

## Safety and Immunogenicity of ND Vaccines Used in Nepal

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### ABSTRACT

*Viral infections, including Newcastle disease (ND), constitute a major health problem in the rapidly growing poultry industry of Nepal. Despite regular vaccination in the commercial farms, ND virus (NDV) outbreaks are frequently reported raising concern over the safety and immunogenicity of live-attenuated vaccines being used. This study was performed to compare the safety and immunogenicity of four commercially available ND vaccines in Nepal after administration at recommended (1X) and higher (10X) dose. There was no safety concern associated with the use of lentogenic live-attenuated ND vaccines even at higher dose. A varying degree of antibody response was observed at recommended and higher doses with the thermostable I-2 vaccine outperforming other groups. A higher dose did not improve antibody response except for the FI vaccine. To prevent widespread outbreaks in future, regular molecular surveillance to identify the circulating strains of NDV together with the periodic evaluation of immunogenicity and protective efficacy of commercial vaccines is necessary in Nepal.*

**Keywords:** I-2 vaccine, Newcastle disease virus, Poultry, Vaccine safety

### INTRODUCTION

Poultry farming is rapidly growing in Nepal [1]. As per the latest livestock statistics, the standing population of fowl is around 83 million which is over 200% greater compared to the population 10 years ago [2]. In Nepal, poultry industry has over a billion-dollar

investment, provides employment to over 150,000 people, and contributes around 4% to the national gross domestic product (GDP) and 8% to the national agricultural GDP (AGDP) [3]. Poultry farming, including the backyard poultry, is an important aspect of income generation in rural areas while chicken meat and eggs serve as the major source of protein in Nepalese kitchen [3].

Viral infections, including avian influenza and Newcastle disease (ND) are the major health problems in the poultry industry of Nepal [1,4,5]. ND is also known as Ranikhet and is caused by Newcastle disease virus (NDV), aka avian paramyxovirus serotype 1, which is a single-stranded RNA virus with non-segmented genome [6]. Based on the pathogenicity in chickens, NDV is classified into very virulent (velogenic), moderately virulent (mesogenic), and avirulent (lentogenic) strains [7]. Clinical signs of NDV infection in chickens vary according to the virus pathotypes. This can range from asymptomatic enteric infection caused by lentogenic NDV to velogenic neurotropic ND characterized by severe neurological and respiratory signs [8]. The World Organization for Animal Health (WOAH) has listed ND as a List A disease for its ability to spread rapidly even beyond the national borders and cause significant socioeconomic and public health consequences [9].

Vaccination against ND is commonly practiced in the commercial farms while its use is limited in the backyard poultry of Nepal [10]. ND vaccination strategy is conventional and mostly utilizes the live-attenuated F, LaSota, I-2 or R2B strains administered through intraocular route, in drinking water, or in muscles [3,11]. First vaccination at 5-7 days is followed by periodic boosts in laying hens [11]. Despite the use of vaccines, ND outbreaks are frequent in Nepal. There were 90 reported outbreaks of ND in Nepal in 2018, that affected over 74,000 chickens and resulted in the deaths of over 7,000 birds [3]. In 2021, there were several outbreaks of ND in the poultry farms of Nepal and outbreaks were reported even in the vaccinated flocks[12]. Despite many years of ND vaccination practice in Nepal, comparative immunogenicity studies of commercial vaccines are very limited. Further, ND outbreaks in the vaccinated farms raised concern over safety of the live-attenuated vaccines. Hence, this study was performed to determine the safety and immunogenicity of four (LaSota, B1, F and I-2) commercially available and commonly used ND vaccines in Nepal after administering at recommended (1X) or higher (10X) doses.

## **MATERIALS AND METHODS**

### **Study Design, Vaccination and Sample Collection**

This study was carried out at the Veterinary Standards and Drug Regulatory Laboratory (VSDRL) Budhanilkantha, Kathmandu from July to September 2021. VSDRL is the regulatory body for the approval of veterinary vaccines in Nepal. Approval for this research was obtained from VSDRL on 24th June 2021 (letter number: 2077-78/49).

