COMPARATIVE STUDIES ON THE EFFECTS OF LA SOTA AND KOMAROV VACCINE ANTIBODIES ON ORGAN DISTRIBUTION, PERSISTENCE AND SHEDDING OF KUDU 113 VIRUS IN CHICKENS

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ABSTRACT

Vaccines derived from the lentogenic and mesogenic strains are used for the protection of clinical ND in chickens. No information is available on the protection offered by these vaccines against virus replication and shedding in Nigeria. This study compared the abilities of La Sota and Komarov vaccines to protect chickens against replication and shedding in chickens challenged with the velogenic Kudu 113 virus. Five hundred and fifty day old chicks were assigned into four groups; unvaccinated and unchallenged (G1), unvaccinated and challenged (G2) La Sota vaccinated and challenged (G3) and Komarov vaccinated and challenged (G4). Batches of birds in G2, G3 and G4 were taken at specified intervals for challenge with Kudu 113. Virus distribution and persistence in organs and shedding in the faeces of the infected birds were assessed. Both vaccines did not prevent viral replication and shedding but significantly reduced the distribution and shedding of Kudu 113. Komarov vaccine showed higher reduction of viral replication and shedding when compared with La Sota. Peak distribution and shedding in both cases were seen between days 4 and 7 PC and virus persisted in the faeces of infected chickens for a maximum of 14 days in G4 and 21 days in G3.

Keywords: La Sota, Komarov, Kudu 113, distribution, persistence, shedding.

INTRODUCTION

Newcastle disease (ND) is a disease of chickens, turkeys, pigeons and other avian species caused by a filterable virus known as Newcastle disease virus (NDV) (Dortmans *et al.*, 2012; Ashraf and Shah 2014). The virus is a single stranded negative sense RNA virus belonging to the genus *Avulavirus*, in the family *Paramyxoviridae* (ICTV, 2012; Lamb *et al.*, 2013; Munir *et al.*, 2012; Miller *et al*, 2013). The disease is a worldwide problem, causing severe economic implications, with morbidity and mortality in susceptible birds reaching up to 90 – 100% in the severe forms of the disease in unvaccinated birds (Al-Habeeb *et al.,* 2013; Ashraf and Shah, 2014). ND is endemic in many countries of Africa and Asia and velogenic strains are involved in severe forms of the disease. ND is controlled worldwide by routine vaccination and appropriate vaccination with subsequent effective immune response is known to be the only measure to avoid the disease outbreaks (Shabbir *et al.*, 2013). Although vaccination against ND have been practiced for many years in some countries, frequent outbreaks of the disease in vaccinated flocks are common occurrence, thus making ND a serious threat to the poultry industry (Okwor and Eze, 2013). Mazengia, (2012) and Miller *et al.,* (2013) suggested that in addition to the use of poor and inadequately stored

vaccine, outbreaks of ND in vaccinated flocks could also be due to differences in pathogenicity of ND, the strain of ND vaccine virus and also that of the infecting virus. Moreover, studies have shown that the currently available vaccines induced better protection against viruses that were isolated in the past epizootics that were seen in the 1950's than against most of the viruses currently circulating in poultry (Kapczynski *et al.,* 2006; Bwala *et al.*, 2012; Cornax *et al*., 2012). It has also been shown that chickens vaccinated against ND and challenged later with virulent strains of the virus may remain clinically normal but showed virus in the tissues (Miller *et al*., 2009; Bwala *et al*., 2012; Kapczynski *et al*., 2013). Vaccination makes the flock more refractory to infection and reduces the quantity of virus shed by the infected birds (Nishizawa *et al.,* 2007; Kapczynski *et al*., 2013). Research has shown that viraemia is important in transmitting the virus to target organs following infection of susceptible chickens but vaccination reduces viraemia and therefore virus shedding in chickens (Kapczynski *et al*., 2006; Nishizawa *et al.,* 2007; Mazengia, 2012; Carrasco *et al*., 2016).

Many field strains of NDV affecting chickens in Nigeria with severe clinical manifestations are of the velogenic pathotype (Okwor *et al*., 2008; Ibu *et al*., 2009; Igwe *et al*., 2014). Kudu 113 virus have been characterised as a velogenic pathotype (Echeonnwu *et al*.,

1993; Shittu *et al.*, 2016). In Nigeria, lentogenic vaccines such as Hitchner B1 and La Sota and mesogenic vaccine such as Komarov are routinely used to vaccinate chickens against the disease (Oladele, 2006, Bwala *et al.*, 2011). The ability of a vaccine to minimize clinical signs and lesions, produce high circulating antibodies, and reduce virus shedding upon challenge and infection are good indices of quality and potency. However ND vaccines are most of the time routinely tested only for their effectiveness to induce clinical protection and this 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 and currently vaccine efficacy is also tested by the abilities of the antibodies developed in chickens as a result of vaccination to reduce viral shedding when challenged (Miller *et al.*, 2007; Miller *et al.,* 2009; Miller *et al*, 2010). There is no information on the shedding of Nigerian isolates of NDV in vaccinated chickens. This work comparatively evaluated La Sota and Komarov induced vaccinal antibodies in their abilities to affect the distribution, persistence and shedding of Kudu 113 virus in chickens. The results obtained may serve as a starting point in establishing the antigenic relatedness of the vaccines to the Nigerian velogenic strain of the virus and guide in reassessing the role of the vaccines in NDV control.

MATERIALS AND METHODS

Experimental Birds: Five hundred and fifty white cockerels were obtained at day old from a reputable hatchery in Nigeria. The birds were reared under the deep litter system with commercial feed and water given *ad-libitum* throughout the experimental period**.** The chickens were not vaccinated in the hatchery. Vaccinations of the birds against infectious bursal disease were done on days 14 and 28 of age and that against fowl pox on day 35 of age while medications against coccidiosis were carried out at days 21 to 23 of age according to local demands.

