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Field vaccinated chickens with low antibody titres show equally insufficient protection against matching and non-matching genotypes of virulent Newcastle disease virus

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ABSTRACT

Newcastle disease (ND) is a severe threat to the poultry industry and is caused by virulent strains of Newcastle disease virus (NDV). Many countries maintain a vaccination policy, but NDV is rapidly evolving as shown by the discovery of several new genotypes in the last decades. We tested the efficacy of the currently used classical commercial ND vaccine based on the genotype II strain VG/GA, applied under standard field conditions, against outbreak strains. Field vaccinated broilers were challenged with four different viruses belonging to genotype II, V or VII. A large proportion of field vaccinated broilers showed suboptimal immunity and the protection level against early and recent NDV isolates was dramatically low. Furthermore, there were no significant differences in protection afforded by a genotype II vaccine against a genotype II virus challenge compared to a challenge with viruses belonging to the other genotypes. This study suggests that the susceptibility of vaccinated poultry to NDV infection is not the result of vaccine mismatch, but rather of poor vaccination practices.

1. Introduction

Newcastle disease (ND) is one of the most important infectious diseases of poultry and has the potential to cause devastating economic losses. Its causative agent is Newcastle disease virus (NDV), also known as avian paramyxovirus type 1, which is able to infect over 240 species of birds (Kaleta and Baldauf, 1988). Because ND can have a severe economic impact, outbreaks have to be notified to the World Organization for Animal Health (OIE) (Communities, 1992). To prevent ND, prophylactic vaccination is applied in many countries worldwide (Alexander, 2009).

The most predominant ND viruses currently circulating worldwide are genotype V and VII viruses (Miller et al., 2010). Available vaccines induce protection against morbidity and mortality from a challenge with virulent NDV strains, but several studies have shown that they do not prevent infection and virus shedding, which may result in silent spread to other vaccinated birds (Kapczynski and King, 2005; Miller et al., 2009). This might be due to insufficient immunity as a result of antigenic divergence between the vaccine strains and the circulating field strains (Hu et al., 2009; Kapczynski and King, 2005; Miller

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et al., 2007, 2009; Qin et al., 2008; van Boven et al., 2008). Vaccines should provide optimal protection even to viruses that have drifted away antigenically from classical ND vaccines based on genotype I and II strains.

In a recent study we showed that a live vaccine that was antigenically adapted to match a genotype VII strain did not provide increased protection compared to a classical genotype II live vaccine. Indeed, chickens that were vaccinated with a classical vaccine, were fully protected against mortality and disease as well virus shedding in the majority of birds when challenged with a genotype VII virus, even with a supposedly suboptimal vaccine dose (Dortmans et al., 2012). Since the immunity of field vaccinated chickens, as monitored by the Dutch Animal Health Service Deventer, The Netherlands (Fabri, 2012), is relatively poor, these data suggest that not antigenic variation but rather poor flock immunity due to inadequate vaccination practices is the major factor responsible for spreading of virulent NDV field strains in a vaccinated population.

The objective of this study was to test the efficacy of a genotype II strain-based commercial vaccine applied under standard field conditions after challenge with different virulent field viruses. To test this, selected field-vaccinated broilers with suboptimal immunity were challenged. Four well characterized viruses belonging to genotypes II, V and VII were used to infect the vaccinated broilers. In addition to the standard observation of morbidity and mortality, also viral shedding was evaluated.

2. Materials and methods

2.1. Cells and viruses

QM5 cells (Antin and Ordahl, 1991) were grown and maintained as previously described (Dortmans et al., 2009).

Strain APMV-1/chicken/U.S.(TX)/GB/48 (Te/48) was received from the University of Wisconsin, USA. This neurotropic velogenic virus has an intracerebral pathogenicity index (ICPI) of 1.8 and belongs to genotype II. Because from PCR analyses the purity of this seed batch became doubtful (Kant et al., 1997) this virus was plaque purified on chicken embryo fibroblast cultures and a new working stock was prepared by inoculating 11-day-old embryonated specific pathogenic free (SPF) eggs. Sequence analysis of part of the F gene fully matched with the published sequence (Paldurai et al., 2010).

