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Research article

Effects of hitchner b1 vaccine antibodies on organ distribution, persistence and shedding of velogenic Newcastle Disease Virus (kudu 113) in chickens

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Abstract

Newcastle disease (ND) is endemic in Nigeria. Control is by vaccination and one of the commonly used vaccines against the disease is Hitchner B1 vaccine. Kudu 113 virus is a velogenic strain of Newcastle disease virus (NDV) that have been characterised. ND is very common among vaccinated flocks with rapid spread or transmission. This study evaluated the effects of Hichner B1 induced humoral antibody response on the Kudu 113 viral distribution, persistence and excretion from experimentally infected chickens. Four hundred chickens were procured at day old and randomly assigned into three groups: unvaccinated and unchallenged (G1), unvaccinated and challenged (G2) and vaccinated and challenged (G3). Vaccination was done on day 2 of age and batches of birds in G2 and G3 were taken at specified intervals for challenge. Virus distribution and persistence in organs and shedding in the faeces of the infected birds were assessed by isolation studies and quantitative haemagglutination assay. Vaccination did not prevent viral replication but significantly reduced the distribution and shedding of Kudu 113. Peak distribution and shedding was seen between days 4 and 7 post challenge (PC) and virus persisted in the faeces of infected birds for a maximum of 21 days.

Keywords: Hitchner B1; Kudu 113 virus; chickens; distribution; virus shedding

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Introduction

ND is an infectious, highly contagious and pathogenic avian viral disease caused by a paramyxovirus (Capua and Terregino, 2011; Dortmans et al., 2012). Since the first recognition of the disease in 1926, ND has spread rapidly throughout most countries of the world (Grund et al., 2014). Many countries of the world report ND in domestic species and many of these have assumed endemic status with outbreaks occurring year after year (Miller et al., 2013).

In countries where ND is endemic and poultry is reared on commercial basis, vaccination and bio security are the major means of controlling the disease (Okwor et al., 2010). In Nigeria, there are still reduced production and mortalities among broiler and layer populations from ND outbreaks, even in fully vaccinated birds. Because of the frequent vaccination

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failures in the field, one therefore doubts the efficacy or potency of the commercially available vaccines. The inability of the commercially available vaccines to protect birds optimally from the pathologic consequences of this disease has been reported (Kapcyznski and King, 2005). Some newly emerging strains have been reported to be of great concern and have been suggested to have the ability to overcome vaccination barriers (Bwala et al*.,* 2011). The vaccine strains are phylogenetically in the same genotype as viruses isolated earlier, but are phylogenetically divergent from strains causing the recent outbreaks (Miller et al*.,* 2010; Dortmans et al., 2011). Analysis of the recent NDV isolates from vaccinated flocks has led several researchers to suggest that antigenic variation may be responsible for the continuing outbreaks of ND (Courtney et al., 2012; Diel et al., 2012; Miller et al., 2013).

ND vaccines are routinely only tested for their effectiveness to induce clinical protection which does not provide information about the level of shedding and consequently transmission of the challenge virus. Interest in the amount of velogenic NDV shed into the environment by vaccinated birds has arisen as a potential indicator of vaccine efficacy (Miller et al*.,* 2009). According to Miller et al. (2013), further investigation as to the best vaccine for individual situations, focusing not only on prevention of clinical disease and mortality, but also on decreasing the amount of virus shed from vaccinated birds is an important consideration in countries with endemic ND.

In Nigeria, Hitchner B1 vaccines are used widely in the vaccination of chicks against ND. Kudu 113 virus was isolated at NVRI and biological characterisation showed that it belongs to the velogenic pathotype (Echeonwu et al., 1993). Studies in Nigeria have shown that most field isolates causing problems in commercial and village chickens belong to the velogenic pathotype (Okwor et al., 2010). Investigations have also shown that in Nigeria, outbreaks of velogenic ND in vaccinated flocks are common (Okwor et al., 2013). Clinical protection trials have been carried out on Kudu 113 infections in chickens in Nigeria (Ezema et al., 2009). There is no information on the distribution and shedding of velogenic viruses in vaccinated birds. This study examined the distribution and persistence of Kudu 113 virus in the visceral organs of birds vaccinated with Hitchner B1. It also examined the shedding of the virus in the faeces of the vaccinated birds. The determination of the distribution, persistence and shedding of this virus in vaccinated birds at different antibody levels will be very useful in understanding better, the epidemiology and transmission of this disease with the resultant institution of appropriate control measures.