Experimental Design: The chicks were randomly divided into 4 groups. Group 1 (G1) had 100 chicks that were not vaccinated and were also not challenged (unvaccinated and unchallenged). Group 2 (G2) had 150 chicks that were not vaccinated but challenged (unvaccinated and challenged). Group 3 (G3) had 150 chicks that were vaccinated with La Sota and challenged (La Sota vaccinated and challenged). Group 4 (G4) had 150 chicks that were vaccinated with Komarov and challenged (Komarov vaccinated and challenged). Because vaccination with Komarov requires prior primary vaccination, birds in G3 and G4 were given Hitchner B1 vaccine on day 2 of age as primary vaccine before La Sota and Komarov were given respectively on day 42 of age as the experimental vaccines. Birds in G3 were giving Hitchner B1 in order to achieve uniformity with birds in G4. On days 0, 21, 49, 77 and 105 post vaccination (PV) of birds in G3 and G4 with La Sota and Komarov respectively, serum samples were collected randomly from 10 birds in the four groups for serology. On days 21, 49, 77 and 105 PV of birds in G3 and G4 with La Sota and Komarov respectively, 30 birds each were randomly collected at each specified time after blood sampling from G2, G3 and G4 and taken to a distant location and challenged with velogenic NDV, Kudu 113 (constituting 1st, 2nd, 3rd and 4th batch challenge studies from the groups). On days 0, 2, 4, 7, 11 14, 21, and 28 post challenge (PC) of each batch, cloacal samples were collected from 20 chickens in G1 (negative control) and the challenged G2, G3, and G4 for challenge virus isolation in embryonated chicken eggs, followed by quantification using haemagglutination (HA) assay. Geometric mean titre (GMT) for the HA values obtained for the groups on the specified days were calculated and this represented the virus shedding in the faeces of the birds. On days 0, 2, 4, 7, 11, 14, 21, 28 PC of each batch two chickens were also taken randomly each from G1, G2, G3 and G4 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, followed by quantification using HA assay. HA positivity was determined for each organ on a specified day. The proportions of the organs positive for NDV on a specified day were expressed in percentages depicting the organ distributions of the virus for the specified day. The duration of isolation or the persistence was taken as how long the organs and the cloacal samples remained positive. The haemagglutinating agents were identified as NDV using monospecific antiserum to NDV prepared in chickens using Haemagglutination inhibition (HI) test.

Vaccines and Vaccination: The Hitchner B1, La Sota, and Komarov vaccines used were live vaccines procured from the National Veterinary Research Institute (NVRI), Vom in Plateau State, Nigeria. The viability of the stock vaccine was checked using HA test (OIE, 2012). The infective dose of the vaccine was also determined by virus titration through infectivity of 11 day old chick embryo (Alexander and Senne, 2008) and calculation using the method of Reed and Muench (1938). The infectivity of 11-day old chicken embryo was used as the index. Serial 10-fold dilutions (10^{-1} - 10^{-10}) of the vaccines were prepared in phosphate buffered saline containing 2,000 iu/ml procaine penicillin, 2mg/ml streptomycin sulphate, and 50ug/ml gentamycin sulphate. Each dilution was inoculated into five, 11-days old ND antibody free embryonated chicken eggs. This embryo age was chosen to allow for better or full development of the allantoic cavity. Each embryo was inoculated with

0.2ml of the dilution through the allantoic cavity route. Five eggs were inoculated with 0.2ml of PBS only and kept as control. The eggs were incubated for 72 hours after inoculation and the allantoic fluid harvested. The allantoic fluid was tested for NDV using HA test and confirmed to be NDV with HI test using monospecific NDV antibody prepared in chickens. The 50% egg infective dose (EID_{50}) was calculated using the method of Reed and Muench, (1938). The estimated HA titre of the Hichner B1, La Sota and Komarov vaccines used were 256, 256 and 128 and the 50% Embryo Infective Dose (EID_{50}), $10^{7.0}$/ml, $10^{7.0}$/ml, and $10^{6.5}$/ml respectively.

Hitchner B1 vaccine was administered by diluting a vial containing 200 doses with 10 ml of sterile water for injection and inoculating each bird by intraocular instillations in both eyes using a standard dropper. La Sota was administered by diluting a vial containing 200 doses with 200ml of sterile water for injection and carefully inoculating each bird with 1ml through the oral route by drenching. Komarov was given by diluting 1 vial containing 200 doses with 10ml of water for injection and inoculating each bird with 0.2ml through intramuscular injection in the thigh muscle.

Haemagglutination (HA) test: This followed the standard procedure outlined in OIE, (2012). To each well of the microtitre plate starting from well no. 1, 50µl of PBS was added across the wells in the row using a multichannel micropipette. Then, 50µl of the reconstituted antigen was added to well no. 1, mixed using the multichannel micropipette and a serial two fold dilution made from this well through well no. 12. The 50µl from the last well was discarded. To each well and starting from well no.1, 50µl of 0.5% RBC suspension was added.   Positive control was set up by adding 50µl of 0.5% chicken RBC suspension to 50µl of known antigen (LaSota). Negative control was set up by adding 50ul of 0.5% chicken RBC to 50ul of PBS. The plate was incubated at room temperature for 40 minutes. Positive results were recorded for those that showed complete agglutination of the RBC (no red button) while negative result was recorded for those that showed no agglutination of the RBC (smooth settling or presence of red button). The reciprocal of the highest dilution that showed complete agglutination of the RBC was recorded as the HA titre of the antigen.

