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Efficacy of Live Attenuated Vaccines against Newcastle Disease in Commercial Broilers

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Abstract

Proper vaccination using quality live and inactivated vaccines may significantly reduce incidence and severity of outbreaks of Newcastle disease (ND), a highly contagious disease of poultry. In this work, the efficacy and interference with maternally derived antibodies (MDA) of live vaccine based on the Hitchner B1 strain of ND was tested in commercial broilers. Vaccine was applied via either spray or oral route at day 1 (spray route) or day 7 (oral route). Birds were challenged by either intramuscular injection or by eye-drop application of a virulent strain of NDV 2-5 weeks after vaccination. Broilers vaccinated via spray route showed 90-100% protection when challenged with the virulent ND virus (vNDV) already 2 weeks after the vaccination, while protection among orally vaccinated hatchmates was 60-80%. By the 5th week protection reached 90-100% in both groups. The performance of ND vaccine was not affected by the MDA. Protection of vaccinated birds was significantly higher than in non-vaccinated controls throughout the study. Intramuscular challenge consistently resulted in lower protection rates than the eye-drop challenge route.

Conclusion: Vaccination of 1-7 days old broilers with live attenuated ND vaccine provides significant protection against field vNDV, despite the presence of MDA. An intramuscular challenge route may not be the optimal practice for studies involving vNDV as it does not imitate the natural infection route and significantly undervalues the contribution of local and cellular immunity.

ABBREVIATIONS

ND: Newcastle Disease; NDV: Newcastle Disease Virus; vNDV: Virulent Strain of Newcastle Disease Virus; MDA: Maternally Derived Antibodies; SPF: Specific Pathogen-Free; $TCID_{50}$: Tissue Culture Infective Dose; i.m.: Intramuscular; LD_{50}: Lethal Dose, 50%; HI: Haemagglutination Inhibition; SD: Standard Deviation

INTRODUCTION

Newcastle disease (ND) is considered to be the fourth most significant disease of poultry in terms of the number of livestock units lost, after the highly pathogenic avian influenza, infectious bronchitis, and low pathogenic avian influenza. It is amongst the geographically most widespread animal diseases along with rabies and Bovine tuberculosis [1]. Being highly contagious and pathogenic, it causes substantial losses in the poultry industry worldwide. ND is caused by highly pathogenic strains of Newcastle disease virus (NDV) which belong to avian paramyxoviruses type 1. It is estimated that over 230 species of birds are susceptible to infection with NDV [2-4].

A favorable epizootiological situation, good biosecurity measures, and appropriate use of high quality live and inactivated vaccines have nearly eliminated losses due to ND in many areas. For example, in nearly all EU countries ND has been kept under control for decades despite the fact that virulent NDV (vNDV) strains are circulating in the wild [3,5]. However, in affected areas, especially in Africa and parts of Asia, mortality from ND outbreaks may reach 100% in unprotected poultry flocks, thus incurring high economic losses from mortality and condemnation of carcasses [4,6-8].

Since the beginning of their commercial use in the 1940s, live and inactivated vaccines based on attenuated NDV strains have been extensively used worldwide to control ND. Although vaccination does not prevent shedding of the virulent virus, it successfully protects birds from morbidity and mortality caused by vNDV [9].

Today's live vaccines are based on attenuated strains and may be applied by a variety of ways convenient for mass application. Nearly a hundred live NDV vaccines are marketed worldwide today [10]. Vaccines containing lentogenic strains such as LaSota [11] and Hitchner B1 [12] are widely used as they provide solid systemic and local immunity alongside their excellent safety profile [13,14]. However, no universally applicable vaccination protocols exist as vaccine efficacy is influenced not only by vaccine handling and quality, sanitary status of the birds, genetic

factors, hygienic practices, and general epizootiological situation, but also to a large extent by the level of maternally derived antibodies (MDA) in the vaccinated flock [15].

MDAs transmitted from the hen`s plasma to the offspring provide protection of young birds against pathogens during the first weeks of life [16]. Due to the heterogeneity of the parent flocks, offspring normally have heterogeneous MDA status. This may greatly influence the vaccine uptake, especially if vaccine(s) are applied at an early age when MDA concentration may still be very high [17,18].

In the present study we have performed an efficacy study of Avishield® ND B1, live attenuated vaccine against ND based on the Hitchner B1 strain, in MDA-positive commercial broilers. To compare two potential challenge routes, birds received vNDV via either intramuscular route, as prescribed by the European Pharmacopoeia protocol [19], or via eye-drop route which seems more appropriate as it resembles the natural infection pathway. Results demonstrate the vaccine is efficacious despite the presence of circulating MDA. Eye-drop and intramuscular challenge routes resulted in significantly different protection rates thereby raising the question on the adequacy of the currently adopted vNDV challenge protocol.