Day old-layer chicken (n=97) of (Brown Nick, H&N International) were purchased from a commercial hatchery and raised with ad libitum feed and water in the research facility of VSDRL. They were raised in a group of 10 in a partitioned chamber. ND vaccines of the lentogenic strains; viz. LaSota (Pestikal LaSota SPF, Genera Croatia), B1 (Himmvac Newcastle B1, KBNP, INC.), F (RaniVax Plus Vet Initial, Incepta Vaccine Ltd.), and I2 (Jovac NDV I2, Jordan Bio Industries Center); were purchased from commercial sources and cold chain (2-80C) was maintained as suggested by the producer until vaccination was carried out. Fifty animals were used for recommended dose (1X) vaccine study while remaining 47 were used for high-dose (10X) vaccine study. In regular dose vaccine study, birds (n=10/group) were immunized with live-attenuated vaccines of lentogenic strains (LaSota, B1, F, or I-2) through supraconjunctival route at 10th day using the dose recommended by the producer. In high dose (10X) vaccine study, birds (n=7-10/group) were immunized with the same lentogenic strains (LaSota, B1, F, or I-2), but with a dose 10 times greater than the recommended. For that, the vaccine vials were diluted as per the producer's instruction; recommended dose (1X) birds were given single dose (1 drop) while the high dose (10X) group were given 10 drops. In both regular and high dose studies, 10 birds were used as unvaccinated control.

To evaluate safety of the vaccines and doses, birds were monitored once daily during the time of offering feed to animals for 21-days post-vaccination for any ND-related enteric (i.e., greenish and watery diarrhea), respiratory (viz., sneezing, gasping, nasal discharge, and coughing), and neurological (viz., depression, muscle tremors, droopy wings, circling, and paralysis) signs and mortality [13].

To measure antibody response, blood was collected from the wing vein and transferred in a clot activator tube. Blood tubes were centrifuged at 4000 rpm for 5 minutes, serum samples were collected and stored at -80°C.

### **Measurement of antibody response**

For the detection of the antibody titers in the serum samples, indirect Enzyme-linked Immunosorbent Assay (ELISA) was performed using the ID screen Newcastle disease indirect ELISA kit (IDvet, Grabels, France). Two different dilutions of serum were used to test antibody levels. This kit has been used previously to determine antibody titers against NDV [14]. Titers above 641 were considered as having positive level as recommended by the company.

### **Data Analysis**

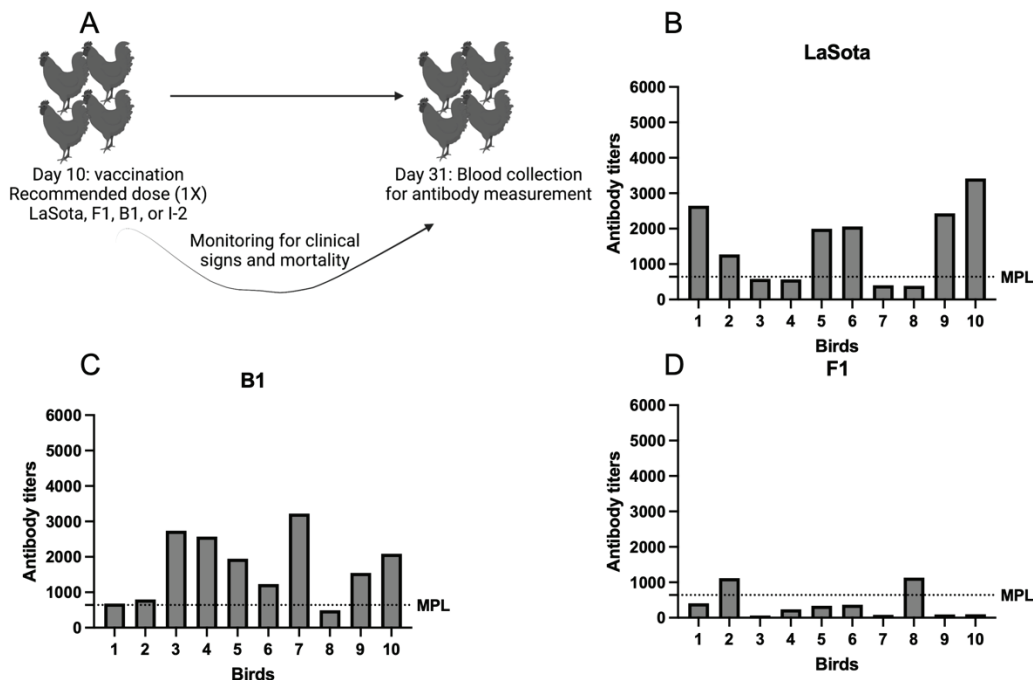
Data were analyzed using GraphPad Prism software version 9.4.1. (GraphPad, CA, USA). Antibody levels among vaccine groups after recommended or higher dose vaccination were compared using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Antibody levels in same vaccine group after recommended