Haemagglutination inhibition (HI) test: The serum samples were collected using the standard procedure as outlined by Ison *et al.,* (2005). The serum samples collected were subjected to serological testing for antibodies against NDV using HI test. The allantoic fluids harvested were also confirmed to contain NDV as the haemagglutinating agent using HI test. Four Haemagglutination units (4HAU) of the antigen were used in the HI test. The method described in OIE, (2012) was used. La Sota was used as the antigen. Using micropipette 0.5ul of phosphate buffered saline (PBS) was dispensed into each well of microtitre plate. Then 0.5ul of serum or allantoic fluid harvest was placed into the first well of the plate and twofold dilution of the serum made across the plate. 0.5ul of the antigen containing 4 HAU was added to each well and the plate left for 30 minutes. 0.5ul of 0.5% chicken red blood cells was added to each well and mixed gently by tapping. The plate was kept at room temperature for 40 minutes. Positive and negative controls were also set up. The HI titre was read as the highest dilution of serum that caused complete inhibition of 4 HAU of the antigen. The inhibition of agglutination was assessed by observation of the formation of smooth RBC buttons and tilting the plate for RBC streaming in the wells at the same rate as the positive control wells.

Virus and Challenge: The NDV inoculum used, Kudu 113 was a velogenic strain of NDV obtained from NVRI. 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^{0}C (min) 120 and virus elution rate > 26. (Echeonwu *et al.,* 1993). The viability and infective dose of the virus supplied was determined following the same protocol as described for vaccine virus. The HA titre of the allantoic fluid suspension of the challenge virus was 128. The inoculum used contained median embryo infective dose (EID_{50}) of $10^{6.5}$/ml. Each challenged chicken was inoculated through the intramuscular route with the challenge dose of 0.2ml of the virus inoculum containing $10^{6.5}EID_{50}$/ml.

Collection and Processing of Cloacal Swabs and Tissue Samples: Cloacal swabs were collected individually and randomly using sterile swab sticks by carefully and gently inserting the cotton wool end through the anal opening into the cloaca and rotating it gently against the mucous membrane of the cloaca with efforts made to pick some faecal materials with the swab. After removal, the swab was put in a tube containing 2mls of PBS with 10,000 iu/ml procaine penicillin, 10mg/ml streptomycin sulphate and 250ug/ml gentamycin sulphate. The materials in the cloacal swabs which were collected in PBS containing antibiotics were transferred into centrifuge tubes, balanced and centrifuged at the rate of 3000 x g for 15 minutes at 4^{0}C. The supernatant was collected. The tissue samples were collected individually and aseptically organ bases in sterile bottles during post mortem and 2.0 grams of each organ type were weighed out and homogenized using pestle and mortar with the aid of a measured quantity of sterile sand. The tissue homogenate was suspended in PBS containing antibiotics to make a 20% W/V suspension. The suspension was transferred to the appropriate centrifuge tube and centrifuged at 3000 x g for 15 minutes. The supernatant was collected. The

supernatants in both cases were used in the virus isolation through the allantoic cavity of 11-day old embryonated chicken eggs.

Embryonated Egg Inoculation: Eggs that were free of antibodies against NDV were used in the isolation studies. Fertile eggs were collected from layers that were not vaccinated against Newcastle disease and reared under strict biosecurity measures. Few samples of the eggs were randomly collected, their yolk harvested and certified to have undetectable levels of NDV antibodies by HI test (Saidu *et al.*, 2006). The egg inoculation was done following the procedures as described in OIE (2012). The inoculation site was swabbed with 70% alcohol and the alcohol allowed to evaporate. The site was tapped gently with an egg shell punch, already disinfected with 70% alcohol and left to dry. Using a syringe and needle, 0.2ml of the inoculum was taken up and introduced into the allantoic cavity. This was done by passing the needle vertically though the site and penetrating approximately 16mm into the egg to reach the allantoic cavity. The inoculum was deposited in the allantoic cavity. The needle was then withdrawn from the egg and the site sealed with melted wax. The inoculated egg was then returned into the incubator and the temperature of 35°C – 36°C and humidity of 60% were maintained. The eggs were incubated for 72 hours and the allantoic fluid harvested used for HA assay.

Preparation of monospecific antiserum and Identification of the Allantoic Fluid Isolate: The presence of a haemagglutinating virus was detected using HA test and confirmation of the agent to be NDV in the allantoic fluids harvested were done using HI test (Orsi *et al.*, 2010; OIE, 2012). The monospecific antiserum used in the HI was prepared in chickens as described in Grimes, (2002), Ibu *et al.,* (2008) and Alkhalaf, (2009). Twenty pullets 14 weeks old were vaccinated with La Sota. Two weeks after this vaccination, another booster vaccination was given. This was followed after another two weeks by a third vaccination. Two weeks after the last (third) vaccination, serum samples were taken from the birds and the HI titre was tested. Serum from birds with a titre of 2^5 (32) and above were taken and pooled together and the HI titre of the pooled samples was determined and used for the HI test**.**

Statistical analysis: Descriptive statistics such as percentages (for virus distributions in the organs) and GMT (for virus titres in the faeces) were used to compare virus distributions and sheddings in the groups and expressed as percent organ distribution and geometric mean HA titres respectively. The mean organ distribution, which is the sum of the percent organ distributions on the sampling days for a given batch challenge divided by the number of sample days in the batch challenge experiment, was calculated for each challenged group and used to further compare virus distribution in the groups. One way analysis of variance (ANOVA) was used to determine statistical differences in the HA virus titres (virus shedding) in the faeces (cloacal swabs) of chickens in each challenged group at the specified periods post challenge and also to compare virus titres (virus shedding) among G2, G3 and G4 (Cleophas and Zwinderman, 2012). Differences were considered statistically significant when p   0.05.

RESULTS

Clinical signs: Birds in G1 did not show clinical sign of ND or any other disease throughout the experimental period. Birds in G2 showed typical signs of ND in all the challenged batches with an incubation period of 3 days in all batches. Typical signs observed in G2 were loss of appetite, loss of body condition, dehydration, greenish diarrhoea and high mortalities of 100% in the first three challenged batches and 84% in the last challenged batch. Birds in G3 did not show clinical signs of ND in the first three batches but showed mild signs of cough in the fourth batch. Birds in G4 did not show clinical signs in all the challenged batches of birds.