MATERIALS AND METHODS

Chickens

Specific pathogen-free (SPF) chickens (Babcock) were hatched from eggs sourced from certified NDV-naive SPF flocks, provided by Prophyl Animal Health Ltd. (Mohács, Hungary). MDA-positive commercial broilers (ROSS308) were obtained from a local hatchery. Anti-NDV antibody titer was measured for each individual chick at Day0 to confirm the MDA status. The broiler group was further subdivided into 5 experimental subgroups (see Experimental design) to be vaccinated once or twice via either oral or spray route with the 5th group being non-vaccinated MDA-positive controls. Birds divided into experimental groups were kept in separated units in conventional animal rooms on litter until the start of the challenge when they were moved to separate units of the Biosafety Level-3 facility. Sufficient care was taken to avoid cross infection between groups. All experimental groups were enrolled in the experiment at the same time to reduce the number of control chickens used at each challenge day. Special biocontainment features were provided as are required in research involving high consequence livestock pathogens. All animal experiments were approved by the Institutional Animal Welfare Committee of Prophyl Animal Health Ltd. The permission for conducting animal trials was issued by the Hungarian Government Office for Baranya County, Food Chain Safety and Animal Health Directorate (Ref. number: II-I-001/1306-012/2012).

Vaccines

Vaccines Avishield® ND B1 and Avishield® ND were provided by Genera Inc. (Croatia). Vaccines are based on lentogenic Hitchner B1 (Avishield® ND B1) and LaSota(Avishield® ND) strains characterized by markedly low ICPI values; 0.02 for Avishield® ND B1 and 0.18 for Avishield® ND. Both vaccines comply with the OIE and Commission Decision 93/152/EEC concerning the virulence of vaccine strains, and with the requirements of the European Pharmacopoeia and Directive 2004/28/EEC concerning the quality, safety and efficacy of live poultry vaccines. Vaccines were reconstituted in an appropriate volume of water for injections, depending on the inoculation route (see below), to provide =$10_{6.0}$ 50% Tissue Culture Infective Dose ($TCID_{50}$) units per dose, which is the minimum dose recommended for vaccination.

Experimental Design

Day-old commercial broiler chickens were divided into three main groups for vaccination via either spray (A) or oral route (B) or to remain as unvaccinated controls (C). Each of the first two groups were further subdivided into two additional subgroups which received either a single dose of Avishield® ND B1 vaccine (A1 and B1) or one dose of Avishield® ND B1 followed by one dose of Avishield® ND three weeks later (A2 and B2). Broilers vaccinated via spray route received vaccine(s) at day 1 (A1) or days 1 and 22 (A2) while orally vaccinated birds received vaccine(s) at day 7 (B1) or days 7 and 28 (B2). To assess the onset and duration of immunity, birds vaccinated only once were challenged 2 and 5 weeks after vaccination while birds vaccinated twice were challenged 2 weeks after the second vaccination. At each challenge point, birds were further split into two groups of 10 birds to be challenged via either intramuscular (i.m.) or eye-drop route. Control groups of twenty SPF chickens and twenty non-vaccinated broilers were included at each challenge point and were challenged via either i.m. or eye-drop route.

Overall, 28 individual groups with 10 chicks per group were included in the test (Table 1).

Challenge

The chickens were challenged with $10_{5.0}$ LD_{50}Herts 33/56 NDV (Wey bridge 33/56) strain by either intramuscular or eye-drop route. Intramuscular route was performed by the method prescribed by the European Pharmacopoeia [19] while the eye-drop challenge dose was given in two drops of suspension (one in each eye) with a total volume of 50 µL. The challenge-exposed chickens were observed for at least 14 days for general appearance and clinical signs of Newcastle disease.

Serology

Haemagglutination inhibition test (HI) was used to measure the level of anti-NDV antibody titre. HI tests were performed in microplates using twofold dilutions of serum, 1% chicken red blood cells and 4 haemagglutinating units of vaccinal LaSota NDV, following the method of Allan and Gough [20]. Titres were expressed as $\log_2$ value of the highest dilution which caused inhibition of the haemagglutination.

Statistical analysis

Data was analyzed using GraphPad Prism version 7 for Windows (GraphPad Software, San Diego, California, USA). Fisher`s exact test was used to compare levels of protection between the groups. Paired t-test was used to compare differences in protection against vNDV challenge of birds challenged via i.m or eye-drop route.