and higher dose vaccinations were compared using unpaired T-test. Similarly, changes in frequencies of positive (having antibodies above minimum protective level, MPL) after higher dose vaccination in same vaccine group was compared using Chi-square test. Data were considered statistically significant at  $p < 0.05$ .

## RESULTS

To test the safety of commercial ND vaccines, all the birds vaccinated at recommended or higher dose were monitored daily for 21 days post vaccination. Birds were monitored for mortality and development of any clinical signs that leads to the suspicion of ND. We did not observe any mortality during the experiment. Similarly, none of the birds showed any clinical signs that lead to the suspicion of ND even at 10-times higher doses than recommended.

After vaccination with recommended dose (1X) (**Figure 1A**), 60% (6/10) birds in LaSota (**Figure 1B**), 90% (9/10) in B1 (**Figure 1C**), 20% (2/10) in F1 (**Figure 1D**), and 100% (10/10) birds in I-2 (**Figure 1E**) vaccine group had antibodies above the minimum protective level (MPL). While comparing antibody levels across the vaccine groups after recommended dose vaccination, I-2 vaccine group showed significantly greater antibody titers than LaSota, B1 and F1 vaccine groups (**Figure 1F**). Antibody levels between LaSota and B1 groups were comparable. The F1 vaccine group had the lowest antibody response which was significantly lesser than all other three vaccine groups (**Figure 1F**).



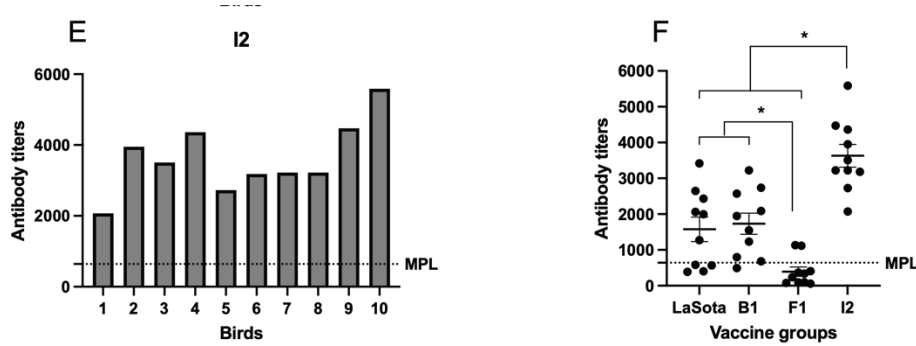
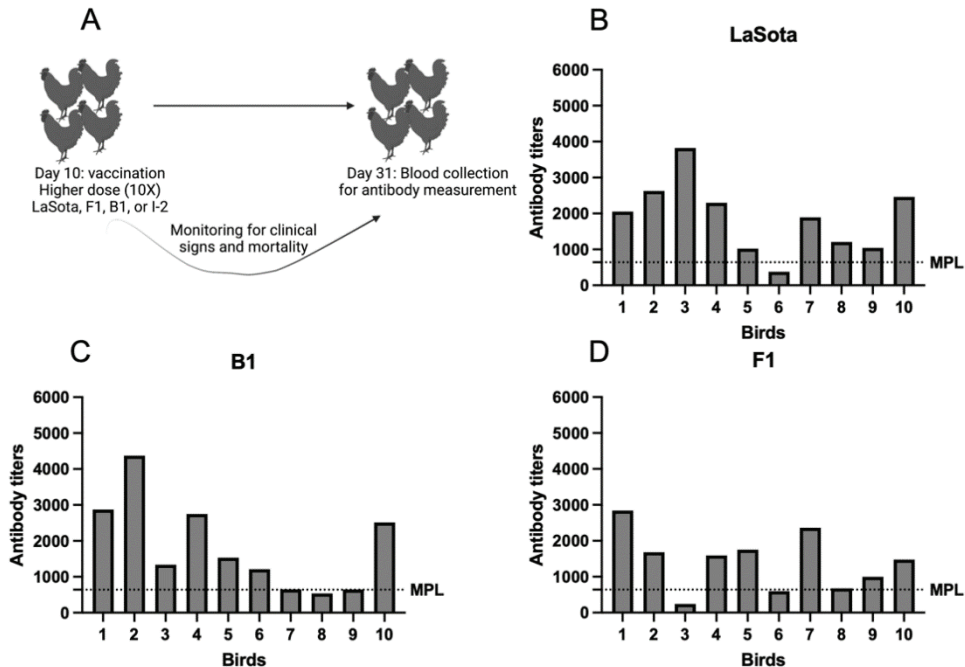