Serology: The serum samples collected on days 0, 21, 49, 77 and 105 PV in G1 showed GMT values of 0, 1.9, 0, 0 and 0 respectively. For chickens in G2, the GMTs were 1.9, 0, 0 and 0 respectively while the GMTs recorded for chickens in G3 were 0, 222.9, 55.7, 6.5 and 0. The GMTs recorded for chickens in G4 were 0, 332.8, 128.8, 55.7 and 8.0 respectively (Table 1).

Virus distribution and persistence in the organs: No virus was isolated from the organs of chickens in G1. Virus was isolated in the organs of the challenged G2, G3 and G4 birds. Virus was isolated from chickens in G3 from day 2 to day 14 PC during the 1st and 2nd batch challenges and from day 2 to day 21 PC during the 3rd and 4th batch challenges. For chickens in G4 the isolation started on day 2 PC and persisted up to day 11 PC during the 1st and 2nd batch challenges but during the 3rd and 4th batch challenges, viruses persisted up to day 14 PC (Table 2). Virus persisted in the tissues of birds in G2 as long as the bird survived and showed clinical disease. Birds in G2, G3 and G4 showed mean organ distribution as shown in Table 2. Highest mean organ distribution of the virus during the four batch challenges was seen in G2 that was not vaccinated. There was appreciable reduction in the mean organ distribution of the virus during the four batch challenges in G3 that were vaccinated with La Sota. It was lowest in G4 that were vaccinated with Komarov during the four batch challenges. In the three groups and in all challenges, peak virus isolations were on days 4-7 PC (Table 2).

Virus shedding: No virus was isolated from the cloacal swabs of chickens in G1.There was virus shedding in the faeces of chickens in G2, G3 and G4 as shown by the GMT obtained (Table 3, Figure 1). Chickens in G3 showed virus shedding with individual HA titres that ranged from 8 – 64. In all challenges, there were significant differences (p < 0.05) in the viral sheddings up to days 4-7 PC. Chickens in G4 had individual HA titre that ranged from 2 -64. In all the challenges in G4 analysis showed significant differences (p < 0.05) in virus shedding up to days 4-7 PC. Higher antibody levels at the time of challenge significantly (p < 0.05) reduced virus shedding. Virus was shed precipitously in the faeces of birds in G2 as long as the bird survived with an individual HA titre that ranged from 16 - 256. Analysis comparing the differences in the mean HA titres recorded on days 4-7 PC among the groups during the first

challenge experiments showed statistical differences (p < 0.05) among the means, with better protection against virus shedding by Komarov than LaSota.

Table 1. Serum HI antibodies levels on days 0, 21, 49, 77 and 105 days post vaccination.

Groups	0	21	49	77	105
			Days Post Vaccination		
Group 1	NDA	1.9	NDA	NDA	NDA
Group 2	NDA	1.9	NDA	NDA	NDA
Group 3	NDA	222.9	55.7	6.5	NDA
Group 4	NDA	33.8	128.8	55.7	8.0

NDA = No Detectable Antibody

The specified days PV reflects the days the different batches of birds in groups were challenged.

Table 2. Percent organ distribution and persistence of virus in tissues of batches of chickens in G2, G3 and G4 after challenge with velogenic NDV.

	Days PC																					Mean Organ Distribution		
	2			**4**			**7**			**11**			**14**			**21**			**28**					
Challenges	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
1st batch Challenge	80	60	56.7	100	90	80	100	86.7	93.3	NS	76.7	20	NS	60	0	NS	0	0	NS	0	0	93.3	53.3	35.7
2nd batch Challenge	83.3	76.7	43.3	100	100	86.6	100	93.3	90	NS	73.3	40	NS	50	0	NS	0	0	NS	0	0	94.4	56.2	37.1
3rd batch Challenge	80	80	73.3	100	100	86.6	100	96.7	93.3	100	86.7	56.6	NS	76.6	10	NS	10	0	NS	0	0	95.5	64.3	45.7
4th batch Challenge	73.3	83.3	76.6	100	100	86.6	100	100	96.6	100	93.3	80	100	76.6	40	100	23.3	0	NS	0	0	82.2	68.1	54.3

NS = No Sample: no chicken survived.

Table 3: Geometric mean titre (GMT) values of NDV shed in the faeces of batches of chickens in G2, G3 and G4 after challenge with velogenic NDV and isolation in embryonated eggs.

