

Safety and Efficacy of *In Ovo* Administration of an Experimental Reovirus Vaccine in Commercial Broiler Chickens

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Abstract: An experimental vaccine was prepared by mixing commercial reovirus vaccine with antibody. Vaccines were inoculated into 18 day-old commercial broiler embryos at 0.1 of the recommended dose. At 3, 6, 9, 12 and 15 days post *in ovo* vaccination (PIOV), serum was collected and antibody against reovirus analyzed by an enzyme-linked immunosorbent assay (ELISA). At the same time, spleens were collected and vaccine virus detected by inoculating chicken embryo fibroblasts (CEF). At day 15 PIOV, chickens were challenged with a virulent reovirus S1133 strain. At day 25 PIOV, birds were euthanized and weighed. Efficacy was based on safety, antibody reaction, and percent (%) protection. Reovirus vaccine alone (vac) or complexed with antibody (AB) did not affect hatchability, morbidity, and mortality. Best protection was with the 2 experimental vaccine groups (73% for vac + 1/16 dilution of AB) and (64% for vac + 1/4 dilution of AB) provided by the vaccine mixed with 1:16 dilution of antibody. No vaccine resulted in an increase in antibody; however, titers of all challenged groups rose after challenge. Vaccine virus was detectable at day 3 PIOV in group 3 (vac + 1/16AB) and 4 (vac) chickens. In contrast, vaccine virus detection was delayed until day 9 PIOV in group 2 (vac + 1/4AB) chickens. The experimental antibody complex vaccine serves as a good starting point for development as a commercial vaccine.

Key words: Reovirus, *in ovo* vaccination, virus-antibody complex

Introduction

Reoviruses are common in commercial broiler breeder and broiler flocks. These viruses can cause various diseases, which include viral arthritis (or viral tenosynovitis), malabsorption syndrome, and chronic respiratory disease (Fahey and Crawley 1954; Kerr and Olson, 1969; Kibenge and Wilcox, 1983; Page, *et al.*, 1982).

Vaccination is important to control diseases caused by reoviruses. Both attenuated and inactivated vaccines are utilized in the poultry industry (Van der Heide *et al.*, 1976 and 1983) to provide active immunity in the breeders or passive immunity for progeny as maternally passed antibody. Reovirus vaccination of the broiler breeding stock is common for protecting progeny. However, maternal antibody levels are not uniform in progeny. Chickens with low maternal antibody are susceptible to field strains of reovirus early in life. Therefore, reovirus vaccination of progeny is often necessary in areas where early challenge exists. Conversely, high levels of maternal antibody could interfere with the efficacy of vaccine in the progeny (Naqi, *et al.*, 1983 and Wyeth and chettle, 1990). *In ovo* vaccination with a vaccine-antibody complex product might prevent this problem.

In ovo vaccination is popular due to increased speed and reduced labor costs. An immune infectious bursal disease virus complex vaccine (IBDV-Icx) has been developed for *in ovo* vaccination of 18 day-old embryos. IBDV complex vaccine was obtained by mixing specific IBDV antibody with live IBDV vaccine

virus. Chickens that received this immune complex vaccine were protected from virus challenge and it was less sensitive to maternal antibodies (Haddad, *et al.*, 1997). However, no reovirus vaccine has been developed for *in ovo* use, since current vaccines are too pathogenic for embryos and can interfere with the replication and efficacy of Marek's Disease (MD) vaccine, if used simultaneously (Rosenberger, 1983). An experimental vaccine virus antibody complex against reovirus was utilized for *in ovo* immunization in commercial broiler embryos that had maternal antibody. The safety and efficacy of the complex vaccine were determined.

Materials and Methods

Animals: Commercial broiler eggs from an Alabama broiler breeder flock were incubated and candled for fertility when they were 10 days-old. On day 18 of incubation, embryos were vaccinated by *in ovo* with a commercial reovirus vaccine or with a complex of vaccine combined with dilutions of antibody. All birds were fed and watered *ad libitum* and reared in isolation units maintained with filtered air under positive pressure. All were reared and handled according to Auburn University's Institutional Animal Care and Use Committee's Guidelines.

