Duration of Immunity of Live and Inactivated Newcastle Disease Vaccines in SPF Chickens Following a Single Administration

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ABSTRACT

Background:

Newcastle disease (ND) continues to be a major constraint on the extensive production of poultry in the rural communities, as well as on the more semi-intensive and intensive production carried out by smallholders in the urban and peri-urban communities, of many low- to middle-income countries (LMICs). A number of studies have demonstrated the benefits of vaccination with helping to control this disease but few have addressed the comparative efficacy of live and inactivated vaccines, especially their duration of immunity following just a single administration, under controlled conditions.

Methods:

Here, we report on the efficacy of two live and two inactivated commercially available ND vaccines, administered to 6-week-old specific pathogen free (SPF) chickens, by assessing the serological antibody response, and protection against mortality following virulent challenge with a recent velogenic ND virus (NDV) isolate at 24, 40 and 53 weeks after vaccination.

Results:

A single administration of either of the live or inactivated vaccines induced high levels of protective circulating antibody which peaked 4 weeks after vaccination and was sustained for at least 53 weeks post-vaccination. Reasonably high levels of protection against mortality, following challenge, were also demonstrated from 24 weeks up to 53 weeks after vaccination.

Conclusions:

These data demonstrate that in SPF chickens, kept under controlled laboratory conditions, commercially available live and inactivated ND vaccines can provide protective immunity following a single administration for at least one year. Further studies should be carried out in more intensively managed production systems using single-aged flocks to confirm our results. These studies should primarily be based on sero-monitoring. For extensively reared village and backyard chickens, which are kept in multi-aged flocks, it is strongly recommended that the current advice on the use of ND vaccines, requiring multiple vaccinations each year, is followed.

KEYWORDS: Newcastle disease, Vaccination, Duration of Immunity, Chickens

INTRODUCTION

Poultry, especially chickens, is an important resource for people living in rural communities of LMICs and involved in extensive production, as well as for those living in urban and peri-urban areas and involved in more market-orientated semi-intensive and intensive smallholder production[1].

One of the major constraints to the production of these birds is Newcastle disease (ND), endemic in much of Africa, Asia and Central and South America. Outbreaks of the disease in susceptible flocks regularly result in mortalities of 50 to 100%. Observing good management practices and efficient bio-security to help prevent and control the spread of ND, and other diseases such as highly pathogenic avian influenza (HPAI) with which it shares many of the clinical signs, can be unrealistic, especially in extensive and semi-intensive smallholder production, and as a consequence there is often little alternative to vaccination[2]. Since the pioneering work of Spradbrow and others[3] much progress has been made with optimising the use of vaccines to help control ND. However, there are still many challenges with designing appropriate vaccination programmes for flocks reared in the rural setting, which contain birds (typical flock size of approximately 20 birds) of various ages, as well as for those reared under more semi-intensive conditions (typical flock size of approximately 100 birds), and intensive production, sometimes referred to as emergent commercial production (typical flock size of approximately 1,000 birds; upper limit of up to 2,000 layers / 5,000 broilers) which contain birds of a similar age. Indeed, the rearing of birds under more intensive production has become more important recently with the intensification of agriculture in many LMICs[4,5]. For these more market-orientated producers, having access to affordable, good quality veterinary services, vaccines and pharmaceuticals are essential for producing high quality birds at a competitive price for the urban market[6,7]. Empirical evidence suggests that emergent small-scale poultry producers in LMICs often face problems of survival in a competitive market[7], causing them to rationalise their inputs. Vaccines with a rapid and long duration of immunity would have fewer technical and economical constraints (e.g., lower logistical cost and higher chance for compliance with vaccination schedules and coverage) for the benefit of smallholders who are required to operate on slim profit margins.

The Global Alliance for Veterinary Medicines (GALVmed), together with partners, has conducted laboratory studies designed to investigate the comparative efficacy of live and inactivated ND vaccines, in particular their apparent duration of immunity (DOI). An initial laboratory efficacy study in SPF chickens (unpublished) had demonstrated a DOI, based on challenge, of up to 30 weeks after a single administration of vaccine. Here, we describe a follow-up laboratory study, using a similar experimental design to the first study, in which the DOI has been demonstrated, based on serology and challenge, up to 53 weeks after a single administration of vaccine.