		Days PC																					
		2			**4**			**7**			**11**			**14**			**21**			**28**		
Challenges	Statistic	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4	G2	G3	G4
1st batch	Mean±SE	41.2	14.0	7.4	108.4	24.0	15.6	108.8	24.4	16.6	0±	18.8	2.3	0±	10.4	0±	0±	0±	0±	0±	0±	0±
Challenge	GMT	$\pm8.1^a$	$\pm4.8^a$	$\pm2.9^a$	$\pm15.7^b$	$\pm5.8^b$	$\pm5.9^b$	$\pm16.0^b$	$\pm6.0^b$	$\pm6.0^b$	0.00^c	$\pm5.7^a$	$\pm1.6^c$	0.00^c	0.00^c	0.00^c	0.00^d	0.00^c	0.00^c	0.00^d	0.00^c
		16.0	4.9	3.0	84.4	16.0	8.6	90.5	14.9	9.8	NS	9.5	1.4	NS	4.0	0	NS	0	0	NS	0	0
2nd batch	Mean±SE	43.2	17.6	5.3	133.6	29.6	13.6	123.2	23.6	15.8	0±	20.1	3.9	0±	6.8	0±	0±	0±	0±	0±	0±	0±
Challenge	GMT	$\pm8.7^a$	$\pm6.5^a$	$\pm2.8^a$	$\pm20.2^b$	$\pm4.1^b$	$\pm3.9^b$	$\pm17.8^b$	$\pm4.3^b$	$\pm4.1^b$	0.00^c	$\pm6.8^a$	$\pm1.8^c$	0.00^c	$\pm3.1^c$	0.00^d	0.00^c	0.00^d	0.00^d	0.00^c	0.00^d	0.00^d
		18.4	8.6	2.6	84.4	26.0	8.6	97.5	17.1	10.6	NS	9.2	2.0	NS	3.0	0	NS	0	0	NS	0	0
3rd batch	Mean±SE	28.0	15.2	10.2	120	32.0	18.4	117.2	32.0	18.0	56.0	21.2	0±	0±	14.4	0±	0±	0±	0±	0±	0±	0±
Challenge	GMT	$\pm6.0^a$	$\pm5.2^a$	$\pm3.6^a$	$\pm18.1^b$	$\pm7.9^b$	$\pm5.8^b$	$\pm20.1^b$	$\pm7.8^b$	$\pm5.6^b$	$\pm17.8^c$	$\pm6.7^b$	0.00^c	0.00^d	$\pm5.1^a$	0.00^c	0.00^d	0.00^d	0.00^c	0.00^d	0.00^d	0.00^c
		17.3	8.0	4.9	90.5	27.9	13.9	78.8	27.9	13.9	55.7	14.9	0	NS	8.0	0	NS	0	0	NS	0	0
4th batch	Mean±SE	34.4	18.4	12.0	104.4	33.2	19.6	101.6	25.6	25.6	83.2	21.2	8.2	10.4	14.2	2.0	2.0	3.4	0±	0±	0±	0±
Challenge	GMT	$\pm9.2^a$	$\pm4.8^a$	$\pm3.2^a$	$\pm17.5^b$	$\pm7.2^b$	$\pm6.5^b$	$\pm17.5^b$	$\pm6.8^b$	$\pm6.7^b$	$\pm13.4^b$	$\pm6.0^a$	$\pm3.0^a$	$\pm6.2^a$	$\pm6.8^a$	$\pm1.6^c$	$\pm1.6^c$	$\pm1.9^c$	0.00^d	0.00^d	0.00^d	0.00^d
		9.8	11.3	6.1	73.3	29.5	10.6	73.3	22.6	21.1	64.4	14.9	5.3	4.0	9.2	1.4	1.4	1.7	0	NS	0	0

Different superscript indicate significant difference within group means along the row at $p < 0.05$ (one-way ANOVA)
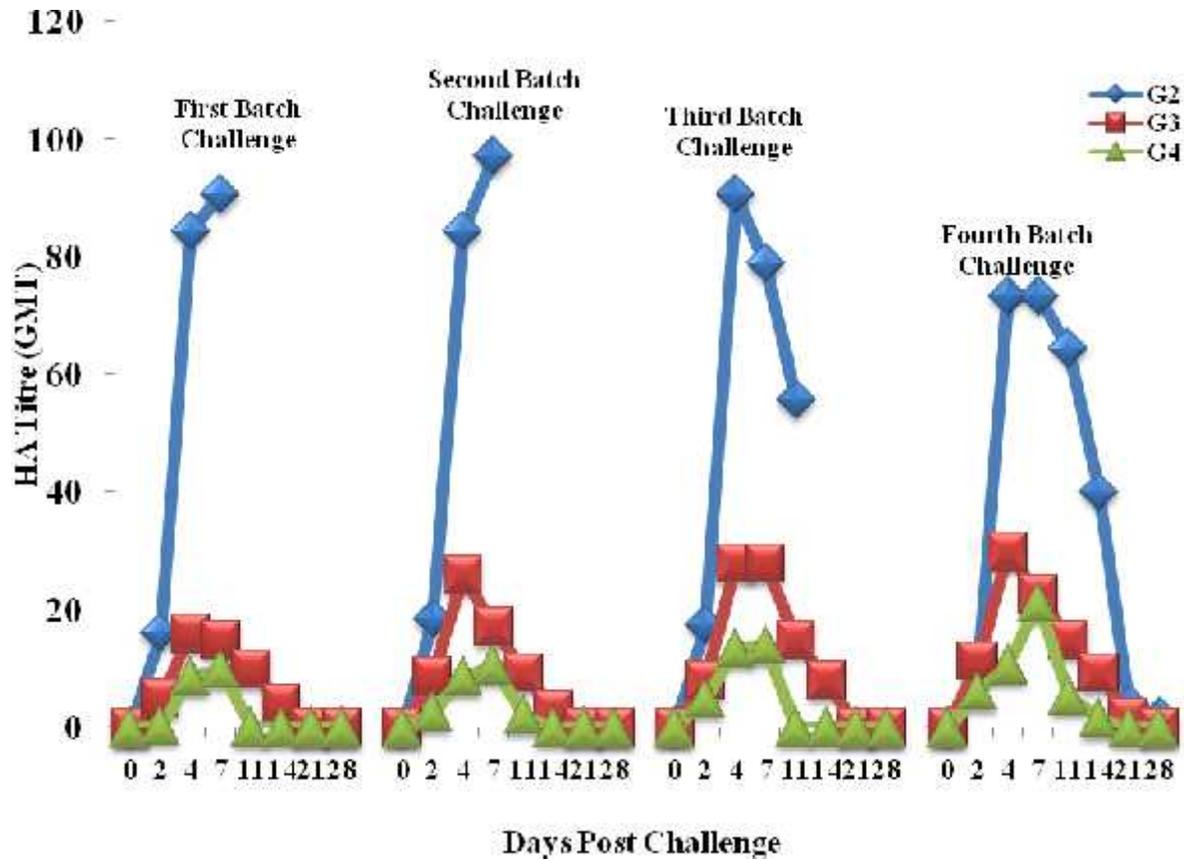
NS = No Sample: no chicken survived.

