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Efficacy of prepared inactivated Newcastle disease virus vaccine using different oil adjuvants

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ABSTRACT

Emulsions have served as common adjuvants in veterinary medicine, especially in poultry. Newcastle disease (ND) is a serious worldwide risk to the production of poultry due to its widespread distribution and substantial economic impact. The point of this study was to find out how well a Newcastle Disease Virus (NDV) vaccine that was given under the skin worked when combined with Coral Biotechnology's new water-in-oil (W/O) emulsion adjuvants. In this study, two inactivated NDV vaccines were studied; the first one is a 1:1 combination of the Lasota strain and genotype VIId that was formulated using CORALVAC RZ 528, and the second one is a commercial inactivated NDV (Lasota strain and genotype VIId) vaccine with montanide ISA 71 adjuvant. To evaluate the effectiveness of the proposed vaccines against 6 log 10 EID50/0.5 ml of the genotype VIId Newcastle disease virus Egyptian isolated strain (GenBank accession number: JX647839). Challenge trials were carried out in specified pathogen-free (SPF) chicks. Every group that received vaccinations showed complete protection. In contrast to the non-vaccinated control group, the combination vaccine produced the greatest hemagglutination inhibition (HI) titers six weeks after vaccination and markedly reduced. In conclusion, all inactivated NDV vaccine formulations prepared with CORALVAC RZ 528 and Montanide ISA 71 effectively protected chicks from morbidity and mortality and the incorporation of cost-effective adjuvants into poultry vaccine production offers a promising strategy for developing vaccines that elicit strong immune responses and remain economically feasible

1. INTRODUCTION

Velogenic strains, classified as serotype 1 (APMV-1) avian paramyxoviruses, trigger Newcastle disease (ND). This virus is part of the genus Orthoavulavirus, located within the subfamily Avulavirinae of the Paramyxoviridae family (Rima et al., 2019). There are 5 types of NDV strains based on how bad the disease is: lentogenic, mesogenic, velogenic neurotropic, velogenic viscerotropic, and asymptomatic enteric (Alexander et al., 2008). The virulence of NDV strains is influenced by the presence of two basic amino acids, lysine (K) or arginine (R), at positions 112-113 and 115-116 within the fusion (F) protein cleavage site. Additionally, a phenylalanine residue at position 117 is essential for intracellular cleavage by proteases. In contrast, lentogenic strains like the La Sota strain possess a monobasic cleavage site, enabling extracellular cleavage by proteases. This restricted cleavage pattern limits the replication of lentogenic strains to specific tissues (Pedersen et al., 2004).

Miller et al. (2013) have reported that strains of NDV related to one serotype yet exhibit antigenic plus genetic variability. Global surveillance has revealed that genotypes V and VII are currently the most prevalent (Miller et al., 2010). Regardless of the implementation of intensive vaccination in Egypt, outbreaks of NDV continue to occur frequently, resulting in substantial losses within infected poultry flocks. These persistent outbreaks may be attributed to several factors, including all categories of serious vaccination regimens, the continual appearance of new NDV pathotypes, and the ongoing evolution of the virus through mutations (Hussein et al., 2014, and Radwan et al., 2013).

Previous studies have highlighted the potential for some commercially available live NDV vaccines to cause infections in Egypt (Aly, 2012). In contrast, inactivated NDV vaccines are generally considered safe, effective, and economically viable for controlling disease outbreaks. However, the serological response and vaccine efficacy of inactivated vaccines can be significantly influenced by the antigen content within the formulation (Hu et al., 2011).

Water-in-oil (W/O) emulsions, acting as depots, facilitate the gradual and sustained release of antigens within the body (Stills, 2005). This controlled release enhances the immune response by providing a prolonged exposure to the antigen. Inactivated vaccines are generally considered safe due to their inability to replicate, undergo genetic recombination, or revert to a virulent form. The use of oil emulsion adjuvants is often favored when the goal is to stimulate both cellular and humoral immune responses (Aucouturier et al., 2001; Chandrasekar et al., 2020). In this study we compared the immune response for two W/O inactivated Newcastle disease viruses with different oil adjuvants, CORALVAC RZ 528 and Montanide ISA 71.

