



Research paper

Onset and long-term duration of immunity provided by a single vaccination with a turkey herpesvirus vector ND vaccine in commercial layers



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ARTICLE INFO

Keywords:

Newcastle disease

Vaccination

Recombinant vaccine

rHVT-ND vaccine

ABSTRACT

The onset and duration of immunity provided by a recombinant ND vaccine using HVT virus as vector (rHVT-ND) was followed up to 72 weeks of age in commercial layer chickens after single application or as part of two different vaccination regimes including conventional live and killed ND vaccines. Efficacy of the different vaccination programmes was checked, from 3 to 72 weeks of age, by serology as well as by challenges with a recent velogenic NDV isolate belonging to genotype VII. Assessment of protection was done based on the prevention of clinical signs and reduction of challenge virus shedding via the oro-nasal and cloacal routes.

Single vaccination with the rHVT-ND vaccine at one day of age provided complete or almost complete (95–100%) clinical protection against NDV challenges from 4 weeks of age up to 72 weeks of age when the latest challenge was done. Shedding of challenge virus both by the oro-nasal and cloacal route was significantly reduced compared to the controls. Booster vaccination of rHVT-ND vaccinated birds with conventional ND vaccines significantly increased the level of anti-NDV serum antibodies and further reduced the oro-nasal excretion of challenge virus.

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1. Introduction

Newcastle disease (ND) is one of the most important diseases of poultry and other bird species and is a global threat

Abbreviations: APMV-1, avian paramyxovirus serotype 1; dpch, days post-challenge; dpi, days post-infection; EDS, egg drop syndrome; ELD₅₀, median embryo lethal dose; FP, fowl pox; HAU, haemagglutinating unit; HI, haemagglutination inhibition; HVT, Turkey herpesvirus/herpesvirus of turkeys; IB, infectious bronchitis; IBD, infectious bursal disease; MDA, maternally derived antibodies; ND, Newcastle disease; NDV, Newcastle disease virus; p.v., post-vaccination; rHVT-ND vaccine, recombinant HVT ND vaccine; rHVT/F, recombinant HVT vaccine expressing F gene of NDV; SPF, specified pathogen free.

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to commercial poultry production. Velogenic strains of ND virus (NDV) cause a devastating disease of poultry throughout Asia, Africa, Middle East, Central and South America till today. NDV, also known as avian paramyxovirus serotype 1 (APMV-1) virus, is a member of the genus *Avulavirus* in the *Paramyxoviridae* family. NDV strains are classified into velogenic (highly virulent), mesogenic (medium virulent) and lentogenic/apathogenic (mild or non-virulent) pathotypes on the basis of their pathogenicity for chickens (Cattoli et al., 2011). The molecular basis for pathogenicity of NDV is mainly determined by the amino acid sequence of the protease cleavage site of the F protein, but other proteins (e.g., V and HN) also contribute to the determination of virulence (Dortmans et al., 2011). The principal antigens that elicit protective immune response are HN and F (Kumar et al., 2011).

Considerable genetic diversity has been detected among NDV strains, but viruses sharing geographical and/or epidemiological relations tend to fall into specific lineages or clades. Phylogenetic analysis revealed that two major separations occurred during the history of ND. An ancient division in the original reservoir (wild waterfowl species) led to two basal sister classes, class I and II. Ancestors of only class II viruses colonized the chicken populations and subsequently converted to virulent forms. Division continued to occur in the secondary host (chicken) resulting in the branching-off class II viruses, compromising the recent velogenic genotypes (Czeglédy et al., 2006). Different genotypes of class II show geographical region specific occurrence and temporal distribution with apparent links to well defined epizootics (Miller et al., 2010). In the past decades, there has been a major shift in the genotypes of NDV strains that have been identified as prevalent in poultry.