Figure 1: Comparison of geometric mean HA titres and therefore the level of protection against virus shedding by the vaccines during the 1st , 2nd ,3rd and 4th batch challenges in G2, G3 and G4.

DISCUSSION

The results of this investigation showed that vaccination with La Sota or Komarov did not prevent the replication of Kudu 113 virus in the tissues of the vaccinated birds. It also did not prevent the shedding of the virus in the faeces of the infected birds. These findings with Kudu 113 virus is in agreement with that reported for other isolates of velogenic NDV in others parts of the world (Kapczynski and Kings, 2005; Miller *et al.,* 2007, Van Boven *et al.*, 2008; Miller, 2008; Miller *et al.,* 2009; Rue *et al.*, 2011; Carrasco *et al*, 2016

The result however showed that vaccination significantly reduced viral replication and shedding. When compared with the control, the two vaccines significantly reduced virus shedding in the vaccinated chickens. According to Miller *et al*., (2009) vaccination will significantly decrease the amount of virus shed in saliva and faeces of vaccinated birds when compared to non-vaccinated birds. Miller *et al*., (2013) reported that the amount shed will depend on the immunity of the host, the host species infected, the amount and virulence of the challenge virus, the dose and type of ND vaccine and the

time between vaccination and challenge.. Our study showed that both vaccines used significantly reduced viral shedding in vaccinated birds when compared with unvaccinated birds. However Komarov showed better protection against viral shedding when compared with La Sota. It may therefore suggest that Komarov vaccine matched Kudu 113 than La Sota vaccine phylogenetically. Miller, (2008) showed that increasing the antigenic relatedness of live NDV vaccines virus to the likely virulent challenge virus would improve the efficacy of the vaccine by decreasing the amount of challenge virus shed from vaccinated poultry.

In our study, we were able to detect viruses in the tissue of challenged birds from day 2 PC which increased significantly with peak at days 4 - 7 PC. Previous studies have demonstrated peak virus isolation in vaccinated birds after challenge from day 2 – 5PC (Carrasco *et al*., 2008; Miller *et al*., 2009; Morales *et al*, 2011). In unvaccinated birds virus isolation continued and persisted as long as the animal showed clinical signs of the disease and the virus was shed precipitously in their faeces. For the vaccinated birds, there was significant reduction in viral shedding, though the amount of viral shedding in their faeces was appreciable enough

to posse transmission risks. With La Sota vaccination, virus was isolated from day 2 to day 21 PC and the number positive for virus in tissues and faeces significantly reduced. With Komarov vaccination, virus was isolated from day 2 to day 14 PC and the number positive for virus in tissues and faeces showing better reduction than those vaccinated with La Sota showing that birds vaccinated with ND vaccine Kamorov showed better reduction or protection against virus replication and shedding when challenged with the velogenic virus.

Reduction in viral replication and shedding were higher when the circulating antibody levels were high in both cases. The higher the antibody levels in the chickens as seen during the 1st batch challenges, the more the protection against replication and shedding. It has been shown that a high threshold of humoral immunity can reduce viral replication, independent of genotype of the vaccine (Cornax *et al*., 2012; Choi *et al*., 2013; Guo *et al*., 2014). The authors demonstrated that higher levels of NDV antibodies are required to stop viral replication when compared to what is required to induce clinical protection. Transmission studies indicate that vaccinated birds with low or undetectable antibody titres may be protected against disease and mortality but that infection and transmission may still occur and quantitative analyses show that Newcastle disease virus is highly transmissible in poultry with low antibody titres (Van Boven *et al*., 2008). . As a consequence, herd immunity can only be achieved if a high proportion of birds have a high antibody titre after vaccination.

The use of mesogenic vaccines especially in secondary vaccination of poultry have been reported to offer the best protection in both clinical disease and mortality and in reduction of virus shedding (Miller *et al.,* 2007; Esaki *et al*, 2013). 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 when compared with B1 and La Sota strains (Miller *et al.,* 2007). 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 the more closely related the vaccine is to the likely field strains the more specific neutralizing antibodies produced and consequently the decrease in 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). It is important to know the characteristics and virulence of the velogenic NDV circulating in a country and which vaccines provide the largest decrease in velogenic NDV shed so that less virus is shed into the environment. In the field, multiple factors may decrease the effectiveness of vaccination thus making the antibody specificity more important. 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 velogenic NDV. Reduction of viral shed from vaccinated birds infected with NDV could potentially minimize the impact of an outbreak and help to prevent spread of disease.

This study suggests that the mesogenic vaccine, Komarov, is ~~closely more~~ more closely related to the velogenic virus under study than the lentogenic vaccine, La Sota. Komarov vaccines where applicable will show more ability to reduce viral shedding and therefore better enhance the role of vaccination in ND control by reducing virus transmission from infected birds. In Nigeria (and possibly in other countries with velogenic ND) where ND is endemic with velogenic field stains being common and where the use of velogenic vaccine is allowed, the use of Komarov will reduce the amount of virus shed into the environment and enhance ND control. Where Komarov cannot be used (as in some countries or age or production groups of birds) strategic and frequent application of La Sota vaccine to maintain high circulating antibodies will reduce virus shedding.

REFERENCES

Alexander, D.J. and D.A. Senne (2008). Newcastle disease, other avian paramyxoviruses, and pneumovirus infections. In: Saif, Y.M., A.M. Fadly, J.R. Glisson, L.R. McDougald, L.K. Nolan, and D.E. Swayne, (Eds.). Diseases of Poultry. 12th Ed. Iowa State University Press; Ames. (USA). 75–116 pp.

Al-Habeeb, M.A., M.H.A. Mohamed, and S. Sharawi (2013). Detection and characterization of Newcastle disease virus in clinical samples using real time RT-PCR and melting curve analysis based on matrix and fusion genes amplification. Vet. World. 6(5): 239-243.

