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EFFECT ON IMMUNE RESPONSE AND VIRUS SHEDDING IN THE CHICKEN VACCINATED AGAINST INACTIVATED LOCAL STRAIN OF NEWCASTLE DISEASE VIRUS GENOTYPE VII

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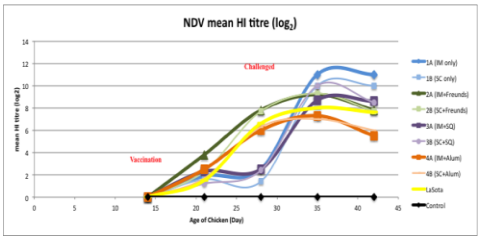
Graphical abstract



Abstract

The effectiveness of the new inactivated vaccine develop from local velogenic strain of Newcastle disease virus (NDV) Genotype VII and commercial vaccines LaSota were compared by determining the immune response and virus shedding of vaccinated chickens. Ten different groups of chicken consists of ten each, were vaccinated with and without adjuvant of the inactivated vaccine via intramuscular and subcutaneous, respectively. Three different adjuvants were used which include Oil-emulsion Complete Freund's, Oil-emulsion Squalene-based, and aluminium hydroxide gel (Alum). As a comparison, a group of chicken was vaccinated with commercial vaccine and control group was not vaccinated. With 100% survival rate and highest mean haemaglutination-inhibition (HI) titre of $\log_2$ 6, the inactivated vaccine with adjuvant Alum and Oil-emulsion Complete Freund's surpassed the LaSota. In addition, the virus shedding was significantly reduced and comparable to LaSota vaccinated chicken. Whereas, without adjuvant, the chicken HI antibody titre is below $\log_2$ 4 after vaccination and only 20-30% were survived. Based on the post-mortem findings on the survived chicken from each vaccinated group, their internal organs appeared normal and no sign of haemorrhage or pathognomonic signs of Newcastle disease (ND). Conclusively, vaccinated chicken are effectively protected from morbidity and mortality against virulent genotype VII challenge with the addition of adjuvant into inactivated local strain of NDV genotype VII vaccine. Thus, the development of inactivated local NDV genotype VII vaccine is a promising candidate to control the current ND endemic in Malaysia.

Keywords: Newcastle Disease Virus, Genotype VII, inactivated vaccine, adjuvant, immune response, virus shedding

1.0 INTRODUCTION

Newcastle disease (ND) is one of the most important diseases of poultry worldwide and probably has the most impact on the world's economy than any other animal disease [1]. The causative agent, NDV or Avian paramyxovirus-1 (APMV-1), belongs to the genus Avulavirus, within the family Paramyxoviridae and has a negative-sense, single stranded RNA genome [2]. By pathotyping, NDV strains are classified into the highly pathogenic (velogenic), moderately pathogenic (mesogenic), and lowly pathogenic (lentogenic). Some lentogenic strains of NDV are avirulent, whereas velogenic forms are further classified as viscerotropic and neurotropic types based on clinical manifestations and lesions [3]. Based on NDV nucleotide sequence, 9 genotypes of Class I and 11 of Class II NDV (I to XI) strains have been identified [4]. Genotypes V, VI, and VII from Class II are virulent viruses which also the predominant genotype circulating worldwide and of these, genotype VII has been highlighted given it was associated with many of the most recent outbreaks in Asia, Africa, and the Middle East [5].

In Malaysia, intensive vaccine programs have been implemented but ND outbreaks and sporadic cases have occasionally occurred even in well-vaccinated farms. A major epidemic of ND has occurred in Peninsular Malaysia from 2000-2001 with peaking 84 outbreaks in 2001 which cause substantial losses [6, 7]. Isolates of low virulence from NDV vaccine strains of genotypes I and II such as LaSota and HitchnerB1 are the most common type of vaccines being used worldwide including Malaysia [5]. According to Kapczynski and King (2013), vaccination will reduce the mortality in ND-infected flocks but it failed to stop the spread of disease, regardless of the vaccine, route or frequency of use. Therefore, it has been suggested that the development of improved vaccines and vaccination strategies are needed to induce protection against infection and inhibit virus shedding [8]. Several studies showed that the used of commercial vaccines can induce protection against morbidity and mortality when challenge with velogenic NDV strains, however they do not prevent the infection and virus shedding [9, 10, 11, 12]. Miller *et al*. (2013) found that the ND challenge virus will induced high haemaglutination - inhibition antibody titres and significantly reduced the amount of virus shed in oral secretions after been vaccinated with the homologous NDV compared to the heterologous vaccines [13]. Therefore, the objective of this study was to compare the effect on immune response and the efficacy of virus shedding in chicken vaccinated with non-adjuvant and adjuvant of local strain NDV genotype VII after challenge with homologous virus strain compared to the commercial vaccine.