METHODS AND MATERIALS

Experimental Design

Four hundred and twenty SPF White Leghorn chickens were enrolled onto the study. Three hundred and forty were allocated randomly on a first-caught basis to four treatment groups, G1–G4, and vaccinated when six weeks of age. The identity of the vaccines was coded (at the request of the vaccine manufacturers') by treatment group, type of vaccine and vaccinal strain (Table 1). Eighty birds were kept as unvaccinated controls (G5). Each group was housed separately to avoid any possible vaccine contamination by shedding from the live vaccine groups.

Eighty-five birds were vaccinated with one dose/0.1 ml per bird of one of the two commercially available live attenuated vaccines by the oculonasal route; on each occasion the dose was shared

between both the nares and the eyes [it is appreciated that under typical field conditions each bird would normally receive one dose of vaccine in a lower volume, between *ca*. 30–50 µl, and that the whole dose would usually be administered intra-ocularly or, in some cases, intranasally]. On the same day, 85 birds were vaccinated with one dose/0.5 ml per bird of one of the two commercially available inactivated vaccines by the subcutaneous route delivered under the skin of the neck.

Challenge and post-challenge observations

From each group, 20 birds were randomly selected on the same day 24, 40 or 53 weeks after vaccination and transferred to biosafety level 3 animal rooms for challenge. For the challenge, each bird was infected intra-nasally (5.0 $\log_{10}$ EID_{50}/0.1 ml/bird) with a velogenic NDV field isolate, "Goose Paramyxovirus" GPMV 171/06/SA, originating from South Africa and belonging to sub-genotype VII.1.1 (former genotype VIId). Each vaccinated group was kept separately, but in the same room, to which five of the unvaccinated controls were added.

These birds were observed daily for general appearance, behaviour, morbidity and mortality throughout the duration of the study including clinical signs of ND during a 10-day period following each challenge. Any birds showing severe clinical signs of ND were euthanized on welfare grounds. All birds that were found dead or had to be euthanized were subjected to a thorough *post-mortem* examination.

Serology

To monitor the antibody response to vaccination, blood samples were taken from 30 birds on the day of vaccination, and from approximately 20 birds from each vaccinated group at 4, 8 and 15 weeks after vaccination and then again immediately before each challenge at 24, 40 and 53 weeks after vaccination. Blood samples were also taken from 20 unvaccinated controls at the same time points to confirm the absence of extraneous NDV infection.

In order to reduce any variation in the results, sera collected before and after vaccination were tested over a period of two days after the completion of the in-life phase of the study. All sera were inactivated at 56°C for 30 minutes before measuring antibody levels against NDV by a haemagglutination inhibition (HI) test (La Sota antigen, 4 haemagglutinating units). Samples with a $\log_2$ titre ≥3 were deemed to be positive (Vilmos Palya, personnel communication) and protective[8].

Statistical analysis

Average antibody titres, $\log_2$ transformed, were summarised at each measurement point. Protection against mortality was calculated at each challenge time point and 95% exact confidence levels calculated. Log_2 antibody titres, at each of the three challenge time points, were analysed using a linear regression with study group as the independent categorical variable. Log_2 antibody titres at the final challenge time point (53 weeks after vaccination) were analysed using a linear regression with protection against mortality as a binary explanatory variable (not including G5). Similarly, protection against mortality was assessed using a binary logistic regression with the same study group variable (not including G5), at each of the three challenge time points. The models were checked to confirm assumptions were valid. Where the overall differences among groups were significant at the 5% level (Chi-squared p-value) specific comparisons were conducted to compare live and inactivated vaccines. Tukey adjustment was applied for multiple comparisons. R version 4.0.3 was used for all analyses.

RESULTS

Results of the serum antibody responses of birds after vaccination are presented together with supplementary assessment of protection against mortality following challenge under controlled laboratory conditions.