Table 1: Study design outline and protection rates of vaccinated MDA-positive broilers and respective controls after challenge with vNDV.

MDA status	Vaccination route	Vaccination regime	Challenge with vNDV							
			Day 14		Day 21		Day 35		Day 42	
			i.m.	eye-drop	i.m.	eye-drop	i.m.	eye-drop	i.m.	eye-drop
MDA positive	Spray	D1*: Hitchner B1**	90****	100			70	100		
		D1: Hitchner B1 D21: LaSota***					90	90		
	Oral	D7: Hitchner B1			60	80			100	90
		D7: Hitchner B1 D28: LaSota							80	90
	Non-vaccinated control		10	40	0	10	10	30	0	0
SPF	Non-vaccinated control		0	0	0	0	0	0	0	0

* "D" followed by a number denotes the vaccination day
** Avishield® ND B1 vaccine, dose of $10^{6.0}$TCID$_{50}$ per chicken
*** Avishield® ND vaccine, dose of $10^{6.0}$TCID$_{50}$ per chicken
****Results are expressed as percentage of healthy birds after 14 days of observation post infection. Birds showing general symptoms and nervous disorders of Newcastle disease such as ruffled feathers, depression, tremor and paralysis or birds which died during observation period were considered affected.
Abbreviations: MDA: Maternally Derived Antibodies; vNDV: Virulent Strain of Newcastle Disease Virus; SPF: Specific Pathogen-Free; i.m.: Intramuscular

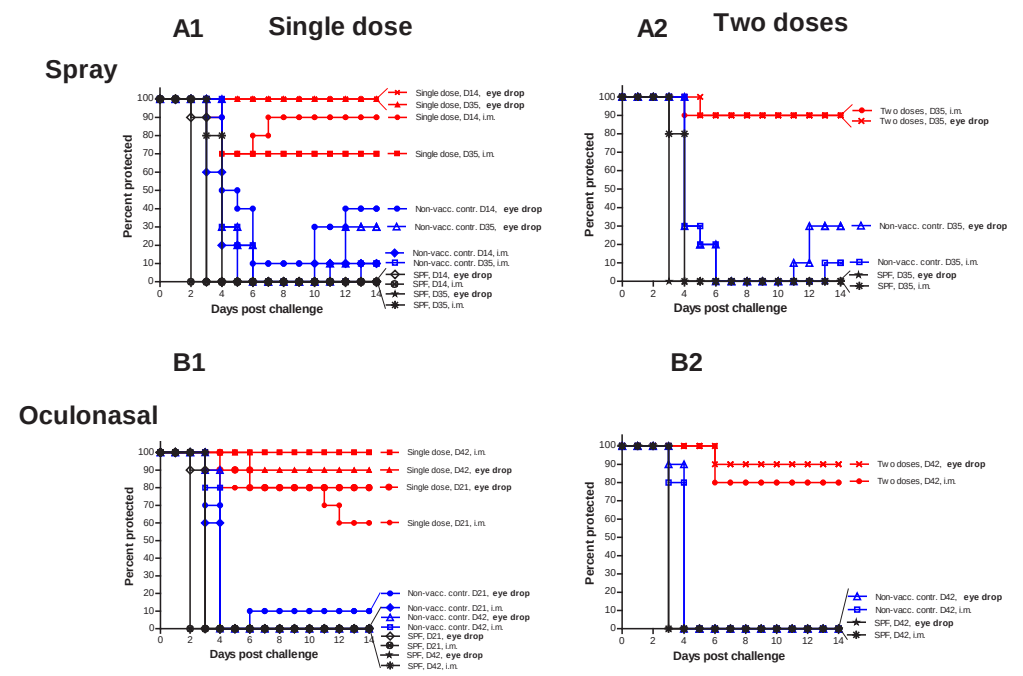

Figure 1 Incidence of clinical signs during the observation period following the challenge with vNDV. Birds showing general symptoms and nervous disorders of Newcastle disease: ruffled feathers, depression, tremor and paralysis or birds which died during observation period were considered affected. Vaccinated MDA-positive broilers: red lines; non-vaccinated MDA-positive broilers: blue lines; non-vaccinated MDA-negative controls (SPF chickens): black lines. "D" followed by a number denotes the challenge day.
Abbreviations: MDA: Maternally Derived Antibodies; vNDV: Virulent Strain of Newcastle Disease Virus; SPF: Specific Pathogen-Free; i.m.: Intramuscular.