2.0 EXPERIMENTAL

2.1 Virus Preparation

The NDV isolates were propagated by inoculation in the allantoic cavity of 9-10 day old embryonated chicken eggs. The virus suspension was measured for its infectivity titre via mathematical technique devised by Reed and Muench (1983) [14]. Then, the attenuation of viruses was performed with BEI, Binary Ethylamine, following the standard protocol for vaccine preparation [15].

2.2 Vaccination Study

One hundred of two-week old SPF chicken were obtained from SPF chicken house, Veterinary Research Institute, Malaysia, and maintained in isolation units with feed and water administered *ad libitum*. The chickens were separated into eight vaccination groups of ten chickens each. Chicken in group 1A and 1B received 0.1 ml of $10^{8.8}$ EID_{50} inactivated NDV genotype VII vaccine without adjuvant via intramuscular and subcutaneous, respectively. Chickens in group 2A and 2B were injected with 0.4ml of $10^{8.8}$ EID_{50} NDV genotype VII with adjuvant oil-emulsion Complete Freund's (Sigma Aldrich) intramuscularly and subcutaneously, respectively. For group 3A and 3B, the chickens received 0.2ml of $10^{8.8}$ EID_{50} NDV genotype VII with adjuvant oil-emulsion Squalene oil-in-water (AddaVax) also via intramuscular and subcutaneous. Whereas chickens in group 4A and 4B were administered 0.2ml of $10^{8.8}$ EID_{50} NDV genotype VII with aluminium hydroxide gel or Alum (AddaVax) intramuscularly and subcutaneously.

Two other groups are chicken vaccinated with commercial vaccine LaSota and chicken without vaccination (control). All groups of chicken were vaccinated once and challenged through intramuscular with $10^{4.3}$ EID_{50} of virulent NDV genotype VII two weeks after vaccination.

2.3 Serological Test and Virus Isolation

Sera of the chicken were taken weekly via intravenous prior and after vaccination, and subject to haemaglutination-inhibition (HI) assay for antibody titre evaluation [16]. Chickens were challenged with the virulent NDV genotype VII two weeks after vaccination. Then, virus isolation was performed from tracheal and cloacal swabs on day 2, 4, 7, and 10 days post-challenge. The swabs were collected into 1ml of Tryptose Phosphate Buffer (TPB) with a final concentration of streptomycin (700 µg/mg), penicillin (1500 u/mg), and kanamycin (50 µg/ml). After filtration, the swabs were inoculated into SPF eggs to determine the positive samples and evaluate the virus shedding. Finally, all of the surviving chickens were culled and subjected to post-mortem for further investigation.

3.0 RESULTS AND DISCUSSION

There are no survivors from chickens in unvaccinated control group after 3 days been challenged with virulent NDV genotype VII. For both groups of chicken vaccinated with the local strain of NDV genotype VII without adjuvant, there was 30% survival rate with their weekly mean HI antibody titre are below $\log_2$ 4 after vaccination (Table 1, Figure 1). Using squalene-oil-in-water type as adjuvant rendering chicken's survival rate by 70% and 50% for intramuscular and subcutaneous, respectively with weekly mean HI antibody titre are also below $\log_2$ 4 before challenged. Meanwhile, the chickens vaccinated with LaSota, inactivated NDV genotype VII vaccine with adjuvant Complete Freund's and adjuvant Alum are 100% survived after challenged with the same virus strain. Their weekly mean HI antibody titre are not less than $\log_2$ 6 starting second week after vaccination. The survival rate and weekly mean HI antibody titre was in par with the chicken vaccinated with commercial vaccine LaSota.

The virus shedding for chickens vaccinated without adjuvant and with squalene-oil-in-water type adjuvant are slightly reduced, meanwhile there are no sign of virus shedding from the chickens in the Alum and LaSota vaccination group (Table 2 and Table 3). Based on post-mortem on the survived chickens from all groups, their entire internal organ appeared normal and showed no sign of hemorrhage or pathognomonic signs of ND.

Table 1 Number of chickens survived after each injection

Group / Day	Two-week old	After Vaccination	After Challenged
1A	10	10	3
1B	10	10	3
2A	10	10	10
2B	10	10	10
3A	10	10	7
3B	10	10	5
4A	10	10	10
4B	10	10	10
LaSota	10	10	10
Control	10	10	0