Figure 1: Antibody response after (A) recommended dose (1X) vaccination with (B) LaSota, (C) B1, (D) F1, and (E) I-2 vaccines. (F) Antibody levels among vaccine groups were compared using one-way ANOVA followed by Tukey’s multiple comparisons test. MPL refers to the minimum protective level and asterisk (\*) refers to significant differences between the indicated groups at  $p < 0.05$ .

After vaccination with higher dose (10X) (**Figure 2A**), 90% (9/10) birds in LaSota (**Figure 2B**), 90% (9/10) in B1 (**Figure 2C**), 80% (8/10) in F1 (**Figure 2D**), and 100% (7/7) birds in I-2 (**Figure 2E**) vaccine group had antibodies above the minimum protective level. The I-2 vaccine group induced antibody levels significantly greater than other three vaccine groups even after the higher dose vaccination (**Figure 2F**). At higher dose, antibody levels among LaSota, B1, and F1 groups were statistically similar (**Figure 2F**).



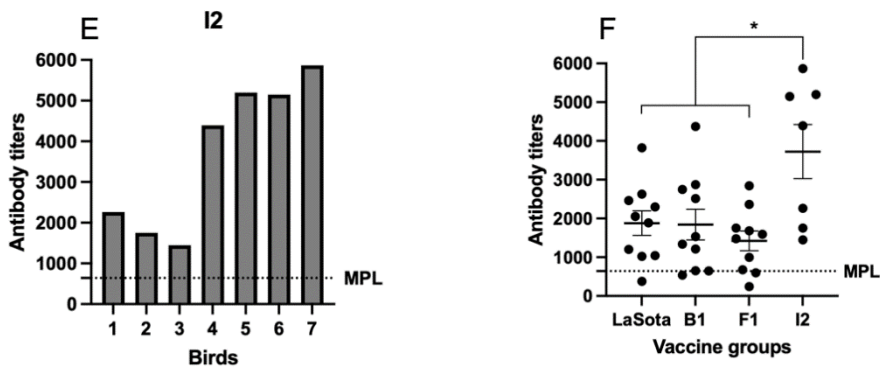


Figure 2: Antibody response after higher (10X) dose vaccination (A) with (B) LaSota, (C) B1, (D) F1, and (E) I-2 vaccines. (F) Antibody levels among vaccine groups were compared using one-way ANOVA followed by Tukey's multiple comparisons test. MPL refers to minimum protective level and asterisk (\*) refers to significant differences between the indicated groups at  $p < 0.05$ .