Serology

Sera taken immediately before vaccination were confirmed to be negative for NDV antibodies. The average antibody titres against NDV for each group of birds following a single administration of vaccine when the birds were 6 weeks of age are shown in Figure 1. All vaccinated groups showed a substantial increase in titres after vaccination, peaking at 4 weeks at a level greater than the protective threshold of $\log_2$ titre ≥3[8]. After the peak, titres slowly declined although they remained close to the protective threshold for a further 49 weeks in all vaccinated groups until the end of the study 53 weeks after vaccination. Statistical analysis was carried out on the results after each challenge. On week 24, G1 showed significantly lower $\log_2$ titres than both inactivated vaccines (vs. G3 $p \leq 0.001$, vs. G4 $p=0.015$) however, G2 was only significantly lower than G3 (vs. G3 $p=0.007$, vs. G4 $p=0.667$). There were no significant differences between the two live vaccines ($p=0.382$) or between the two inactivated vaccines ($p=0.262$). On weeks 40 and 53 the only significant differences observed were between each of the vaccinated groups and the control ($p<0.001$ for all) with no significant differences observed between vaccines (p-value range from p=0.180–1.000).

The percentage of birds having an antibody titre equal to, or above, the protective threshold of $\log_2$ ≥3, on week 53 (the final challenge time point) was: G1, 80%; G2, 79%; G3, 90% and G4, 85%. At this same time point, there was a significant difference in the HI antibody titre levels between protected and non-protected birds ($p \leq 0.001$) but no significant differences among the vaccinated groups. Protected birds had a significantly higher HI antibody titre (mean=4.6, 95% confidence interval 4.3–5.0) than non-protected birds (mean=0.9, 95% confidence interval 0–2.1).

Survival during the 10-day period after challenge

All control birds (G5) died, or were euthanized, by day 10 after challenge for each of the three challenge periods. For the week 24 challenge 35 to 60% of the vaccinated birds (G1–G4) died, but for the week 40 challenge only one bird did not survive (G2) and for the week 53 challenge only one to three birds in each of the vaccinated groups either died or were euthanized.

Protection against mortality

The protection of birds against mortality following challenge on weeks 24, 40 and 53 are shown in Figure 2. It shows moderate protection for all four groups at week 24, very high levels of protection for all four groups at week 40 and high levels of protection for all four groups at week 53. A similar picture was seen for the protection against morbidity following each challenge (data not shown).

Overall, there were no significant differences between any of the vaccinated groups at any challenge time point. On week 40, across the four vaccinated groups there was only one non-protected bird, with no significant differences among the four groups ($p=0.422$). Similarly, the difference among the vaccinated groups on weeks 24 and 53 were not significant ($p=0.326$ and $p=0.641$, respectively).

DISCUSSION

SPF chickens were vaccinated when 6 weeks of age with a single administration of either a live or an inactivated commercially available ND vaccine and then challenged 24, 40 or 53 weeks later with a recent velogenic NDV field isolate chosen for being one of the most prevalent ND virus strains in many parts of the world[9]. All unvaccinated control birds either died or had to be euthanized on welfare grounds when showing severe clinical signs of ND during a 10-day period following each challenge. At 4 weeks after vaccination, serology confirmed 'vaccine take' in all four vaccinated groups. Together, these criteria confirmed the validity of the study. The efficacy of each vaccine was determined primarily on comparative serology and protection against mortality following challenge.

High levels of protective circulating antibody were induced in all four vaccinated groups, which peaked at 4 weeks after vaccination and were sustained for at least 53 weeks after vaccination. It has been reported that levels of protection based on serum antibody levels can be over-estimated for vaccines formulated with a La Sota strain of virus when the same strain is also used as the antigen in the HI test[10]. However, similar levels of protection were also demonstrated using an ELISA (Biochek NDV ELISA) (data not shown) – a technique which correlates well with the HI antibody test[11] – although the percentage of birds still having a positive protective titre by ELISA (≥1,159, as defined by Biochek) at 53 weeks after vaccination was slightly lower in both groups given either of the live vaccines compared with those given either of the inactivated vaccines (55–60% vs. 80–85%).

