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Development of an improved vaccine evaluation protocol to compare the efficacy of Newcastle disease vaccines

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ABSTRACT

While there is typically 100% survivability in birds challenged with vNDV under experimental conditions, either with vaccines formulated with a strain homologous or heterologous (different genotype) to the challenge virus, vaccine deficiencies are often noted in the field. We have developed an improved and more stringent protocol to experimentally evaluate live NDV vaccines, and showed for the first time under experimental conditions that a statistically significant reduction in mortality can be detected with genotype matched vaccines. Using both vaccine evaluation protocols (traditional and improved), birds were challenged with a vNDV of genotype XIII and the efficacy of live heterologous (genotype II) and homologous (genotype XIII) NDV vaccines was compared. Under traditional vaccination conditions there were no differences in survival upon challenge, but the homologous vaccine induced significantly higher levels of antibodies specific to the challenge virus. With the more stringent challenge system (multiple vaccine doses and early challenge with high titers of vNDV), the birds administered the homologous vaccine had superior humoral responses, reduced clinical signs, and reduced mortality levels than those vaccinated with the heterologous vaccine. These results provide basis for the implementation of more sensitive methods to evaluate vaccine efficacy.

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1. Introduction

Newcastle disease (ND) is one of the most important diseases affecting poultry world-wide. It is caused by virulent strains of Newcastle disease virus (vNDV), also known as avian paramyxovirus serotype 1 (APMV-1) [1,2]. NDV belongs to the genus *Avulavirus* of the family *Paramyxoviridae* [1,2]. The virus genome consists of a single-stranded, negative sense, non-segmented, RNA molecule with approximately 15.2 kb which encodes six structural proteins: nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion (F), hemagglutinin-neuraminidase (HN), and RNA polymerase (L) [1,2]. Genome sequence analysis of multiple NDV isolates allowed their classification into two major classes (class I and II). Class II is subdivided into at least eighteen genotypes (I to XVIII), and contains most of the vNDV strains circulating in poultry around the world [3–5]. According to the OIE, virulent strains are defined as those NDV containing an F protein cleavage site with at least three basic amino acids between position 113 and 116, and a phenylalanine at position 117, or an intracerebral pathogenicity index $\geq$0.7 [6]. Infection with vNDV in countries with endemic disease results in significant economic losses to the poultry industry due to decreased growth rates and to drop in egg production in vaccinated birds, or due to high levels of mortality in naïve or poorly vaccinated birds. Control of ND requires implementation of expensive culling measures, preventive vaccination and biosecurity measures to prevent the disease from spreading [1,7].

Several studies have concluded that classical live or inactivated vaccines made of viruses of genotype I or II (heterologous), when administered to healthy birds in adequate doses, are capable of

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Abbreviations

ND	Newcastle disease
vNDV	virulent Newcastle disease virus
NDV	Newcastle disease virus
APMV-1	avian paramyxovirus serotype 1
HI	hemagglutination inhibition
SPF	specific pathogen free
PK33	vNDV isolate Chicken/SPVC/Karachi/NDV/33/2007
LS-wt	NDV LaSota
rLS	recombinant LaSota
SEPRL	Southeast Poultry Research Laboratory
USDA	United States Department of Agriculture
ARS	Agricultural Research Service
CEF	chicken embryo fibroblasts
ECEs	embryonating chicken eggs
RT-PCR	reverse transcription polymerase chain reaction
MVA/T7	recombinant modified vaccinia virus Ankara expressing the T7 RNA polymerase
MOI	multiplicity of infection
pFLC-LaSota	recombinant plasmid containing the NDV LaSota complete genome
rLS-PK33	NDV vaccine candidate containing F and HN from genotype XIII virus into the LaSota backbone
HA	hemagglutination assay
BHI	brain heart infusion
EID_{50}	median embryo infective dose
ICPI	intracerebral pathogenicity index
MDT	mean death time
VI	virus isolation
dpv	days post vaccination
dpc	days post challenge.

preventing clinical disease and death caused by infection with vNDV strains from most of the current circulating genotypes, but do not completely prevent viral replication and shedding [8–14]. Previously, advantages of the use of genotype matched (homologous) vaccines have been demonstrated only at the level of control of viral shedding [15]. Studies performed in our laboratory, and by others, demonstrated decreased oropharyngeal virus shedding after challenge when the genotype of the vaccine virus was homologous to the genotype of the challenge virus compared to vaccines that did not match the genotype of challenge virus [11,16–18]. In addition, it was demonstrated that decreasing shedding of the challenge virus can potentially reduce horizontal transmission of vNDV [18]. Unfortunately, *Title 9* of the Code of Federal (9 CFR) regulations does not take into account the determination of viral shedding after challenge as part of the NDV vaccine evaluation process as it does with other vaccines such as avian infectious bronchitis vaccine [19].

