Seroimmune responses to strategic vaccination in chickens against Newcastle disease using commercially available vaccines

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SUMMARY

Evaluation of the Newcastle Disease (ND) antibody level after different vaccination strategies using I-2 and La Sota Vaccines was experimentally conducted on broiler chicken using standard HI test. Three vaccination strategies employed were 12.5%, 25% and 50% of the chickens were vaccinated; positive and negative control groups were used. At 12.5% vaccination strategy, for I-2 vaccine, 12.5% of the chickens were sero-converted to protective level (HI titre results≥log3base 2) and was not significantly different ($p>0.05$) to the negative control group. For La sota vaccine 62.5% of the chickens seroconverted to protective level and was not significantly different ($p>0.05$) compared to control positive group.At this strategy, La sota vaccine has proved to do better than I-2 vaccine in activating humoral immune response.At 25%vaccination strategy, I-2 vaccine has shown that, 75% of the chickens were seroconverted to protective level, the results was significantly different ($p<0.05$) to the negative control group. La sota likewise, 75% of the chickens were seroconverted to protective level which was significantly different ($p<0.05$) to the negative control. At this strategy, both vaccines have the same activation. At 50% vaccination strategy, for I-2 vaccine, 81% of the chickens were seroconverted to protective level and for La sota Vaccine, 94% of the chickens were seroconverted to protective level. For both vaccines their results were significantly different ($p<0.05$) to negative control. At this strategy, both vaccines have similar effect in humoral immune response activation. Therefore vaccinating 25% and above of the chickens will results in a flock immunity in intensive farming.

Key words: Chicken, Newcastle disease, vaccination, La sota

It is estimated that Tanzania had about 36.2 million chickens by 2008, out of which almost 95% are Free Range Village Chickens (FRVC) and the rest are exotic breeds (RLDC, 2011) and most of these village chickens are kept mainly in the rural areas by women and children. Village poultry production plays an important contribution to household food security and income generation (Goromela, 2009; Goromela 2009). The proportion of traditionally raised birds kept in Tanzania, majorities are chicken (94.1%) followed by ducks and geese (5.3%), guinea fowl (0.4%) and turkeys (0.2%) (Melewas, 1989). The growth rate of the chicken industry has been increasing at the rate of 2.6% per year since 2003 (Msami,2008; Msami 2008). The rapid increase of the chicken industry has been influenced by the increased demand of chicken meat and eggs as source of animal protein due to increasing fast food vendors in urban settings (Gyles, 1989) and taken as an opportunity by people both in urban and rural settings. In Tanzania chicken industry is divided into the traditional and the commercial sectors. Indigenous free range local chickens dominate in the traditional sector. Chickens are poorly managed, poorly housed and some roost on trees, no feed supplementation and hardly given veterinary attention and if happens traditional medicines are practices. Chicken management in a family is the responsibility of women and children and men show little interest in chickens except in some areas where chicken sale fetch higher selling price especially in areas where traders from big cities and town come to buy chickens for urban consumption. Village chicken supplies 100% of all the chicken meat and egg demands for rural people and about 20% of urban demand (Ministry of Livestock Development, 2006).

The effort in developing the chicken industry is directed to both the commercial and traditional sectors (Melewas, 1989). Importation of hatching eggs and day old chicks for commercial purposes and production and increased used of Thermo stable Newcastle disease vaccine for village chickens are all effort to develop the chicken industry in the country (Ministry of Livestock Development, 2008). This is because the industry provides employment to people both in urban and rural settings and provides income to government through levy. Therefore, Tanzanian government through Ministry of Livestock Development (MLFD) has been encouraging chicken keeping for income generation and therefore poverty alleviation (Ministry of Livestock Development, 2006).