Since there was no safety concern after recommended or higher dose vaccination, we next wanted to understand if higher dose vaccination could induce greater antibody responses than recommended dose. Higher dose vaccination did not increase antibody titers in LaSota (**Figure 3A**), B1 (**Figure 3B**) and I-2 (**Figure 3D**) vaccine groups. However, a higher dose vaccination induced significantly higher level of antibody response in F1 vaccine group (**Figure 3C**). Subsequently, we wanted to understand if higher dose vaccination increased the frequencies of birds that produced antibodies above the minimum protective level or not. Though higher dose vaccination with LaSota increased the frequency of birds with antibodies above MPL from 60% to 90%, it was not statistically significant (**Figure 4A**). Difference was not observed in B1 vaccine group (**Figure 4B**). However, in F1 vaccine group the frequency of birds with antibodies above MPL changed from 20% during recommended dose vaccination to 80% after higher dose vaccination and it was significantly improved (**Figure 4C**).

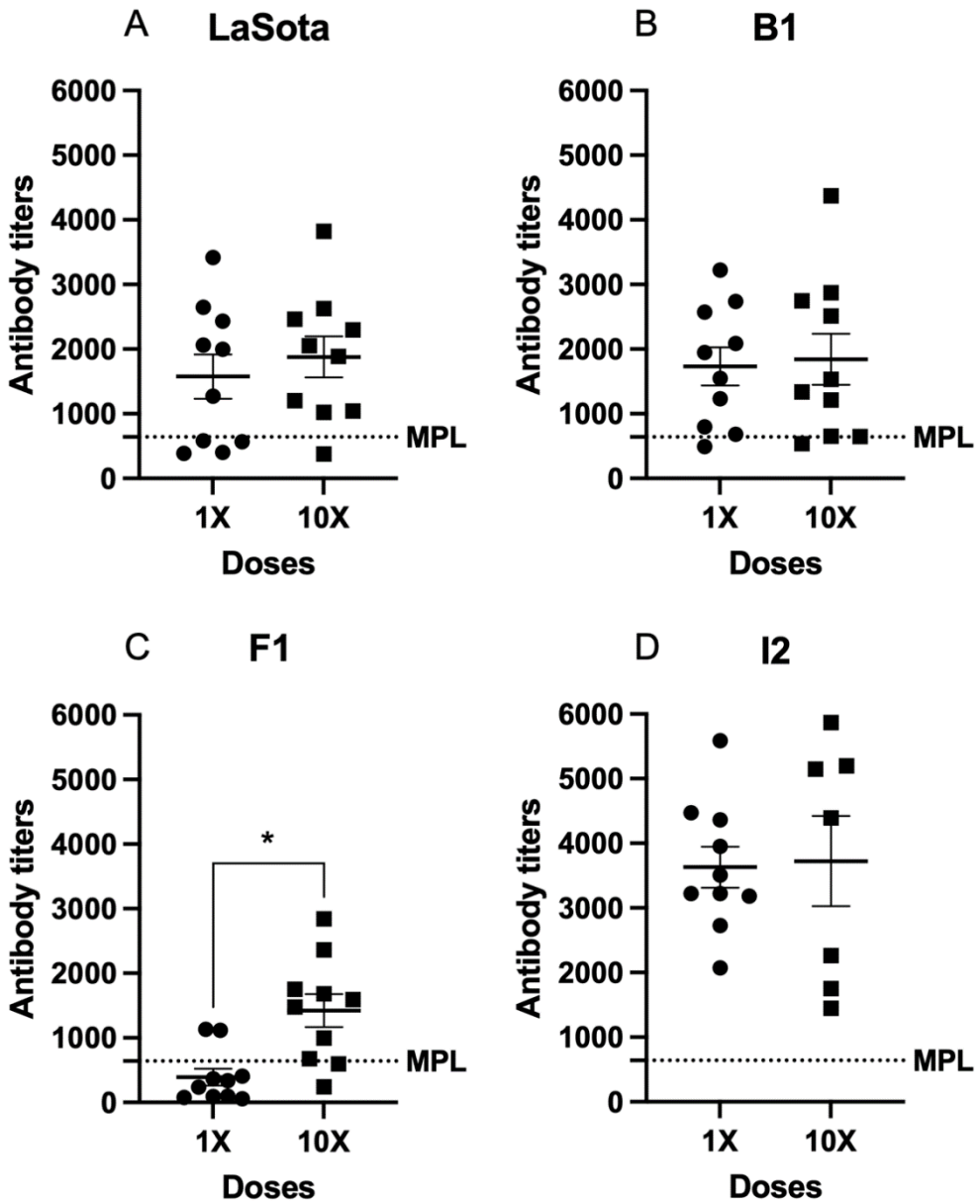


Figure 3: Antibody response after recommended (1X) and higher (10X) dose vaccination with (A) LaSota, (B) B1, (C) F1, and (D) I-2 vaccines were compared using unpaired T-test. Asterisk (\*) refers to significant differences between the vaccine doses at  $p < 0.05$ .

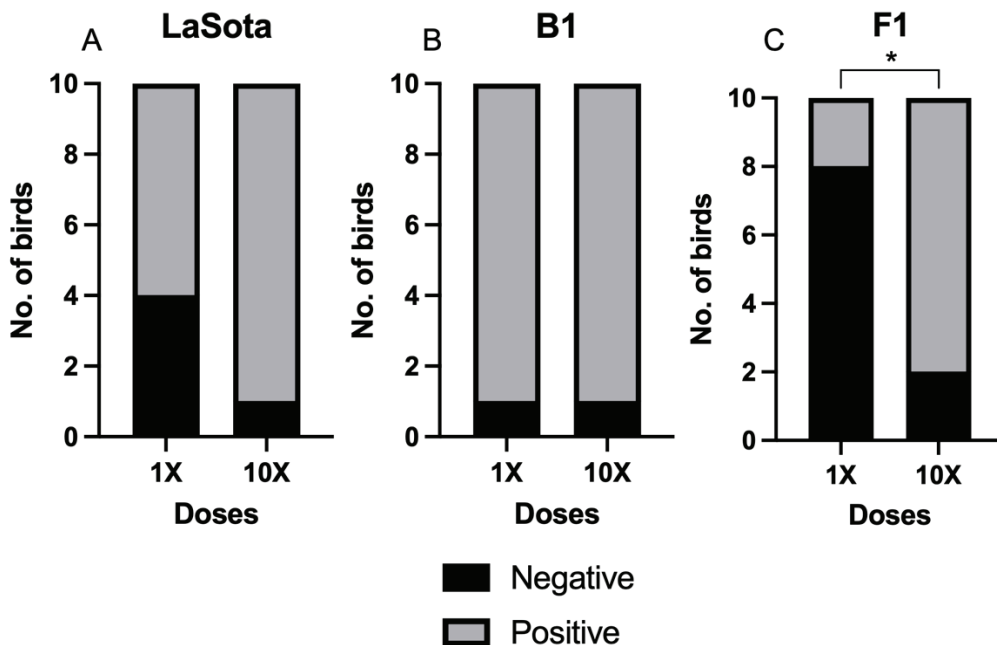