Materials and Methods

Experimental Birds: Four hundred day-old white cockerels were obtained from a reputable hatchery in Nigeria and used in the study. The vaccination history of the parent stock was not given any vaccine in the hatchery. The birds were reared under the deep litter system with commercial feed and water given *ad-libitum* throughout the experimental period. Prophylactic vaccinations against infectious bursal disease and fowl pox and medication against coccidiosis were carried out according to local demands and prescriptions.

Experimental Design: The four hundred day old chicks were randomly divided into three groups. Group 1 (G1) had 100 birds that were not vaccinated and not challenged. Group 2 (G2) had 150 birds that were not vaccinated but challenged while group 3 (G3) had 150 birds that were vaccinated and challenged. Birds in G3 were vaccinated at day 2 of age through the intraocular route. Thereafter and on days 21, 49, 77 and 105 post vaccination (PV), serum samples were collected randomly from 10 birds in the three groups for serology. After serum sampling on days 21, 49, 77 and 105 PV, 30 birds were randomly collected from G2 and G3 and taken to a distant location and challenged. On days 2, 4, 7, 11 14, 21, and 28 PC cloacal samples were collected from 20 chickens in G1, and the same number each from the challenged G2 and G3 for challenge virus isolation in embryonated chicken eggs, and subsequently, identification and quantification using haemagglutination (HA) assay. Geometric HA values (GMT) for the groups on the specified days were calculated and this represented the virus sheddings in the faeces of the birds. On days 2, 4, 7, 11, 14, 21, and 28 two chickens were taken randomly each from G1, G2, and G3 and sacrificed humanely. Samples of the spleen, proventriculus, bursa of Fabricius, thymus and cecal tonsil were collected for challenge virus isolation in embryonated chicken eggs, and subsequently, identification and quantification using HA assay. The proportions of the organs positive for NDV were expressed in percentages depicting the organ distributions of the virus and the duration of isolation, the persistence. Haemagglutinating agents were tested for specific inhibition using monospecific antiserum to NDV.

Vaccine and Vaccination: Hitchner B1 vaccine was used. The viability of the stock vaccine was checked using HA test (OIE, 2012). The infective dose of the vaccine was determined by virus titration through infectivity of 11 day old chick embryo inoculation (Alexander and Senne, 2008) and calculation using the method of Reed and Muench (1938). The estimated HA titre of the vaccine used was 128. The 50% Embryo Infective Dose (EID_{50}) of the vaccine was $10^{7.0}$/ml.

Serology: The serum samples collected were subjected to serological testing for antibodies against NDV using Haemagglutination inhibition (HI) test (OIE, 2012). ND LaSota strain was used as the antigen. 4 Haemagglutination units (HAU) of the antigen were used in the HI test.

Challenge virus: The NDV inoculum was a Nigerian velogenic field isolate known as Kudu 113. The pathogenicity indices were, MDT 49.6, ICPI 1.56, IVPI 2.18, and % adsorption to chicken brain cell 97.66, thermostability of haemagglutinin at 56^0C (min) 120 and virus elution rate > 26. (Echeonwu et al., 1993). The stock virus used had HA titre of 128 and median embryo infective dose (EID_{50}) of $10^{6.5}$/ml.

Virus isolation: The virus multiplication in tissues and shedding in faeces of chickens after challenge were studied using virus isolation in 11 day old embryonated chicken eggs and thereafter quantitative HA assay on the harvested allantoic fluid. The egg inoculation was done following the procedures as described in OIE (2012).

Identification and confirmation of the allantoic fluid isolate: The presence of a haemagglutinating virus and confirmation of the agent to be NDV in the allantoic fluids harvested were demonstrated using HA and HI tests respectively (OIE, 2012). The monospecific antiserum used in the HI was prepared in chickens following the methods described by Grimes, (2002).

Statistical analysis: One way analysis of variance (ANOVA) was used to determine the significant differences in the mean virus titres of chickens in the groups and descriptive statistics such as GMT and percentages were used to compare virus distributions and sheddings in the groups (Cleophas and Zwinderman, 2012). Differences were considered statistically significant when $P \leq 0.05$.