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2. MATERIAL AND METHODS

Ethical approval
Approval for this research was obtained from the Animal Care and Use Committee at the Veterinary Serum and Vaccine Research Institute (VSVRI) located in Abbasia, Cairo, Egypt. All procedures adhered to the guidelines established in the European Communities Council Directive 1986 (86/609/EEC) concerning the protection of animals utilized for experimental and other scientific purposes.
Specific pathogen-free embryonated chicken eggs (SPF ECE)
The SPF ECEs were sourced from the Nile SPF Farm in Fayoum, Egypt. These eggs were utilized for virus propagation, virus titration, and confirmation of complete virus inactivation.
Specific pathogen-free chicks
Sixty (60) SPF chicks 2 weeks old were sourced from the Nile SPF Farm in Fayoum, Egypt. Chicks were utilized for vaccine efficiency tests.

Viruses
1. The velogenic viscerotropic Newcastle disease virus (vNDV) NDV Genotype VIId (GenBank accession number: JX647839) was provided by the Animal Health Research Institute (AHRI) and used in vaccine preparation, challenge, and hemagglutination inhibition tests.
2. LaSota strain was provided from the Veterinary Serum and Vaccines Research Institute (VSVRI) used in vaccine preparation and the hemagglutination inhibition test.

Vaccines
1.*Inactivated NDV vaccine with montanide ISA 71 adjuvant* as a commercial vaccine provided from Veterinary Serum and Vaccines Research Institute (VSVRI).
2. *Inactivated NDV vaccine using CORALVAC RZ 528*
a. Virus propagation: Newcastle disease virus was propagated by inoculating the allantoic cavity of 9-11-day-old SPF ECEs, and the infectivity of the propagated virus is tested by titrating the virus in SPF ECE to calculate the EID_{50} according to the methods outlined in the 2021 WOAH Manual.
b. Virus inactivation: Viral inactivation was performed using a 3.00% (v/v) solution of binary ethylenimine (BEI; Merck), following the protocol described by Razmaraii et al. (2012). The reaction was terminated by adding a 20.00% sodium thiosulfate (Merck) solution at a tenfold excess of the final BEI concentration. To confirm complete inactivation, the inactivated virus (prior to adjuvant addition) was subjected to two consecutive blind passages in SPF ECEs 9-11 days old (100 µl per egg via the allantoic cavity). Mortality and survival for embryos were monitored for up to six days post-inoculation. Allantoic fluid from both dead and surviving embryos was tested for the presence of hemagglutinating activity as described by WOAH, 2021. Complete inactivation was confirmed by the absence of both embryo mortality and hemagglutination activity, as outlined by WOAH, 2021.

Preparation of inactivated NDV vaccine emulsion: The inactivated NDV vaccine was prepared as a 1:1 combination of the Lasota strain and NDV Genotype VIId, formulated using CORALVAC RZ 528 as a water-in-oil emulsion, with a volume ratio of 30:70 for the aqueous and oil phases. The production process followed the standard procedures set by Coral Biotechnology Industry and Trade Incorporated Company.
The sterility and safety evaluation involved assessing the prepared vaccine for microbial contamination, including both fungal and bacterial presence, through culture methods as specified in Chapter 1.1.9 of the WOAH 2024 guidelines.
Experimental design
Sixty (60) SPF chicks 2 weeks old were divided into 3 groups. Forty (40) SPF chicks divided as Group (1) were vaccinated with prepared inactivated NDV with CORALVAC RZ 528, and Group (2) was vaccinated with commercially inactivated NDV vaccine produced by VSVRI adjuvanted with Montanide ISA 71. Both groups were vaccinated by the S/C route with 0.5 ml/chick. Twenty SPF chicks were kept as an unvaccinated group as Group (3).

Assessing the humoral immune response and efficacy of the NDV vaccines in vaccinated chicks: Serum samples were collected weekly for eight weeks after vaccination for the hemagglutination inhibition test (HI) using 4 HA units of each of the La Sota virus and NDV Genotype VIId antigens to determine antibody titers in sera of vaccinated and non-vaccinated chickens according to WOAH 2021.

A challenge test was done 3 weeks post-vaccination on ten chicks from each group. with 106 EID_{50}/0.5 ml genotype NDV VII d intramuscularly and monitored daily for clinical signs.

Quantitation of virus shedding by quantitative RT-PCR
Tracheal swabs (n = 3) from each group were collected on the 3rd, 5th, 7th, and 10th days post-challenge for quantitation of virus shedding. The collected swabs were dispersed in phosphate buffer saline and then stored at -80 °C (Miller et al., 2009).