Strain APMV-1/chicken/U.S.(Ca)/2098/71 (Ca/71) was isolated from chickens during the ND outbreak in California in 1971 and was also received from the University of Wisconsin, USA. This viscerotropic velogenic virus has an ICPI of 1.8 and belongs to genotype V based on the partial F gene (van Boven et al., 2008). This virus was passaged twice by inoculating 10-day-old embryonated SPF eggs to make a new working stock and completely sequenced in the present study.

Strain APMV-1/chicken/NL/152608/93 (NL/93) was isolated during the ND outbreak in The Netherlands in 1992–1993. This isolate was recently sequenced (Dortmans et al., 2012) and phylogenetic analysis had placed this virus in genotype VIIa (Lomniczi et al., 1998).

Strain APMV-1/chicken/ZA/AL495/04 (ZA/04) was isolated from village chickens at Hopewell Village in 2004, during the ongoing outbreak of South Africa (Abolnik, 2007) and was received from Dr. Abolnik of the University of Pretoria, South Africa. This virus has an ICPI of 1.91 and was placed in genotype VIId based on the partial F gene (Abolnik, 2007). For the present study this isolate was passaged once by inoculating 11-day-old embryonated SPF eggs to make a working stock and completely sequenced.

2.2. RNA isolation, RT-PCR and sequencing

Genomic RNA was isolated with a High Pure Viral RNA kit (Roche, Netherlands). First-strand DNA synthesis was carried out using a Superscript™ III Reverse Transcriptase kit (Invitrogen, Carlsbad, CA, USA). Overlapping subgenomic cDNA fragments were generated, purified using a High Pure PCR purification kit (Roche, Netherlands) and subsequently sequenced (de Leeuw and Peeters, 1999). Primer sequences are available upon request. Nucleotide sequencing was carried out using a BigDye Terminator v1.1 cycle sequencing kit and a 3130 genetic analyzer (Applied Biosystems, The Netherlands).

2.3. Phylogenetic analysis and accession numbers

A 375 base pair fragment (nt 47 to 422) of the NDV fusion (F) protein gene of class II genotypes I to VII viruses were aligned and analysed using the ClustalW multiple alignment method. A phylogenetic tree was constructed using MEGA5 software by Neighbor-Joining method (1000 replicates for bootstrap). The evolutionary distances were computed by Pairwise Distance method using the Maximum Composite Likelihood Model (Tamura et al., 2011). The F genes of the following reference strains (GenBank accession no.) were used: Ulster/67 (AY562991); LaSota (AF077761); VG-GA (EU289028); TexasGB/48 (GU978777); Mukteswar (JF950509); Herts/33 (AY741404); Italien (EU293914); Ca/02 (EF520718); Largo/71 (AY562990); Anhinga/93 (AY562986); NDV-P05 (HM117720); 248VB (EF026584); IE/04 (JN986839); AV324/96 (GQ429292); Fontana/72 (AY288992); IT-227/82 (AJ880277); SW/95 (HQ839733); TR-7/97 (AF136784); IT1/00 (AF293350); Cockatoo/90 (AY562985); TW/95-7 (AF083968); NL/93 (JN986837); ND/03/044 (GQ338310); JSD0812 (GQ849007), FP1/02 (FJ872531); ZJ1 (AF431744); SF02 (NC_005036).

2.4. Pathogenicity tests and haemagglutination inhibition (HI) assay

Determination of the intracerebral pathogenicity index (ICPI) in one-day-old chickens and HI assays were performed as described in the European Community Council Directive 92/66/EEC (Communities, 1992). For the HI assays, 8 haemagglutinating units of the following viruses were used as antigen: Ulster, Te/48, Ca/71, NL/93 and ZA/04. The intranasal/intratracheal pathogenicity index was determined using an approach similar to the