Results

Serology: The geometric mean antibody titres on days 21, 49, 77 and 105 PV (days for first, second, third and fourth challenges) in G3 were 97.0, 21.1, 2.3 and 0 respectively. For birds in G1 and G2 (unvaccinated) the corresponding GMT on those specified days were 2.0, 0, 0 and 0 respectively.

Virus distribution and persistence: No virus was isolated from birds in G1. The result showed that during the four challenge studies, virus was isolated from the organs in G2 and G3 (Table 1). G2 showed high organ distribution in all challenges. Peak organ distributions in both groups during all challenges were between days 4 and 7 PC. Virus persisted in the tissues up to and not more than day 21 PC with higher organ distributions when the birds had lowed circulating antibodies.

Virus Shedding: No virus was shed in the faeces of birds in G1. Virus was shed in the faeces of bird in G2 and G3 (Fig. 1, 2, 3, and 4). The level of circulating antibodies affected virus shedding. In all cases in G2, the viral shedding in faeces increased significantly ($P<0.05$) up to days 4 to 7 PC, after which it declined appreciably. Analysis showed statistical difference ($P<0.05$) in virus shedding in all cases in G3 up to days 4 to 7 PC. The result revealed marked reduction in virus shedding in the vaccinated chickens especially during the early challenge experiments. During the first, second and third challenge experiments, there were significant reduction ($P<0.05$) in virus shedding in chickens in G3 when compared with chickens in G2 on days 4 and 7 PC. There were no significant reductions ($P>0.05$) in virus shedding between chickens in the two groups on days 2, 4, and 7 PC during the fourth challenge experiment (Fig. 4).

Discussion

Vaccination with the development of circulating antibodies did not prevent Kudu 113 virus replication and shedding in the challenged chickens. It has been reported that vaccination of poultry against ND will only protect the birds from the more serious consequences of the disease which are mainly clinical signs and mortality, but will not protect them from infection and replication of the virulent strain of the virus (Miller, 2008; Ezema et al., 2009; Miller et al., 2009; Rue et al., 2011). Therefore this study with Kudu 113 virus and previous studies showed that the

Table 1: Total percentage distribution and persistence of virus in tissues of chickens in G2 and G3 after challenge with velogenic NDV at the different challenges

	Days PC													
	2		4		7		11		14		21		28	
Challenge	G2	G3	G2	G3	G2	G3	G2	G3	G2	G3	G2	G3	G2	G3
1st Chall.	80	66.7	100	93.3	100	93.3	=	73.3	=	36.7	=	0	=	0
2nd Chall.	83.3	70	100	96.7	100	93.3	=	80	=	80	=	6.7	=	0
3rd Chall.	80	80	100	100	100	93.3	100	86.7	=	83.3	=	6.7	=	0
4th Chall.	73	80	100	100	100	100	100	93.3	100	93.3	80	53.3	0	0

= no sample, no chicken survived.

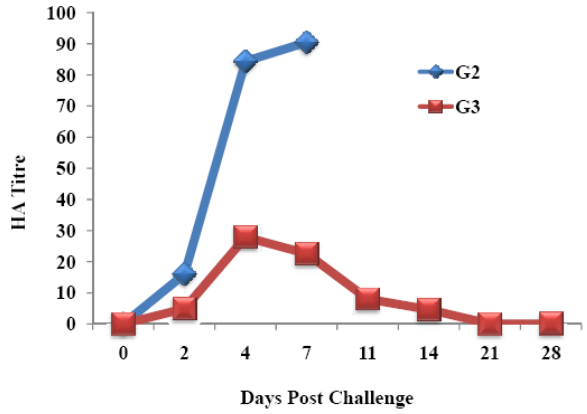

Fig. 1: Comparison of geometric mean HA titres (indicating the level of virus shedding) of chickens in G2 and G3 during the 1st challenge