RESULTS AND DISCUSSION

Serological results

Titer of anti-NDV antibodies was measured in day-old chickens entering the study prior to vaccination. Mean $\log_2$HI titre in participating day-old broilers was 4,1 ± 1,6 (mean ± SD), ranging from 0 to 8, n=200. Anti-NDV antibody titer in chickens vaccinated per os on day 7 prior to vaccination was 2,6 ± 1,7 (mean ± SD), ranging from 0 to 6, n=60. Such heterogeneity in protective titers is commonly seen in commercial broiler flocks [21]. The

average protective titre and homogeneity in the protective levels depend on the vaccines used, immunization schedule, breed and age of the parent flock, etc.

Based on the literature data [22], it is expected that titer of protective antibodies is halved approximately every 4 days. This kinetics model fits with the observed data on the protective levels of unvaccinated challenged commercial broilers which showed little protection against NDV challenge already at day 14, unlike vaccinated hatchmates (Table 1). It needs to be stressed that level of protective anti-NDV antibodies is not always the optimum estimate of protection of birds against NDV challenge. Correlation between serum anti-NDV titer and protection against NDV challenge is usually more reliable in birds vaccinated with inactivated vaccines as the major immunological response to a killed virus is humoral [23,24]. In birds vaccinated with live attenuated vaccines, cellular and local immunity contribute considerably to the protection rate by decreasing disease and transmission potential [25,26]. In the previous work we have shown that even broilers, which after vaccination with live attenuated LaSota-based vaccine show little or no serum antibody response, still exhibit relatively high protection against NDV challenge, presumably as a result of non-humoral and local immunity [27]. Therefore, the often used assessment of flocks` protection against NDV after vaccination with live attenuated vaccines based on the antibody titers may underestimate the actual protection rate.

SPF chickens in the study were confirmed to be seronegative to NDV.

Protection against NDV challenge

Immunity in birds vaccinated with live vaccines against NDV starts to develop very early following vaccination. Neutralizing antibodies can be detected already 6–10 days post infection, while generation of antigen specific cytotoxic T-cells starts at about 7–10 days post infection [25,28,29]. Anticipating potential interference of the vaccine virus with the existing MDA, the first challenge point was set at 14 days post vaccination. Furthermore, expecting that protection rates may peak approximately 3 weeks after primo-vaccination and then gradually decline [27], a group of birds received the second NDV vaccine dose 21 days after the first dose with the aim to test whether the protection rate will be maintained until the end of the production period. Consequently, the remaining challenge tests were performed 35 days after the first dose, which in the case of birds vaccinated two times is 14 days after the second dose. Table 1 summarizes protection rates based on morbidity on the last observation day while Figure 1 shows incidence of clinical signs during the observation period following the challenge with vNDV.

In broilers vaccinated by spray on day 1 protection reached 90-100% already at day 14 and remained high until the last test point at day 35 (70-100%). Consequently, second vaccination given at day 21 did not have much room to improve the already high protection rate and it measured 90% at day 35.

Similar protection rates were measured in oral-vaccinated broilers vaccinated once at day 7 or twice at days 7 and 28. In birds vaccinated once, protection was 60-80% at day 21 (14 days post vaccination) and reached 90-100% at day 42 (35 days post vaccination). Again, the second dose could not significantly improve protection rates and it was 80-90% at day 42 for birds vaccinated twice. Analysis of the protection rates among vaccinated groups did not show statistically significant differences between any data points (Fisher`s exact test, $P<0,05$). It may be speculated that an experiment on larger experimental groups might have revealed fine differences in protection between individual groups. However, the general conclusion is that vaccination with attenuated live virus in the face of MDA was effective and provided high protection already 14 days post vaccination. The protection was persistent for at least 5 weeks post vaccination. Whether the second vaccination dose provides additional benefit in terms of prolonging the duration of immunity remains to be resolved. Based on the previous work with the LaSota based live vaccine, this is likely the case [27].

Both application routes seem to provide equivalent response in terms of level of protection and duration of immunity, with the only difference being that oral vaccination was given to 7-days old chickens vs. day-old chickens in the spray group. It is difficult to ensure that day-old birds drink evenly sufficient amount of vaccine dispersed in drinking water within the required period during which the vaccine remains stable in the solution (usually 2-3 hours). This practice, therefore, may result in suboptimal average vaccination of the flock and high variability in protection levels within flock. Contrary, spray-vaccination disperses vaccine evenly over the flock and requires less time which makes it a better choice for vaccination of day-old chickens, especially when vaccination is performed in a hatchery. As vaccination of older birds by either route seems to provide comparable protection, the choice of the delivery method depends on the usual vaccination practice in a particular farm.