Avian reovirus antibody: Antiserum was obtained by vaccinating SPF white leghorns (Charles River Laboratories, Inc., Charles River, Conn.) with a full dose of a live vaccine (ChickVac from Fort Dodge

Animal Health, Fort Dodge, KN) by subcutaneous injection. At 2 weeks later a full dose of VaVac (Fort Dodge) was given by eye drop. An intramuscular injection with a full dose of an inactivated vaccine (TriReo) from Fort Dodge was given at two weeks after that. When the chickens were 9 weeks old, they were bled and euthanized. Titers (11,704) were determined with an enzyme-linked immunosorbent assay (ELISA) kit (Kirkegaard and Perry Laboratories, 2 Cessna Court, Gaithersburg Maryland). Neutralization activity of serum (1,254) was determined using a virus neutralization (VN) assay in chicken embryo fibroblasts (CEFs).

Avian reovirus vaccines: A commercial avian reovirus vaccine (Synovac from Lohmann Animal Health, Gainseville, GA) containing an attenuated S1133 reovirus was chosen at 0.1 dose. The standard dose contains $10^{4.5}$ tissue-cultured infectious dose (TCID₅₀). The vaccine was used alone or with antibody. Antibody complexed vaccine was obtained by mixing 0.1 dose of the vaccine with antiserum, followed by one-hour incubation at room temperature. Two dilutions of serum (1:4, 1:16) were used. The VN test indicated that a 0.1 dose of the vaccine mixed with a 1:4 dilution of antibody resulted in complete neutralization (no cytopathogenic effect) (CPE) in CEFs.

***In ovo* vaccination:** Eggs were injected through the air space. $10^{3.5}$ TCID₅₀ (0.1dose) of vaccine alone or vaccine combined with antiserum (vac+AB) were injected into the eggs after the egg surface was sterilized and a hole punctured with a 23 gauge, 2.5cm needle. Each group consisted of 25 eggs, which included the no vaccine (control 1A and 1B), vaccine alone (group 4), and vaccine combined with 1:16 (group3), and 1:4 (group 2) AB dilutions. Control eggs were inoculated with 0.1ml of Marek's Disease vaccine diluent (Fort Dodge). Holes were sealed by Duco cement (Walmart, Inc. Auburn, AL) and eggs transferred to the hatcher.

Sample collection and preparation: At 3, 6, 9, 12 and 15 days post *in ovo* vaccination (PIOV), three to four chicks per group were decapitated and serum collected for antibody determination. At the same time, spleens were removed and placed in sterile Dulbecco phosphate-buffered saline (PBS). Spleens were stored at -70°C until further analysis. Spleens were homogenized by pounding in sterile Nasco Whirl Pak bags, with the same volume of PBS. A % weight/volume was used (1g tissue ml⁻¹ PBS). The mixture was centrifuged at 4,000 x G for 20 minutes and pellets discarded. Aqueous phase was used to determine the presence of vaccine virus by inoculating onto secondary monolayers of CEFs. A 1:4 antibiotic

mixture (Penicillin-Strptomycin from Sigma Chemical, Inc.) was added to spleen supernatants and 10-fold serial dilutions made before they were inoculated onto the 96-well plates. CPE was determined after incubation at 37°C for 5 days. Viral titer was analyzed according to Reed and Muench (Reed and Muench, 1938).

Virus challenge: At 18 days PIOV, vaccinated groups (2-4) (including control 1B) were challenged with a virulent S1133 strain in the right footpad. Titer of S1133 was $10^{6.5}$ embryo infectious dose m⁻¹ (EID₅₀/ml). Virus was diluted 1:500 with diluent. Each chicken received 0.1ml. Control was divided into two groups; one received no challenge (1A) and the other was challenged (1B). One week post-challenge, chickens were euthanized by cervical dislocation. Footpad swelling was scored from 0 to 3 as follows: 0 = no change; 1 = slight swelling; 2 = increased swelling; 3 = swelling severe and expand to surrounding tissue. Birds without lesions were considered protected. Efficiency of vaccines (% protection) was determined by ratio of number of birds with a score of 0 compared to the total number of chickens in the same group.