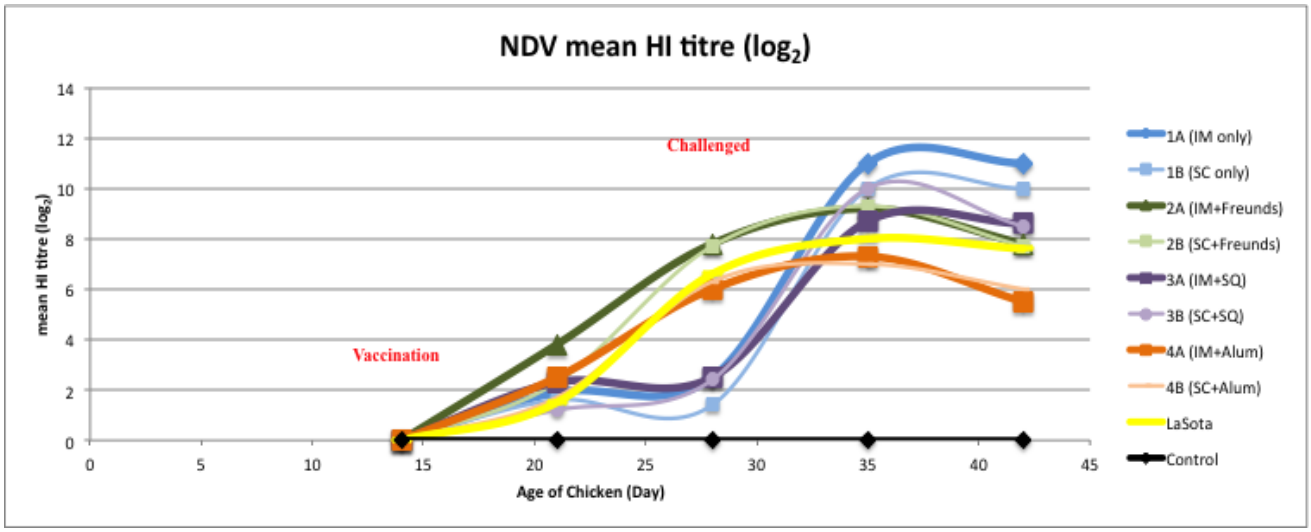


Figure 1 HI titre of two-week old chicken with virus challenge two weeks after vaccination
*IM=Intramuscular; SC=Subcutaneous; SQ=Squalene

Table 2 Percentage number of positive tracheal swab within two week after challenge

Group/Day	2	4	7	10
1A	60	90	0	0
1B	90	100	30	0
2A	0	0	0	0
2B	20	0	10	10
3A	27	87	14	0
3B	9	78	20	0
4A	0	0	0	0
4B	0	0	0	0
LaSota	0	0	0	0
Control	50	NS	NS	NS

*NS = Not Survived

Table 3 Percentage number of positive cloacal swab within two week after challenge

Group/Day	2	4	7	10
1A	90	100	60	60
1B	80	100	0	0
2A	0	20	0	0
2B	0	0	10	10
3A	18	75	86	57
3B	27	100	60	40
4A	0	0	0	0
4B	0	0	0	0
LaSota	0	0	0	0
Control	30	NS	NS	NS

This study showed that non-adjuvant inactivated vaccine of NDV genotype VII did not provide complete protection although it reduced virus shedding. Kapczynski *et al.* (2005) suggested that successful vaccination with protection could be achieved with ND when more than 85% of the flock has antibody titre more than $\log_2$ 4 [9]. Rahman *et al.* (2002) also reported that birds having antibody titre up to 16 ($\log_2$ 4) will fail to prevent the challenge infection against virulent NDV [17]. Both statements above are in accordance with the present study, which showed 100% of survival chicken in LaSota vaccination group and chicken vaccinated together with alum by having antibody titre more than $\log_2$ 4. Meanwhile, only 30% of chickens survived in group vaccinated with NDV genotype VII without adjuvant.

According to Bermudez *et al.* (2008), combination of inactivated vaccine and adjuvant are designed for subcutaneous or intramuscular injection [18]. Adjuvant plays an important role in the development of vaccines especially in improving the chicken immune response [19]. The adjuvant enable continuous exposure of the low level antigen to the immune system cells, thus triggering the high level production of antibody. Antigen was also preserved by adjuvant from rapid degradation and eradication by the host. Generally, the binding of emulsion adjuvant to antigen is more persistent compared to aluminum compounds [20]. In this study, the HI titer for inactivated vaccine with Complete Freund's adjuvant remain high even after six weeks post-vaccination. Whereas, the HI titer for inactivated vaccine with Alum adjuvant begin to decrease slowly at four weeks after vaccination.

4.0 CONCLUSION

Local NDV inactivated vaccine with Complete Freund's and aluminium hydroxyde gel as an adjuvant provide effective protection against velogenic NDV pathotypes as well as lentogenic and mesogenic types. The inactivated vaccine without adjuvant is inadequate to retain the spreading of NDV infection among the flocks. The vaccine with Alum was able to shed the challenged virus completely, hence comparable with the commercial vaccine LaSota. The oil emulsion squalene-based adjuvant was incompatible with the antigen of NDV inactivated vaccine, since only 50-70% of chickens survived and the shedding virus were similar to vaccinated chicken without adjuvant. Conclusively, the development of local NDV inactivated vaccine with Alum adjuvant have great potential to curb the current ND endemic.

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