During spray vaccination a significant portion of the vaccine ends up on feathers or the floor while only a small amount is inhaled and infects susceptible cells [30], initially in the upper respiratory tract. However, in the case of live vaccines, even this amount is sufficient to initiate active propagation of the vaccine virus and trigger the immune response. Some practitioners assume that in addition to the inhaled virus, a certain amount of vaccine delivered onto hatchmates` feathers during spray-vaccination is being taken orally immediately after vaccination, as chickens are kept in close proximity during and 20-60 min after vaccination.

In the case of oral application, the vaccine is delivered mostly to the digestive tract, but the end result in terms of quality of protection seems to be comparable. It is known that NDV spreads throughout the body and may replicate not only in the respiratory tract (trachea, lungs) but also in the spleen, kidneys, caecal tonsils, duodenum, brain, etc. [31].

Above mentioned protection rates were achieved in a controlled laboratory environment ensuring that all birds received the same dose of the vaccine and challenge viruses. This approach is different from the field conditions as it does not take into account potential contribution of the herd immunity. Under field conditions, herd immunity may provide protection to suboptimal-vaccinated or unvaccinated birds, provided that the remaining majority of the flock is well vaccinated [25]. It is estimated that 58%-100% of all birds in a flock need to be

immune to NDV in order to prevent a major NDV outbreak in the field [32].

Non-vaccinated MDA-positive broilers were used as a control of vNDV challenge and as a reference against which a contribution of the vaccine to the overall immunity of the vaccinated chickens was assessed. The protection of the non-vaccinated broilers against vNDV challenge based on the remaining MDA was moderate at the first test point (day 14, protection 10-40%) but it declined during the experiment as MDA vanished from the circulation (Table 1). The protection levels of the non-vaccinated groups were at each test point significantly lower than in the respective vaccinated groups (P<0,05).

No protection against the challenge was observed in any of the control SPF groups, thus validating the challenge model.

Intramuscular vs. eye-drop challenge route

European Pharmacopoeia protocol for vNDV challenge studies proposes intramuscular injection of the challenge virus [19]. This route, however, does not imitate natural infection that occurs via the respiratory or digestive tract.

In natural infection, the virus first faces the local immunity of the respiratory/digestive tract. Only if this first line of defense is breached, the virus would have the chance to propagate in the host. Experimental intramuscular injection, however, delivers the virus directly into the host avoiding this first line of defense, thus largely neglecting the contribution of the non-humoral immunity to the overall protection level in the natural conditions. In this respect, the actual protection of the vaccinated birds under field conditions may be different (higher) than judged from experimental results obtained using intramuscular injection route.

To test whether current challenge protocols give misleadingly low protection results in vNDV challenge studies, half of the birds in each test point were challenged by delivering the same amount of challenge virus via eye-drop route, which generally resembles the natural infection route. Comparison of paired results of protection against vNDV challenge via i.m. vs. eye-drop route (Table 1) clearly shows that birds challenged via eye-drop route are statistically significantly better protected than matching birds challenged via i.m. route (P<0,05). This proves the hypothesis that the challenge route recommended by current regulations for laboratory experiments is not the optimum and that it probably results in underestimation of the potential protection which may be achieved in the field using the tested vaccine.

Clinical signs

No clinical signs of NDV disease were observed in any of the vaccinated chickens prior to the challenge. Seven chickens died during the observation period (before the challenge) due to complications related to retained yolk sac and/or omphalitis. Clinical signs in the challenged birds included usual symptoms related to infection with vNDV such as ruffled feathers, depression, tremor and paralysis which in most cases led to fatality, although a number of vaccinated birds did fully recover by the end of the observation period.

CONCLUSION

Vaccination of commercial MDA-positive broilers by spray or oral route with live attenuated ND vaccine based on the Hitchner-B1 strain was shown to significantly decrease morbidity and mortality caused by virulent NDV. No negative interference of the vaccine with the remaining circulating MDA was observed in the tested broiler flock. Potential interference, however, cannot be excluded in flocks with extremely high MDA protective titers [17,18, 27]. In such a situation, vaccination against NDV may be postponed until MDA levels fall sufficiently to ensure good vaccination uptake and/or a second vaccination may be introduced. As the eye-drop route better imitates the natural infection route, it may be a better choice for studies involving vNDV as intramuscular infection significantly undervalues the contribution of local and cellular immunity.

CONFLICT OF INTEREST

The first author Anto Vrdoljak is employed by Genera Inc., now part of the Dechra Pharmaceuticals PLC Group, manufacturer of Avishield® ND B1 and Avishield® ND vaccines. The co-authors Máté Halas and Tamás Süli are employed by the Prophyl Animal Health Ltd., CRO which performed the animal studies.

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