Figure 4: Frequencies of birds having antibodies above minimum protective levels after recommended (1X) versus higher (10X) dose vaccination with (A) LaSota, (B) B1, and (C) F1, vaccines were compared using Chi-square test. Asterisk (\*) refers to significant differences between the vaccine doses at  $p < 0.05$ .

## DISCUSSION

Vaccination together with stringent biosecurity measures are practiced worldwide to prevent NDV infection in the farm [15]. Most of the commercial poultry farms in Nepal also practice regular vaccination against NDV [10]. Despite vaccination, ND outbreaks are common each year leading to a huge economic loss [1,3]. Use of live-attenuated vaccines even raises concern over their possibility to cause outbreaks specially if there is wrong dosing of vaccines. In this study, for the first time, we compared four different lentogenic strain ND vaccines commercially used in Nepal for their safety and immunogenicity. Single dose immunization of chickens with recommended as well as 10-times higher dose of LaSota, B1, F1, and I-2 vaccines did not cause any clinical signs that can be assigned to ND or mortality. This showed that vaccines using lentogenic strains are safe and unlikely to cause disease outbreaks in the farms even when used at higher doses. Thus, recent outbreaks of NDV in Nepal, including those in the vaccinated farms, is unlikely to be linked to the use of live-attenuated vaccines. Rather, a recent study showed that NDV genotype VIIc (GVIIc) was the probable causative agent for the widespread outbreak of ND in 2021 in Nepal [10]. NDV GVIIc represents a velogenic strain with severe clinical outcomes [16,17]. Studies in other countries have shown