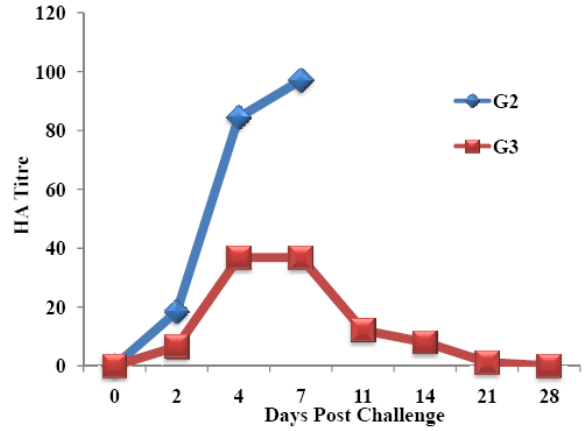

Fig. 2: Comparison of geometric mean HA titres (indicating the level of virus shedding) of chickens in G2 and G3 during the 2nd challenge

available vaccines do not protect against infection as vaccinated birds exposed to virulent NDV will be infected and shed the virus in the faeces and other excretions.

Though Hitchner B1 vaccine used in this study did not stop infection with Kudu 113 virus, it offered protection by significantly reducing distribution and shedding of the virus in vaccinated and challenged chickens. The organ distribution of the virus was higher in the unvaccinated chickens than the vaccinated chickens. Though both the vaccinated and unvaccinated groups yielded virus upon challenge, the titres of virus shedding were significantly higher in unvaccinated chickens than in the vaccinated chickens except at the fourth challenge. The highest or peak shedding titres recorded in unvaccinated chickens during the four challenge periods were respectively, 90.5, 97.0, 90.5 and 73.3 compared with the corresponding 27.9, 36.8, 45.3 and 68.6 in vaccinated chickens. The shedding

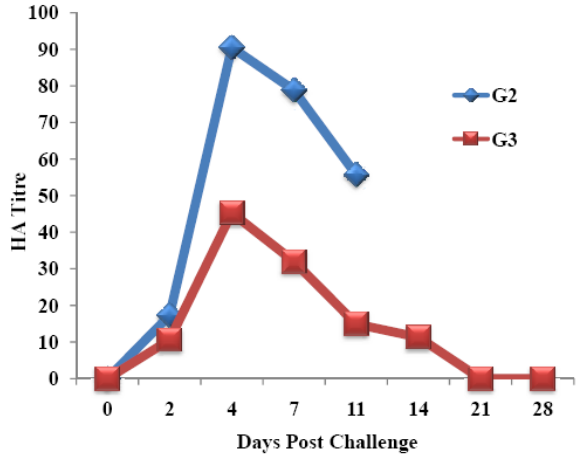

Fig. 3: Comparison of the geometric mean HA titres (indicating the level of virus shedding) in chickens in G2 and G3 during the third challenge

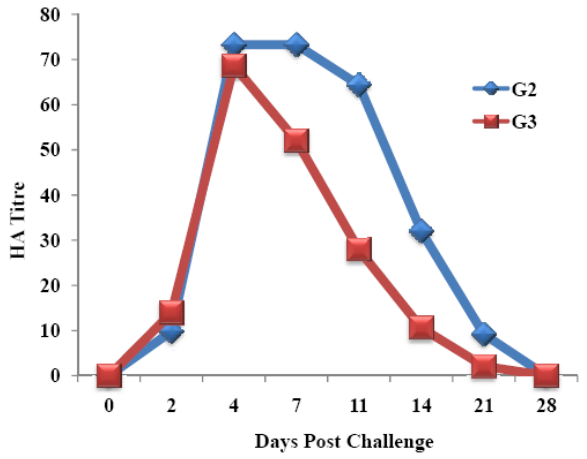

Fig. 4: Comparison of the geometric mean HA titres (indicating the level of virus shedding) in chickens in G2 and G3 during the 4th challenge

during the four challenges in the unvaccinated chickens did not vary much from each other as the circulating antibodies were at undetectable levels. The little variation observed during the fourth challenge (73.3) may be due to age resistance as the birds were older at this period. The vaccinated chickens showed increased shedding with subsequent challenges and this could be due to the lowering levels of circulating antibodies at subsequent challenges. Higher levels of shedding were seen in the vaccinated chickens when the circulating antibodies were low. The virus shedding titre was lowest at first challenge and highest at fourth challenge when the circulating antibody has become low and at this challenge did not differ significantly from the unvaccinated control. Therefore, higher levels of circulating antibodies are needed at the time of challenge for a significant reduction in virus shedding

to be achieved. This observation is in agreement with that made by Miller et al. (2013) who stated that as the level of humoral antibodies increased in vaccinated chickens, the number of infected chickens and the amount of NDV shed decreased. High levels of circulating antibodies are needed to sufficiently neutralise the infective dose and reduce virus multiplication. This therefore points to the need to maintain high levels of circulating antibodies in chickens as this is important in reducing the level of shedding and therefore the transmission of the virus.