Alkhalaf, A.N. (2009). Serological evidence of avian paramyxovirus-2 infection in backyard and commercial poultry birds in Saudi Arabia. Pakistan Vet. J. 29(3): 107-109.

Ashraf, A. and M.S. Shah (2014). Newcastle disease: present status and future challenges for developing countries. African J. of Microb. Res. 8(5): 411-416.

Bwala, D.G., F.O. Fasina, A. Van Wyk and N.M. Duncan (2011) Effects of vaccination with lentogenic vaccine and challenge with virulent Newcastle

disease virus (NDV) on egg production in commercial and SPF chickens. Int. J. Poult. Sci. 10 (2): 98-105.

Bwala, D.G., S. Clift, N.M. Duncan, S.P. Bisschop, and F.F. Oludayo (2012). Determination of the distribution of lentogenic vaccine and virulent Newcastle disease virus antigen in the oviduct of SPF and commercial hen using immunohistochemistry. Res. Vet. Sci. 93(1): 520-528.

Carrasco, A.O., M.C. Seki, T.F. Raso, A.C. Paulillo, and A.A. Pinto (2008). Experimental infection of Newcastle disease virus in pigeons (*Columbia livia*): humoral antibody response, contact transmission and viral genome shedding. Vet. Microbiol. 129: 89-96.

Carrasco, A.O., M.C. Seki, J.L. Benevenute, P. Ikeda and A. A. Pinto (2016). Experimental infection with Brazilian Newcastle disease virus strain in pigeons and chickens. Braz. J. Microbiol. 13(1): 231-242.

Cleophas, T.J. and A.H. Zwinderman (2012). Statistics applied to clinical studies. 5th Ed. Springer. Netherlands. 588 p.

Choi, K.S., S.J. Kye, J.Y. Kim and H.S. Lee (2013). Genetic and antigenic variation of shedding viruses from vaccinated chickens after challenge with virulent Newcastle disease virus. Avian Dis. 57(2): 303-306.

Cornax, I., P.J. Miller, and C.L. Afonso (2012). Characterization of live La sota vaccine strain-induced protection in chickens upon early challenge with a virulent Newcastle disease virus of heterologous genotype. Avian Dis. 56: 464-470.

Dortmans, J.C., B.P. Peeters, and G. Koch (2012). Newcastle disease virus outbreaks: vaccine mismatch or inadequate application? Vet. Microbiol, 160: 17-22.

Echeonwu, G.O.N., C.U. Iroegbu, and A.C. Emenwa (1993). Recovery of velogenic Newcastle disease virus from dead and healthy free roaming birds in Nigeria. Avian Pathol. 22: 283-287.

Esaki, M., A. Godoy, J.K. Rosenberger, S.C. Rosenberger, Y. Gardin, A. Yasuda and K. M. Dorsey (2013). Protection and antibody response Caused by turkey herpesvirus vector Newcastle disease Vaccine. Avian Dis. 57(4): 750-755.

Grimes, S.E. (2002). A basic laboratory manual for the small-scale production and testing of I_2 Newcastle disease vaccine. FAO-APHCA Regional Office for Asia and Pacific. Thailand. 19-72 pp.

Guo, H., X. Liu, Y. Xu, Z. Han, Y. Shao, X. Kong and S. Liu (2014). A comparative study of pigeons and chickens experimentally infected with PPMV-1 to determine antigenic relationship between PPMV-1 and NDV strains. Vet. Microbiol. 168(1): 88-97.

Ibu, J.O., J.O.A. Okoye, D. Eze, F.O.Fasina, E.P. Aba-Adulugba, A.S. Abechi, and I.T. Woma (2008). Antigenic relatedness among Newcastle disease virus isolates from Nigerian feral birds and the LaSota strain. Ach. Vet. Sci. 13(2): 79-84.

Ibu, J.O., J.O.A Okoye, E.P. Aba-Adulugba, K.F. Chah, S.V.O. Shoyinka, E. Salihu, A.A. Chukwuedo, and S.S. Baba (2009). Prevalence of Newcastle disease virus in wild and captive birds in Central Nigeria. Int. J. Poult. Sci. 8(6): 574-578.

ICTV, (2012). Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses. King, A.M.Q, M.J, Adams, E.B, Carstens, and E.J, Lefkowitz Ed: Elsevier, San Diego.

Igwe, A.O., S.W. Ezema , D.C. Eze and J.O.A. Okoye (2014). Experimental Velogenic Newcastle Disease Can Be Very Severe and Viscerotropic in Chickens but Moderate and Neurotropic in Guinea Fowls. Int. J. Poult. Sci. 13(10): 582-590.

Ison, A.J., S.J. Spiegle, and T.Y. Morishida (2005). Poultry blood collection. Ohio State University Extension Factsheet. Vet. Prev. Med. 1900 Coffey Road, Columbus, Ohio. 1-3 pp.

Kapczynski, D.R., and D.J. King, (2005). Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccine upon challenge with highly virulent virus from California 2002 exotic Newcastle disease outbreak . Vaccine. 23(26): 3424-3433.

Kapczynski, D.R., G.N. Mark, and J.K. Daniel (2006). Susceptibility and protection of naive and vaccinated racing pigeons (Columbia livia) against exotic Newcastle disease virus from the California 2002-2003 outbreak. Avian Dis. 50: 336-341.

Kapczynski, D.R., C.L. Afonso and P.J. Miller (2013). Immune responses of poultry to Newcastle disease virus. Develop. and Comp. Immunol. 41: 447-453.

Lamb, R.A. and G.D. Parks (2013). Paramyxoviridae. In: Knipe, D.M. and P.M Howley. (eds). Fields Virology 6th edition. Lippincott Williams and Wilkins, Philadelphia, PA. 957-995 pp.

