations: IB= Infectious Bronchitis, MDA= Maternally Derived Antibodies, ELISA= Enzyme Linked Immunosorbent Assay, - = not tested.

RESULTS AND DISCUSSION

Ciliary activity

Onset of protection was observed already at the first test point, 10 days after vaccination, reaching 50-85% protection depending on the route of administration (Table 1). The differences between levels of protection in the MDA positive and MDA negative groups within each application method were not significant (P=0.0931 for eye-nose drop; P=0.3373 for spray and P=0.2560 for oral administration). It is worth noting that ten-day-old MDA broilers which were left unvaccinated were completely unprotected already at this time point, regardless of high MDA levels at D0 (Table 1). This correlates well with previous findings where it was demonstrated that although MDAs protect chickens when challenged at day old, the protection diminishes rapidly within first weeks of life [9,13].

Protection in vaccinated groups reached 100% by day 21 post vaccination, even in MDA-positive broilers. It is not excluded that complete protection was achieved even prior to day 21. Nevertheless, immunity developed rapidly and apparently faster than reported in previous comparable experiments [12,14,15]. Duration of immunity was tested at 35 days after vaccination where all vaccinated groups showed complete protection. It may be expected that immunity would sustain even longer than the last challenge point at 35 days post vaccination [16,17].

Complete ciliostasis in tracheal explants was observed in all of the non-vaccinated MDA-positive and non-vaccinated MDA-negative groups after challenge with IB M41 (Table 1).

From the above results, it is clear that MDA had no detectable impact on vaccination of day-old broilers via spray/eye-nose-drop route nor on the vaccination of 7-day-old broilers via drinking water. From previous studies, authors also reported successful vaccination of 1-,15- and 20- days old MDA+ chicks; while lower protection and more clinical signs were observed after vaccination of 6 and 10-days old chicks [12]. Contrary in another experiment, vaccination of day-old chicks was ineffective in MDA+ chicks [9].

The tracheal mucosa is the primary target tissue for IBV infection and there is considerable evidence indicating that local

immunity in the upper respiratory tract is an important first line of resistance against IBV infection [18]. It may be therefore expected that spray and oculonasal method of administration would result in better replication of virus and yield better immune response than in orally vaccinated birds. However, in this experiment the same onset, level and duration of protection were achieved by oral vaccination as well, albeit birds were vaccinated on day 7 vs. day 1 in spray/oculonasal groups. Earlier work demonstrated successful vaccination by all three routes of administration even with administration via drinking water for day old chicks [15,19].

Serology

In this study MDA positive and MDA negative broilers were vaccinated with IBV H120 live vaccine by different routes. The mean ELISA anti-IBV titer in MDA broilers on day 1 virus was 1610 (Table 2). Anti-IBV antibodies were detected in only few of the non-vaccinated MDA-positive broilers on day 9 and in only a few of the vaccinated MDA-positive broilers on days 20 and 34 after vaccination (Table 2), while protection against challenge was high in the vaccinated broilers. Poor sero conversion is not unexpected for vaccination with live attenuated IBV vaccines at day of hatch and it was reported before [14,20,21]. Indeed, lack of association between serum antibody levels and resistance to challenge with IBV was often reported [18,22,23]. This is important as serum antibody titers are often taken as a measure of vaccination efficacy or field challenge. However, this method is restricted only to circulating IgG and in the case of IBV, the results are often difficult to interpret [10,11,14]. Local and mucosal neutralizing antibodies seem to be more relevant for the protection against challenge than serum antibodies as they play a critical role in restriction of primary replication of the virus, resulting in the host protection [18]. Furthermore, cell-mediated immunity is an additional protective mechanism of resistance against IBV infection, given that specific cytotoxic T CD8 lymphocytes have been shown to be important in immune-protection against IBV [24-26]. Thus, the most probable mechanism of immune-protection against IBV infection is that local antibodies and cell-mediated immunity mechanisms act in an additive or synergistic way resulting in a more complete protection against IBV infection [18,26].

Unlike Mondal and Naqi (2001), faster depletion rates of MDAs was not observed in vaccinated groups in comparison with unvaccinated chickens.

CONCLUSION

MDA positive chicks can be successfully vaccinated with H120 vaccine at day old by spray and eye-nose drop and at 7 days old via drinking water. No correlation was established between circulating MDAs and achieved protection by vaccination. There were no significant differences in onset, level, and duration of protection post vaccination between chicks with and without MDAs. Onset of protection was achieved already at 10 days after vaccination and reached 100% in MDA+ chicks 21 days after vaccination. Serum antibody levels are not the most reliable measure of the vaccine efficacy as even vaccinated sero negative birds may be well protected by means of local and cell-based immunity.

CONFLICT OF INTEREST

G.J. Boelm and Sjaak de Wit are employed by the GD Animal Health, CRO which performed the animal studies. Lana Ljuma Skupnjak is employed by Genera Inc., now part of Dechra Pharmaceuticals PLC Group, manufacturer of Avishield IB H120.

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