The major hindrance to rural chicken prosperity is Newcastle Disease (ND). The disease may cause 90% of mortality rates and sometimes clears the whole flock during an outbreak (Buza and Mwamuhehe, 2000). Vaccination against the disease has remains the most effective means of controlling ND (Orajaka, 1999). In Tanzania mostly available commercial ND vaccines are La Sota and I-2 vaccine. La Sota, a lentogenic live vaccine is used mainly in commercial poultry sector and having setbacks in application in rural areas due to the problem of heat intolerance of the vaccine strain, large dose presentation, affordability, reliability, transport and cold chain for effective administration of the vaccine (Dias, 2001) The avirulent, thermostable ND vaccine strains I-2 provide rural poultry farmers with an effective, affordable and reliable means of controlling ND in their flocks (Dias, 2001) and have been used widely and effectively in village chickens population in many Asian and African countries (Dias, 2001). The vaccine is currently widely used in Tanzania (Wust, 2010), the vaccine has been accepted as suitable for use in Vietnamese villages (Tu, 1997) and has been used in Nigeria, Kaduna state (Nwanta, 2006). However, apart from the vaccine availability, ND is still a bottleneck to local village production.

In spite of the availability of vaccines against the ND, there is inadequacy of controlling the disease; this could probably be due to failure of following manufacturer's indications to vaccinate individual chicken in a flock, the free range nature of chickens and short time available due to farmers' engagement to other economic activities. Therefore, this study looked for the best vaccination strategy which will ensure highest level of flock protection and at the same time lessen the work of vaccinators or farmers because vaccination so far remains the most effective strategy for controlling Newcastle Disease (Orajaka,1999). The study investigated the extent of horizontal transmission of vaccine virus and protective antibody response to non-vaccinated in contact chickens following strategic vaccination of chickens against

MATERIALS AND METHODS

Study area

The study was conducted in Mpwapwa District at Kikombo basin. Mpwapwa District is one of the six districts in Dodoma Region. Mpwapwa is located 120 kms from Dodoma Regional headquarters and 54 km from Morogoro-Dodoma main road at a village called Mbande. Mpwapwa lies between latitudes 6°00" and 7°30" South of the Equator and between longitude 35°45" and 37°00" East of Greenwich (Council 2009). It borders Kilosa District on the Eastern part, Kongwa District on the Northern part, Chamwino District on the Western area and Kilolo District on the Southern part. The district covers a total area of 7 379 square kilometers about 18.1% of total area of Dodoma Region (Mpwapwa DC, 2009). Kikombo is an area where institutions under the ministry of Livestock and Fisheried Development including National Livestock Research Institute (NLRI), Veterinary Investigation Centre (VIC) and Livestock Training Institute (LITI) are found.

This study area was chosen because of availability of poultry units which needed little modification to suit the present experiment at LITI poultry units, to disseminate the knowledge about chickens rearing, sample collection and processing to LITI students using VIC laboratory facilities, the area is accessible to Dodoma where chickens inputs can be fetched.
The experiment site was located at latitude 6^{o} 20'66''South and longitude 36^{0} 30' 60'' E. with an altitude of 948m above sea level (GPS. Geko 101, Hampshire, UK). During the study period, November 2011 to March 2012, Kikombo experienced the following maximum average dry temperatures of 30.7 ^{o}C, 28.5 ^{o}C, 28.5 ^{o}C, 29.7 ^{o}C, 28 ^{o}C from November to March respectively and average rainfall of 35.6 ml, 244 ml, 214 ml, 136 ml and 123 ml during the same period but with uneven daily rainfall distribution in a month(Centre 2012).

Experimental Study Design and Methodology

House preparation and chicks managements

Cleaning and disinfection of the housing using broad spectrum disinfectants was done using Rhino White disinfectant containing tar acids at 7-8 % (v/v), a product of SAPA Chemical Industries, Dar Es Salaam, Tanzania. Disinfection was done twice, a month before and two weeks before stocking the chicks. Chicken house was divided into non communicating compartments (Figure 2 below) and spacious enough (90 cm wide x 240 cm long x 210 cm high) to accommodate 16 chickens until the end of study period. The non-communicating compartments where enough to prevent cross contact amongst chickens of different experimental groups. Foot bath carrying tar acids 8% (v/v) was placed at the entry point to prevent contaminants brought in by the attendant. Attendant had to wear overcoat and gumboot when attending chickens and not allowed to work for chickens/animals anywhere else to avoid introduction of contaminants.