Data analysis: Body weights and lesion scores were compared by ANOVA and Chi square test used for mortality and hatchability. Statements of statistical significance were based on P < 0.05.

Results

Hatchability and Mortality: Hatchability of control eggs was 98%. Chickens that received vaccine alone (vac) vaccine-antibody complex (vac + AB) had a hatchability that ranged from 97% to 100%. No mortality occurred in any vaccinated group (Table 1).

Table 1: Percent hatchability and mortality of chickens immunized with a vaccine alone or vaccine - antibody complex

Group ^A	1	2	3	4
Hatchability (%)	98	97	100	100
Mortality (%)	2	0	0	0

^AGroup 1. Control; Group2. 0.1 dose of vaccine complexed with 1:4 antiserum; Group 3.0.1 dose of vaccine complexed with 1:16 antiserum; Group 4. vaccine alone.

Table 2: % protection and body weight after S1133 challenge

Group ^A	1a	1b	2	3	4
Body Wt. (G) ^B	835 ± 85 ^{ab}	889 ± 49 ^a	848 ± 94 ^{ab}	780 ± 43 ^b	825 ± 57 ^{ab}
Protection (%) ^C	NC ^b	0	18	72	55

Protection: The two groups 3 (vac + 1/16AB) and 2 (vac + 1/4AB) provided the best protection 72% and

Table 3: Severity of gross lesions after S1133 challenge

Sources of Lesions ^{BC}	Groups ^A				
	1a	1b	2	3	4
0	NC ^D	0/11 ^a	2/11 ^a	8/11 ^b	6/11 ^b
1	NC	3/11 ^a	4/11 ^a	2/11 ^a	2/11 ^a
2	NC	4/11 ^b	5/11 ^b	1/11 ^a	3/11 ^{ab}
3	NC	4/11 ^b	0/11 ^a	0/11 ^a	0/11 ^a
Average	NC	2.10 ^c	1.27 ^b	0.36 ^a	1.00 ^{ab}

^Agroup 1. negative control received no challenge (NC); Group 1b. positive control was challenged; Group 2. 0.1 dose vaccine complexed with 1:4 antiserum; Group3.0.1dose of vaccine complexed with 1:16 antiserum; Group 4. vaccine alone.

^Bsource : 0= no change; 1 = slight swelling; 2 = increased swelling and wrinkles on the planter surface disappeared; 3 = swelling severe and expand to surrounding tissue.

^CNumbers with different lowercase superscripts within the same row differ significantly ($P \leq 0.05$).

^DNC = not challenged

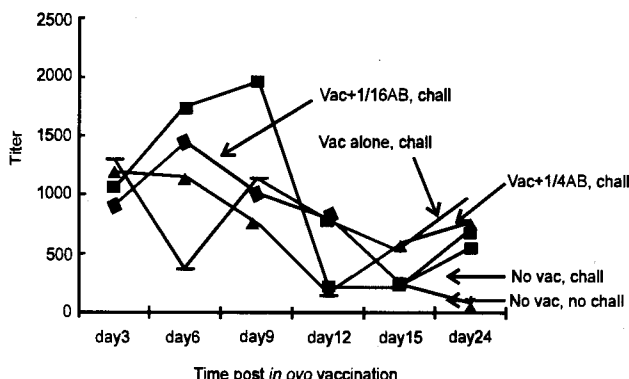


Fig. 1: Anti-reovirus serum ELISA titers from commercial chickens post *in ovo* vaccination