Control of Newcastle disease, in addition to good biosecurity practices, primarily relies on preventive vaccination of flocks and culling of infected and at risk of being infected birds (protection zone). Since all ND viruses belong to a single serotype, thus by definition any NDV strain utilized to prepare a vaccine should induce protection against morbidity and mortality following challenge with any virulent NDV strains (Bwala et al., 2009; Perozo et al., 2012). Most countries, where poultry is raised commercially and where the disease is endemic, rely on vaccination to keep the disease under control. At the present time, most vaccination programmes for ND include the use of live (containing lentogenic or apathogenic NDV strains) or inactivated (killed) vaccines in order to induce a good protective immunity while producing minimal adverse effects in the birds. Both types of vaccine have their advantages and disadvantages, but the occurrence of continuous ND outbreaks in commercial poultry flocks in many part of the world indicate that routine vaccination in the field often fails to induce sufficiently high levels of immunity to control ND. Current ND vaccines widely used in commercial poultry can protect the vaccinated birds from disease and reduce virus shedding, but cannot prevent vaccinated birds from being infected and subsequently shedding the virus, and potentially transmitting it to susceptible birds (Dortmans et al., 2012). The presence of maternally derived antibodies (MDA) also interferes with the establishment of an early and persisting immunity after single or even repeated vaccination during the first 2–3 weeks of life.

A further consideration regarding conventional ND vaccines is that they might induce a better protection against viruses isolated in past epizootics than against the ones causing the recent outbreaks (Hu et al., 2009; Kapczynski and King, 2005; Miller et al., 2009, 2007; van Boven et al., 2008). The newly emerging virulent NDV strains (genogroup V and VII) have been suggested to have the ability to overcome vaccination barriers. While the causes of the apparent vaccine failures in the field have not clearly been identified in most cases, the efficacy of available conventional vaccines is being questioned based on the findings of the above referred papers. On the contrary, results of Dortmans et al. (2012) indicated that poor vaccination practices and or concurrent infection with

immunosuppressive pathogens rather than antigenic variation may be responsible for poor immunity levels.

The shortcomings faced when current ND vaccines and vaccination schemes are used necessitated the search for more potent vaccines, which can be applied more efficiently in the control of ND. A promising approach to achieve the above goals is the development of vector vaccines. The first and foremost advantage for using a vector vaccine is its safety. Some live vaccines used in the poultry industry have some undesirable side-effects, such as horizontal transmission, reversion to virulence and vaccine reactions, any of which may result in disease or production loss (Alexander, 2008). With a vector vaccine, the gene(s) of the donor pathogen is inserted into a 'safe' vector, thus separating the key protective antigen from the live donor organism and its undesirable side-effects.

Herpesvirus of turkeys (HVT) has already been used worldwide both as live vaccine and as vector for recombinant polyvalent vaccine in poultry. Recombinant vaccines against ND using the herpesvirus of turkeys (rHVT) as vector contain and express the protective antigens, typically the F and/or HN glycoprotein (Morgan et al., 1992). HVT-based recombinant vaccine containing the F protein (rHVT/F) elicited immune response and provided protection against lethal challenge with a velogenic strain of NDV (Morgan et al., 1992). As in case of HVT itself (Purchase et al., 1971), long term virus persistence was shown for rHVT also in inoculated chickens (Reddy et al., 1996), and furthermore, the expression of the F gene was measurable even after 30 weeks of a single s.c. inoculation of day-old chickens (Saitoh et al., 2003). Additionally, the immune response evoked by the rHVT/F construct appeared to be less sensitive to interference with MDA, which adds further useful characteristic to this vector vaccine (Morgan et al., 1993). Beyond that, the application of this kind of vaccine proved to be safe since it did not have adverse effects on hatchability or the survival of in-ovo and post-hatch vaccinated specified-pathogen-free (SPF) chickens (Morgan et al., 1992; Reddy et al., 1996).

Efficacy of a commercialized rHVT-ND vaccine (Saitoh et al., 2003) expressing the F protein of the avirulent D26/76 genotype I NDV strain (Sato et al., 1987, GenBank accession number: M24692) has already been shown in SPF layers and in commercial broilers with MDA in previous publications (Rauw et al., 2010; Palya et al., 2012). The aim of the study presented here was to evaluate the onset and duration of immunity of the same vaccine in commercial layers up to 72 weeks of age after single ND vaccination at day old or as a component in ND vaccination programmes including conventional live and killed vaccines.

2. Materials and methods

2.1. Chickens

Layers (Lohmann, Brown Lite) with MDA to NDV and MDV were purchased from a commercial hatchery. Chickens were randomly assigned to four groups according to their vaccination programme against ND. Number of day-old birds was 296, 280, 180 and 223 in groups 1, 2, 3 and 4, respectively. All groups were kept in isolated

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