Under optimal experimental conditions, ensuring the administration of appropriate doses of vaccine and the sufficient time to induce an immune response, no statistical differences in morbidity and mortality rates between homologous and heterologous NDV vaccines have been observed. It has been argued that new NDV vaccines are not necessary because all NDV strains belong to a single serotype. Furthermore, since the current commercial NDV vaccines protect equally well against morbidity and mortality caused by any virulent NDV strain, again, there is no reason to discontinue the use of the NDV vaccine strains formulated with strains that were isolated in the late 1940s [20]. This justification, based on the use of vaccine evaluation protocols that only measured survival under optimal conditions (using a high vaccine dose and challenging after three weeks post vaccination), along with the fact that the currently circulating vNDV stains belong to genotypes for which no natural lentogenic variants exist, have hampered the development of new NDV vaccines.

Failure to control vNDV with current vaccines and vaccination programs in countries where the virus is endemic (Israel, Egypt, China, Pakistan, Korea, South Africa) [14,21–30], challenges the previous justification and underscores the need to improve NDV vaccines, and to re-evaluate the current system for evaluating NDV vaccine efficacy. While results from experimental conditions document the ability of NDV vaccines formulated with NDV strains heterologous to challenge virus to prevent morbidity and mortality, the results in the field are not as convincing. This disconnect between the experimental and the field efficacy of vaccines has encouraged us to develop a more stringent vaccine evaluation protocol, and to demonstrate that it is possible to measure survival differences after challenge in birds vaccinated with homologous vs. heterologous NDV vaccines.

In the present study, we have developed a live attenuated chimeric ND virus that expresses the surface glycoproteins (F and HN) from a recent genotype XIII NDV isolate to document that the NDV vaccine protocol can be improved. Virulent ND viruses from genotypes XIII have been circulating and causing important outbreaks in Pakistan and are very closely related to viruses circulating in Iran and India (unpublished observations, Afonso C. L.). The resulting chimeric vaccine (homologous) was compared to the LaSota vaccine (heterologous) for its effect on preventing clinical signs and virus shedding after challenge following standard and suboptimal dose vaccination schemes on specific pathogen free (SPF) birds.

Because of the rapid mortality caused by vNDV (4–6 days post infection) [12,31,32], it is also important to develop vaccines that induce rapid immune responses. Here we demonstrate that when an experimental evaluation scheme mimics field conditions (early and strong challenges); it is possible to measure significant differences in morbidity and mortality between homologous and heterologous NDV vaccines, being the homologous vaccine significantly more effective.

2. Materials and methods

2.1. Viruses

Virulent NDV isolate Chicken/SPVC/Karachi/NDV/33/2007 (GenBank: GU182331) (PK33) was used in the present study as source of the F and HN genes to generate the chimeric vaccine candidate and as challenge virus. PK33 was isolated in 2007 from commercial poultry in Karachi, Pakistan during a ND outbreak [33–35], and has been classified into genotype XIII [4]. NDV strain LaSota (LS-wt) is used worldwide as a live or inactivated vaccine and was used here as a control vaccine in the immunization-challenge experiments, comparing its performance to that of the new chimeric vaccine candidate developed and tested in the present study. These two viruses, along with a recombinant LaSota (rLS) virus used as a backbone for the vaccine, were obtained from the Southeast Poultry Research Laboratory (SEPRL, USDA-ARS, Athens, GA) repository and propagated in 9–11 day-old specific-pathogen-free (SPF) embryonating chicken eggs (ECEs). The recombinant modified vaccinia virus Ankara expressing the T7 RNA polymerase (MVA/T7) (a gift from Bernard Moss, National Institute of Health) was propagated in primary chicken embryo fibroblast cells (CEF) and used to rescue the chimeric viruses.

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