Mazengia, H. (2012). Review on major viral diseases of chickens reported in Ethiopia. J. Infect. Dis. Immun. 4(1): 1-9.

Miller, P.J., D.J. King, C.L. Afonso, and D.L. Suarez (2007). Antigenic differences among Newcastle disease virus strains of different genotypes used in vaccine formulation affect viral shedding after a virulent challenge. Vaccine. 25: 7238-7246.

Mller, P.J. (2008). Antigenic differences of Newcastle disease vaccine affect viral shedding. Poult. Informat. Profess. 101: 1-3.

Miller, P.J., C. Estevez, Q. Yu, D.C. Suarez, and D.J. King (2009). Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccine formulated with wild- type and recombinant viruses. Avian Dis. 53: 39-49.

Miller, P.J., E. Lucio, and C.L. Afonso (2010). Newcastle disease: evolution of genotypes and the related diagnostic challenges. Infect. Gen. Evol.10: 26-35.

Miller, P.J., C.L. Afonso, J.E. Attrache, K.M. Dorsey, S.C. Courtney, Z. Guo, and D.R. Kapczynski (2013). Effects of Newcastle disease virus vaccine antibodies on the shedding and transmission of challenge viruses. Develop. and Compar. Immunol. 41: 505-513.

Morales, J.A., E. Merino, D. García, R. Ortega, N. Christy, D. Marrufo, R. Cascante1, E. Lucio, M. Cruz1, A.E. Absalon, D.V. Cortes, A. Mariano, and A. Vazques (2011). Development of a recombinant vaccine and its efficacy in a trial with broilers. Proc. 22nd Latin Am. Poult. Congr. 103 p.

Munir, M., M.Z. Shabbir, T. Yaqub, M.A.B. Shabbir, N. Mukhtar, M.R. Khan, and M. Berga (2012). Complete genome sequence of a velogenic neurotropic Avian Paramyxovirus 1 isolated from peacocks (Pavo cristatus) in a wildlife park in Pakistan. J. Virol. 86(23): 13113-13114.

Nishizawa, M., A.C. Paulillo, L.S. Nakachi, A.D. Nunes, J.M. Campioni, and J.L. Doretto, (2007). Newcastle disease in white Peking ducks: response to experimental vaccination and challenge. Rev. Brasil. Ciencia Aviocola. 9: 200-207.

OIE (Office Internatioal des Epizooties), (2012). Manual of diagnostic tests and vaccines for terrestrial animals: mammals, birds and bees. Biological Standards Commission. World Organization for Animal Health, Paris. 1–19 pp.

Okwor, E.C., J.O.A Okoye, and G.O. Echeonwu, (2008). Distribution of a local isolate of velogenic Newcastle disease virus in organs of infected chickens. Nig. Vet. J. 28(3): 19-23.

Okwor, E.C. and D.C. Eze (2013). Newcastle disease in layers: Preliminary studies on the stress associated with onset of lay and initiation of clinical disease. African J. of Microbiol. Res. 7(11): 960-965, .

Oladele, S.B., A.J Nok, P. Abdu, L. Saidu, and K. Esievo, (2006). Comparative studies on serum neuraminidase, free and erythrocyte surface sialic acid, packed cell volume and haemagglutination inhibition antibodies of chickens vaccinated with different Newcastle disease virus vaccines. Veterinar. Arh. 76(5): 391-401.

Orsi, M.A., L. Doretto Jr, S.C. Camillo, D. Reischak, S.A. Ribeiro, A. Ramazzoti, A.O. Mendonça, F.R. Spilki, M.G. Buzinaro, H.L. Ferreira, and C.W. Arns, (2010). Prevalence of Newcastle disease virus in broiler chickens (*gallus gallus*) in Brazil. Brazilian J. Microbiol. 41: 349 – 357.

Reed, L., and H. Muench, (1938). A simple method of estimating fifty percent endpoint. Am. J. Hyg. 27: 493-497

Rue, C.A., L. Susta, I. Cornax, C.C. Brown, D.R. Kapczynski, D.L. Suarez, D.J. King, P.J. Miller, and C.L. Afonso (2011). Virulent Newcastle disease virus elicits a strong innate immune response in chickens. J. Gen.Virol. 92: 931-939.

Saidu, L., Abdu, P. A., and Tekdek, L. B. (2006). Newcastle disease antibodies in parent stock, yolk and chicks. J. Anim. Vet. Adv.-5(6): 503 – 506.

Shabbir, M.Z., S. Zohari, T. Yaqub, J. Nazir, M.A. Shabbir, N. Mukhtar, M. Shafee, M. Sajid, M. Anees, M. Abbas, M.T. Khan, A.A. Ali, A. Ghafoor, A. Ahad, A.A. Channa, A.A. Anjum, N. Hussain, A. Ahmad, M.U. Goraya, Z. Iqbal, S.A. Khan, H. Aslam, K. Zehra, M.U. Sohail, W. Yaqub, N. Ahmad, M. Berg and M. Munir (2013). Genetic diversity of Newcastle disease virus in Pakistan: a countrywide perspective Virol. J. 10: 170-180.

Shittu, I., P. Sharma, T.M. Joannis, J.D. Volkening, G.N. Odaibo, D.O. Olaleye, D. Williams-Coplin, P. Solomon, C. Abolnik, P.J.Miller, K.M. Dimitrov, C.L. Afonso (2016). Complete genome sequence of a genotype XVII Newcastle disease virus isolated from an apparently healthy domestic duck in Nigeria. Gen. Announc. American Soc. Microbiol. doi: 10.1128/ genome A.01/1615.

Van Boven, M., A. Bouma, T.H. Fabri, E. Katsma, L. Hartog, and G. Koch, (2008). Herd immunity to Newcastle disease virus in poultry by vaccination. Avian Pathol. 37: 1-5.