3. RESULTS

Sterility and safety evaluation
Prepared inactivated NDV vaccine using CORALVAC RZ 528 was free from both fungal and bacterial contamination.
Vaccine humoral immune response:
The humoral immune response was assessed in chick groups vaccinated with inactivated ND vaccines. The start antibody titer for Group (1) against LaSota strain and NDV Genotype VIId viruses was (6.5 and 3), respectively; on the other hand, Group (2) showed (6 and 4) at the 3rd week after vaccination. The highest titer was at the 6th week after vaccination (10 and 7.5 for Group 1 and 9.5 and 7 for Group 2). The two vaccinated groups showed a gradual decrease in antibody titer till the 8th week (Fig. 1).

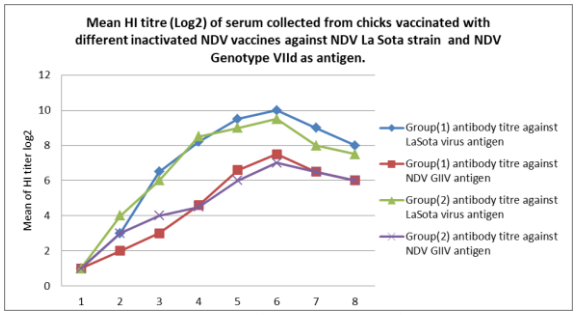

Figure 1: Mean HI titre (Log2) of serum collected from chicks vaccinated with different inactivated NDV vaccines against NDV La Sota strain and NDV Genotype VIId antigens.

Protection percentage: Challenge with NDV virulent virus isolate for vaccinated groups with Inactivated NDV CORALVAC RZ 528 oil as an adjuvant Group (1) and Inactivated NDV vaccine produced by VSVRI Group (2) remained healthy with no mortality, demonstrating 100% protective efficacy in SPF chickens. Non-vaccinated group (3) chickens showed a 100% mortality rate within 3-4 days post-challenge with severe clinical signs.

Virus shedding: Viral shedding from oropharyngeal swabs was assessed by quantifying the viral load (determined by quantitative RT-PCR and expressed as 50% egg infectious dose [EID_{50}]) at 3,5,7, and 10 days post-challenge. Compared to the non-vaccinated group, chickens vaccinated with the prepared vaccine demonstrated a notable and significant decrease. in both the shedding bird's number and the amount of virus shed ($p \leq 0.05$), as shown in Table 1

Table 1. Viral shedding in tracheal swab from vaccinated birds after challenge with NDV strains by real time PCR

Days post challenge	Group (1) Virus shedding titre (Log10)	Group (2) Virus shedding titre (Log10)	Group (3) Virus shedding titre (Log10)
3	1.99 ± 1.2[a]	1.4 ± 1.4[a]	8.66 ± 0.33[b]
5	2.53 ± 0.7[a]	2.20 ± 0.40[a]	NS
7	2 ± 2[a]	1.80 ± 0.60[a]	NS
10	NA	NA	NS

NA: not applicable, NS not survived, EID_{50}: Egg infective dose fifty. a b Different letters indicate significant differences between the groups ($P \leq 0.05$),

4. DISCUSSION

The poultry industry in Egypt faces significant economic losses due to NDV Genotype VIId infections, regardless of the presence of several programs of vaccination across all commercial flocks. These efforts underscore the critical need for the development and implementation of effective protective vaccines (Eid et al., 2022).

This study demonstrated that BPL effectively inactivated NDV at a final concentration of 3% following an 18-h incubation at 30°C. Previous research by Mondal et al. (2005) and Razmaraii et al. (2012) indicated the efficacy of BPL as an inactivating agent, even at lower concentrations. Furthermore, an additional research study reported that Genotype VIId of NDV vaccines stopped by formalin had lower hemagglutination inhibition (HI) titers in comparison with those stopped by BPL (King, 1991).

Quality control assessments conducted on the prepared vaccines, adhering to the guidelines outlined in the WOAH Chapter 1.1.9, confirmed the absence of bacterial and fungal contamination, indicating sterility.