Reasonably high levels of protection against mortality, following challenge, were also demonstrated from 24 weeks up to 53 weeks after vaccination (40–65% at 24 weeks, 85–95% at 53 weeks). The slightly lower level of protection against challenge demonstrated 24 weeks after vaccination was unexpected and cannot be explained fully. An initial laboratory efficacy study in SPF chickens (unpublished) had demonstrated high levels of protection against mortality, between 75–100%, based on challenge infections with the same isolate, 8, 16 and 30 weeks after a single administration of each of the same live and inactivated vaccines. The lower levels of protection seen in this study 24 weeks after vaccination may be due to hormonal changes as the ovaries reach maturity in preparation for the laying period, which occurs between 17–25 weeks of age in an SPF bird[12]. However, this would appear to be unlikely as average HI antibody levels were similar for each vaccinated group at each challenge time point. The contribution of any mucosal-based protective immunity was not measured during this study.

CONCLUSIONS

Overall, these data demonstrate that commercially available live and inactivated ND vaccines can provide up to one-year duration of protective immunity following a single administration in young SPF chickens kept under controlled laboratory conditions. Further studies should be carried out by emergent commercial producers in LMICs that rear intensively managed single-aged flocks to confirm our results where factors such as the presence of maternally derived antibodies, vaccine handling errors (cold chain, usage after reconstitution), poor husbandry practices and breed effects can influence the immune response to vaccination. It is also suggested that such studies should primarily be based on sero-monitoring as the protective HI antibody titre has been well established in the literature.

Finally, it is important to emphasise that the multi-aged structure of flocks reared in the rural setting in many LMICs would likely mean that multiple vaccinations would still be required each

year to provide adequate protection in any new immunologically naïve birds introduced into a flock. Therefore, it is strongly recommended that the current advice on the use of ND vaccines in village and backyard chickens is followed[13].

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AVAILABILITY OF DATA AND MATERIALS:

The authors declare that all datasets generated during the study will be made available in a freely accessible online data repository.

ETHICS APPROVAL AND COMPLIANCE:

The study was conducted at Prophyl Ltd.-Immunolab, Bár, Hungary. All birds were handled in compliance with the provision of Directive 86/609/EEC Hungarian ACT XXVIII/1998, and the study protocol was reviewed by Prophyl's Institutional Animal Welfare Committee.

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Table 1: *Type of vaccine and vaccinal strain used for each treatment group.*

Group	Type of vaccine	Vaccine strain
G1	live	ND I-2
G2	live	ND La Sota
G3	inactivated	ND La Sota
G4	inactivated	ND La Sota
G5	unvaccinated control	—

FIGURE 1 *Average HI log_2 titres (bars show 95% confidence intervals for each challenge on weeks 24, 40 and 53 (168, 283 and 371 days) after vaccination. Log_2 titres ≥3 are protective.*