that vaccination with lentogenic strain, like LaSota, provides poor protection against virus strains of NDV GVII and viruses of this genotype still circulate in the vaccinated farms [16,18,19]. Thus, it is likely that the vaccines being used in Nepal are unable to induce antibody responses cross-protective against the circulating NDV strains leading to many outbreaks in the farms. Only a more rigorous virus challenge study can confirm this which was beyond the scope of this study.

Birds immunized with I-2 vaccine produced significantly greater antibodies than other vaccine groups. The better performance of this vaccine, in part, may be associated with its thermostability. I-2-based NDV vaccines are stable at room temperature for several days [10,20]. Though due care was given to maintain cold chain at our end, it is uncertain whether there was any breakage in the proper cold chain maintenance at any stage of the vaccine storage and transportation chain. This is important as live-attenuated NDV vaccines deteriorate quickly within few hours if cold chain is not maintained [21].

An earlier study from Nepal showed that LaSota strain vaccination in village chickens is immunogenic and efficacious against NDV infection [21]. A recent study also showed that LaSota vaccination was safe and induced a protective level of antibodies in 98% of vaccinated birds at 28 days post-vaccination [22]. Field verification study of ND I-2 vaccine also showed that it can induce protective levels of antibodies in 50-100% of birds at different study locations [23]. Moreover, ND I-2 vaccine was shown to induce protective levels of antibodies up to 90 days post vaccination in village chickens [24]. A recent study showed that thermostable I-2 NDV vaccine in tablet formulation is effective against NDV, including a virulent 2021 outbreak strain with 85% efficacy [10]. These findings, together with ours, highlight the importance of continuous use of NDV vaccination in the farms. However, whether antibodies produced by these vaccines are cross-protective against endemic and emerging NDV strains circulating in the country also need to be tested. Vaccines against ND, including F, LaSota, R2B and I-2, are produced in Nepal [3]. In-country vaccine production is not sufficient and hence ND vaccines are also imported from different countries [1]. A total of about 184 million doses of ND vaccines were imported in Nepal in the fiscal year 2020/21 [25]. Thus, quality assessment of vaccines produced within the country and imported from elsewhere in a regular manner is important to make sure they provide the expected level of protection in the field against the circulating strains.

## **CONCLUSION**

The NDV vaccines being used in Nepal appear safe and produce a varying degree of antibody responses. However, they may not be protective enough against NDV strains circulating in the farms. Regular molecular surveillance to identify the circulating strains of NDV in Nepal and periodic evaluation of immunogenicity and protective

efficacy of commercial vaccines against the circulating strains of NDV is important to prevent its widespread outbreaks in the future.

**Author Contributions:** Conceptualization, S.M., N.U., and P.S.; methodology, S.M., N.U. and P.S.; software, S.D; validation, S.M. and S.D.; formal analysis, S.D.; investigation, S.M., N.U., P.S and S.S; resources, N.U., P.S., S.S., and S.S.; data curation, S.D.; writing—original draft preparation, S.M., S.D.; writing—review and editing, S.M., N.U. and S.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** The work is a part of internship research of S.M. Research was carried out as a routing experiment in Veterinary Standards and Drug Regulatory Laboratory (VSDRL), **Budhanilkantha, Kathmandu**, a national competent body for vaccine testing.

**Data Availability Statement:** Data will be available upon reasonable request.

**Acknowledgments:** We would like to acknowledge Veterinary Standards and Drug Regulatory Laboratory (VSDRL), **Budhanilkantha, Kathmandu** for providing the resources. We would also like to acknowledge Dr. Sujeeta Pokharel and Dr. Sita Rijal from VSDRL for their help during the research.

**CONFLICTS OF INTEREST:** The authors declare no conflict of interest.

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