In poultry vaccine production, the utilization of low-cost adjuvants plays an important role in enhancing the efficacy of vaccines while maintaining affordability. Adjuvants are materials that increase the immune response to certain antigens, thereby increasing vaccine efficacy. Coralvac RZ 528 adjuvants, developed fully by Coral Biotechnology Industry and Trade Incorporated Company (Turkey), incorporate a proprietary surfactant that facilitates vaccine manufacturing. This surfactant enables the simple incorporation of aqueous media into the CORALVAC oil phase at ambient temperature during manual preparation. However, for large-scale production, vigorous stirring and high-shear mixing are required.

The adjuvants utilized in this study are formulated as water-in-oil emulsions. This emulsion type promotes sustained antigen release at the injection site, triggering a prolonged humoral immune response mediated by B lymphocytes (Jansen et al., 2006). The evaluation of humoral immunity against Newcastle Disease Virus (NDV) was conducted by measuring hemagglutination inhibition (HI) antibody titers in chicks that received two distinct inactivated NDV vaccines. Group (1) received a vaccine adjuvanted with CORALVAC RZ 528 oil, while Group (2) received a locally produced vaccine adjuvanted with Montanide ISA 71. At six weeks post-vaccination, both groups exhibited high antibody titers. Group (1) demonstrated higher titers against both the Lasota strain (10 $\log_2$) and NDV Genotype VIId (7.5 $\log_2$) compared to Group (2) (9.5 log2 and 7 $\log_2$, respectively). But no significant difference by static analysis ($p \leq 0.05$) was noticed among the two vaccinated groups.

These findings align with previous research by Zhou et al. (2021), which demonstrated that oil-in-water emulsions, commonly employed in vaccine adjuvants, form microstructures within the body. Upon intramuscular administration, these microstructures act as a depot, facilitating the sustained release of antigens. This controlled release mechanism can stimulate both cellular and humoral immune responses.

Adding emulsions and mineral salts to vaccine formulations can result in a site of injection as a depot effect, which promotes a gradual release of the antigen and prolonged immune system activation. At the same time, incorporating Toll-like receptor (TLR) agonists and other immunostimulatory adjuvants can improve the mobilization of immune cells to the site of injection. area and boost cytokine production, thereby enhancing the overall immune response. (Burakova et al., 2018).

The inactivated vaccine, when adjuvanted with CORALVAC RZ 528 and Montanide ISA 71, demonstrated full protection against morbidity and mortality in all vaccinated chickens during the challenge. In contrast, the group that did not receive the NDV vaccine showed no signs of protective effectiveness. These findings align with previous research indicating the necessity of employing inactivated ND vaccines derived from currently circulating NDV strains in the local region (Wang et al., 2015). Our findings corroborate those of Miller et al. (2013), who observed that all unvaccinated chicks experienced mortality, while subcutaneously vaccinated SPF chicks with a single dose of inactivated NDV vaccine at three weeks of age survived. Following a subsequent challenge with NDV (Miller et al., 2013). The NDV vaccines, which include both genotype VII and LaSota strains, showed a decrease in both the number of birds shedding the virus and the intensity of viral shedding from individual birds. (Miller et al., 2007, and Palya et al., 2012). Our findings demonstrate that the developed vaccines effectively protected against mortality, reduced the number of shedding birds, and significantly lowered viral titers compared to the unvaccinated control group. Viral load had a significant reduction in all vaccinated groups when compared to those who were unvaccinated. These results suggest that the combined vaccine formulation elicits robust neutralizing antibody responses against the circulating NDV genotype VII strain.

The elevated antibody levels detected in HI assays were adequate to trigger protection., regardless of whether the vaccines were homologous or heterologous. This evidence suggests that the level of antibody protection is a critical factor in preventing disease transmission. These results are in line with earlier research that found that heterologous virus vaccines can effectively stop transmission as long as birds have enough time to build up a strong immune response (Van Boven et al., 2008; Miller et al., 2013).

5. CONCLUSIONS

In conclusion, all inactivated NDV vaccine formulations prepared with CORALVAC RZ 528 and Montanide ISA 71 effectively protected chicks from morbidity and mortality in challenge trials, demonstrating 100% protection. These findings indicate that the developed vaccines have the potential to control outbreaks caused by strains of NDV that are currently circulating in Egypt's poultry sector. The incorporation of cost-effective adjuvants like Montanide ISA 71 into poultry vaccine production offers a promising strategy for developing vaccines that elicit strong immune responses and remain economically feasible for widespread use within the poultry industry.

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