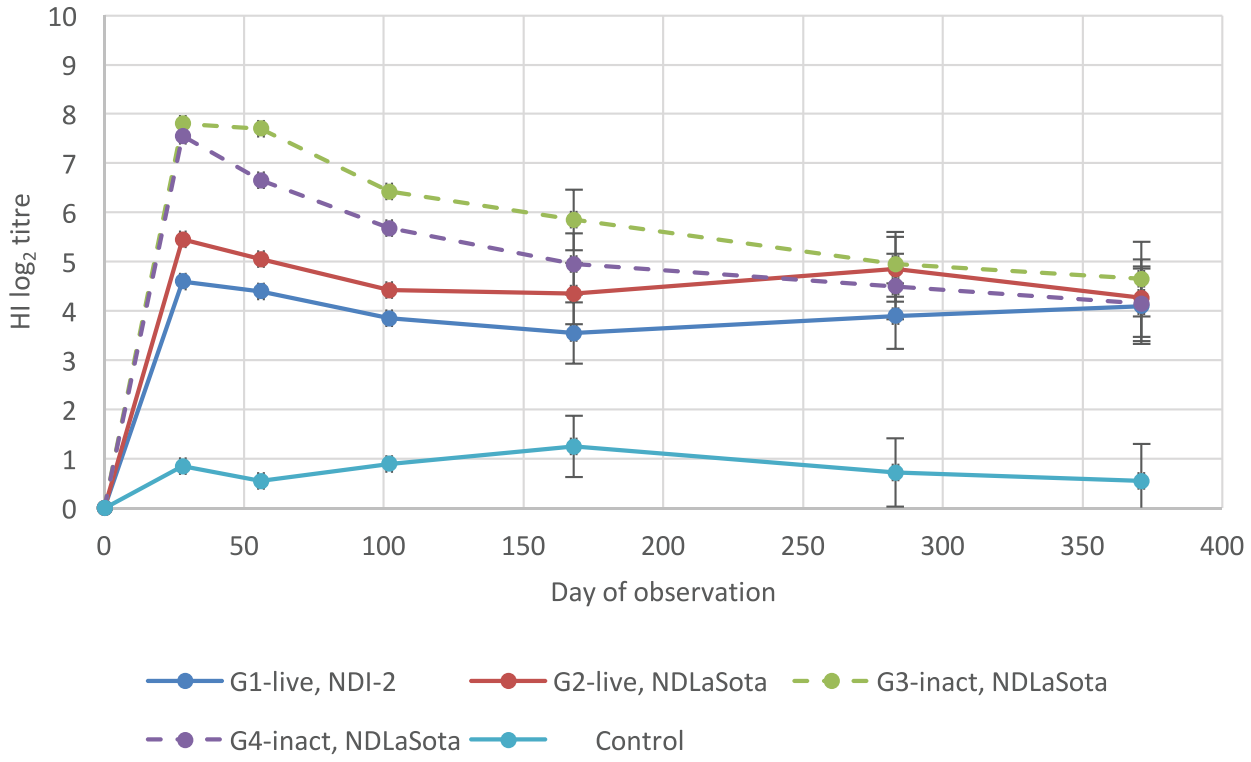
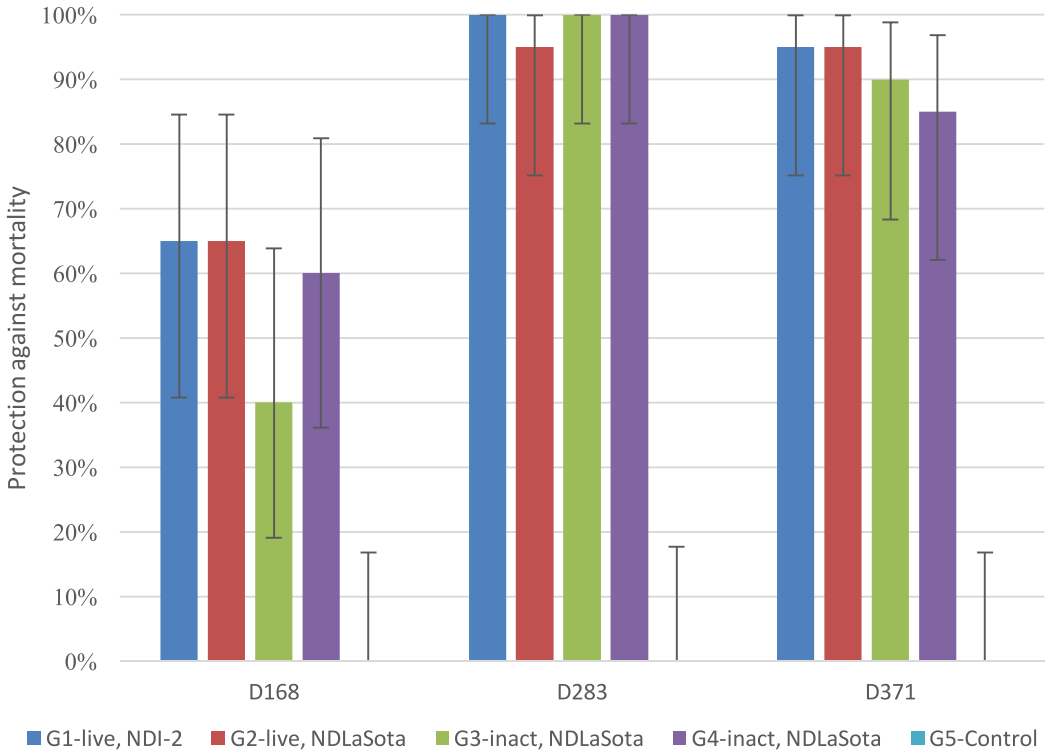


FIGURE 2 *Protection against mortality for each challenge 24, 40 and 53 weeks (168, 283 and 371 days) after vaccination (bars show exact 95% confidence intervals).*



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