Our study in chickens showed that virus was isolated between day 2 PC and day 21 PC depending on the group. In all the cases in the challenged groups, highest virus shedding titres were recorded at between days 4 to 7 PC. The organ distribution for NDV was also highest at between days 4 to 7 PC. In the challenged groups, the virus isolation increased significantly up to this identified peak period after challenge. Virus shedding after challenge in the unvaccinated chickens continued as long as they showed clinical disease while for the vaccinated chickens virus was not isolated beyond 21 days PC. Previous work has shown virus isolation starting in vaccinated birds after challenge from day 2 - 5 PC and up to day 53 PC (Carrasco et al., 2008). Other reports have shown recovery of NDV for up to 10 days and occasionally more than 2 weeks in chickens after vaccination with HB1 strains of ND vaccine and subsequent challenge with velogenic NDV (Joen et al., 2008; Miller et al., 2009; Bwala et al., 2011). It is known that development of circulating antibodies rapidly reduces the amount of virus in the circulation and in the tissue (Alexander et al., 2006). However, the isolation of virus in the cloacal samples up to day 21 PC points out to the fact that virus may continue to replicate and be shed in the gastrointestinal tract. According to Alexander (2008) in the presence of circulating antibodies, the bone marrow and the GIT are good samples to take for virus isolation. We also suggest that the picking of the virus from the contaminated litter may also have contributed to the virus persisting for up to 21 days in the faeces of the chickens. The observation that virus is shed maximally between days 4 to 7 PC suggests that this may represent the period when neutralizing antibodies were still building up and were not enough to suppress viral replication and shedding. It therefore implies that the best period for collecting samples in chicken for NDV isolations in vaccinated and unvaccinated birds is between days 4 to 7 PC and clinical specimens submitted within this period will yield best results.

Since this vaccine practically and significantly reduced virus shedding up to 77 days PV, its efficacy in the control of ND is acceptable and chickens should be vaccinated frequently to maintain high antibody levels. It is widely recognized that because ND isolates are of one serotype, ND vaccines prepared with any NDV lineage if given correctly can protect poultry from clinical disease and mortality from a virulent NDV challenge (Qin et al., 2008; van Boven et al., 2008; Hu et al., 2009). However, long before now, the feasibility of one NDV vaccine being able to protect birds from ND without evaluating the factors for each individual outbreak has been questioned (Miller et al., 2007; van Boven et al., 2008). New forms of NDV being isolated in some parts of the world are able to infect vaccinated chickens and these new viruses seem partially resistant to the antibodies induced by the current vaccines (Bogoyavlenskiy et al, 2009; Miller et al., 2010). Kapczynski and King (2005) showed that current vaccination programmes in commercial broilers are not completely effective at preventing clinical disease and virus shedding after experimental challenge with recent virulent isolate. Therefore, different strategies have been used to produce vaccines that can give better protection from clinical disease, mortalities and virus shedding following contact with velogenic virus in vaccinated birds. The use of live recombinant vaccine or vaccines of the same genotype as the expected field challenge have been shown to protect birds more by the birds showing less virus being shed in the oropharyngeal and cloacal swabs (Miller et al., 2007; Miller et al., 2013). They observed that genotypic differences between vaccine and challenge virus did not diminish ability of vaccines to protect against disease, but genotypic similarity did reduce virus shed and may reduce transmission. Therefore, increasing the genetic relatedness of the ND vaccine virus to the likely virulent challenge virus will produce more specific neutralizing antibodies and decrease the amount of challenge virus shed from vaccinated poultry. Vaccines homologous with the challenge virus will reduce oral and cloacal shedding significantly more than the heterologous vaccines (Miller et al., 2013). Vaccines with the ability to reduce viral shedding would enhance the role of vaccination in ND control by reducing virus transmission from infected birds. Practically, the effectiveness of vaccines is ultimately determined by their ability to control epizootics of ND, therefore vaccine development should be focused on providing vaccines that protect against infection and shedding rather than against disease.

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