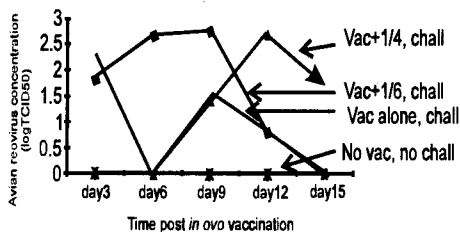


Fig. 2: Avian reovirus detection by virus isolation from spleens of SPF chickens

64%, respectively (Table 2). Severity of lesions in group 3 (vac + 1/16AB) was the lowest (Table 3). Only the body weight of group 3 (vac + 1/16AB)was

significantly different from the positive control chickens ($p \leq 0.05$). None of the non-challenged chickens (group 1A) had lesions, whereas 100% of non-vaccinated challenged chickens (group 1B) had severe lesions (2.10).

Serology: Average maternal antibody in group 1A and 1B at day of age (day 3 PIOV) was 1,450. No vaccine resulted in an increase in antibody titer. Titers in all groups dropped to the lowest on day of challenge and increased after challenge. The non-challenged group (1A) titers continued to drop until they were euthanized (Fig.1).

Detection the vaccine virus: At day 3 PIOV, virus was detected in samples from chickens that received vaccine alone (group 4) or vac + 1/16AB (group 3). In contrast, virus was not detectable until day 9 PIOV in group 2, which received vac + 1/4AB. On the day of challenge, only group 2 (vac + 1/4AB) had detectable vaccine virus (Fig.2).

Discussion

Vaccine, given alone or complexed with antiserum did not affect hatchability and nor cause mortality in chickens. Ratio of vaccine virus and antibody was critical for vaccine efficacy. Protection was highest in groups that received experimental antibody complexed vaccines. This may indicate that immune complexes were more immunogenic than vaccine alone or the antibody in the complex protected vaccine virus from the neutralization effect of maternal immunity. Higher amount of protection in the vac + 1/16AB group compared to the vac + 1/4AB group reduced vaccine replication and thereby reduced vaccine efficacy.

ELISA results showed that no vaccine caused a significant rise in antibody. Antibody levels were similar before challenge in all groups, however, protection in these groups ranged from 55 to 72%. After challenge, titers rose in all challenged groups. Level of antibody was not consistent with protection. Therefore, immunity against reovirus may also be cell-mediated. This agrees with other research (Van Loon, *et al.* 2002), in which reovirus vaccinated broilers had good protection, even though they had no antibodies were detected at the time of challenge.

Vaccination of commercial broiler embryos with vaccine alone or vaccine complexed with antibody resulted in vaccine virus recovery from the spleen of all vaccinated groups and persisted in spleens at least 10 days after hatch. Vaccine virus was detectable at day 3 PIOV in groups 3 (vac + 1/16AB) and 4(vac). This implies that virus propagation occurred early. Virus replication was delayed at least 6 days in group 2 (vac + 1/4AB) chickens, when compared to chickens in group 3

group 3 (vac + 1/16AB) or group 4 (vac). High amount of antibody (1/4AB) in the vaccine could delay vaccine virus replication. A previous study with an IBD antibody complex vaccine showed that the antibody level in the vaccine determined the time of vaccine virus release (Whitfill, *et al.* 1995).

The mechanism of this experimental reovirus antibody complex vaccine has not been elucidated, but previous research on the mechanism of IBDV complex vaccine (Jeurissen, *et al.* 1998) showed that IBDV complex vaccine caused a delay in virus detection of about 5 days and induced more germinal centers in the spleen and larger amounts of IBDV were localized in both splenic and bursal follicular dendritic cells (FDC). Our results showed that reovirus complex vaccine can delay vaccine virus replication in chickens. Further investigation is needed to determine if the mechanism of reovirus complex vaccine is also related to its specific cellular interaction with FDCs in spleen and if the reovirus vaccine has a similar effect on T lymphocytes. Experimental reovirus vaccines when given *in ovo* were safe and efficacious for commercial broilers. Although % protection (73%) did not meet minimum (80%) USDA requirement, the experimental vaccine serves as a starting point for development of a commercial vaccine.

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