Figure 2. A section of chickens in their none-communicating pans

Feed and water were given ad libitum using commercially prepared feed sourced in the market (Igo products, Dar Es Salaam, Tanzania), commercial vitamins supplements (A, B, C, D, E and K) were provided to the chicken through drinking water. Light source was made available to provide heat and light twenty four hours for the first three weeks during brooding (Figure 3 below) and only during night hours thereafter until the end of the study period. Anticoccidial products (Ancoban, Ipswich, UK) were provided to chickens as prophylaxis and treatment during the study period. The deep litter (saw dust) 3 inch was set at the beginning of study and was not changed till the end of the study period.

Figure 3. A Section of chicks during brooding stage

Chickens source and vaccination

About two hundred day old broiler chicks (Figure 3 above) were purchased from a local supplier who sourced them from Interchick®, these chicks of similar age sex and breed came from the parent stock with a history of being vaccinated against ND using live vaccine (Nobilis Clone 30, a product of Intervet, South Africa). The chicks were brooded in the isolation unit for three weeks before being transferred to the experimental site. All chicks were vaccinated against Infectious Bursa Disease (IBD) at the age of two weeks and against Pox at the age of four weeks.

Experimental design

At the age of three weeks, 160 chickens were randomly selected and divided into ten groups of 16 chickens each to be vaccinated using commercially available vaccines either I-2 or La Sota. The first five groups were for I-2 vaccine and the remained five groups were for La Sota vaccine. Each chicken was wing tagged and identified by type of vaccine, group number and specific number of chicken example GL. 1. 1-16 where GL stood for Group La Sota vaccine, 1 stood for 12.5% vaccinated and 1-16 refer specific number of chick within the group.

Yellow colored tags were for none vaccinated and red colored tag for vaccinated ones.

In this experimental trial, the first five groups were vaccinated using I-2 vaccine (CVL product, Dar Es Salaam, Tanzania) batch ND 1107VD111). From group 1 to 5 strategic vaccinations were 12.5% (2 chicks vaccinated), 25% (4 chicks vaccinated), 50% (8 chicks vaccinated), 100% (16 chicks vaccinated) and 0% (None is vaccinated) respectively (Figure 4). I-2 vaccine was given as an eye drop(Young 2002).

The same strategic vaccination was applied for the La Sota vaccine (BIOVAC product, Israel, batch 101414) and was given through drinking water. For both vaccines groups, 100% and 0% vaccination was used as positive and negative controls respectively.

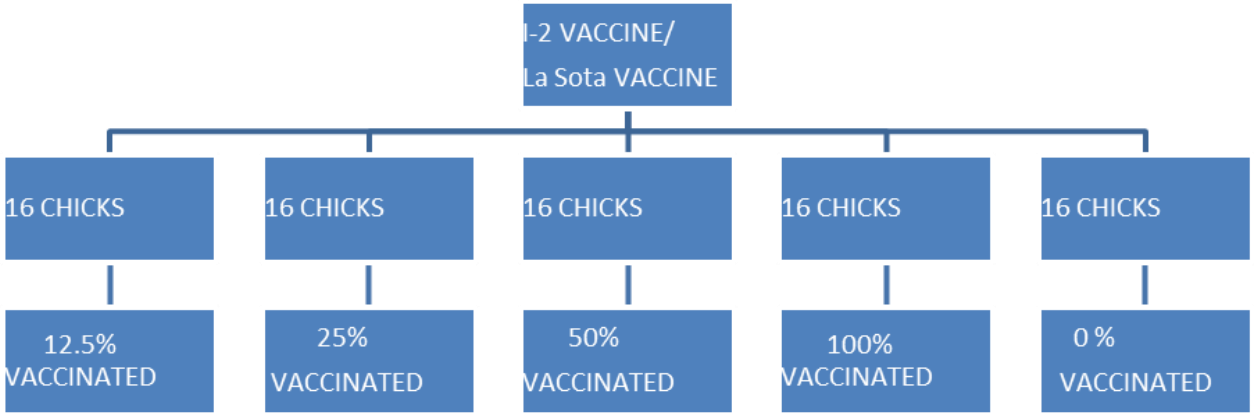

Figure 4. The design of the experimental groups per vaccine used, number of chickens in each group and strategic vaccination in percentage of chickens in a group.

Blood Sample collection

From all the chickens, the first blood sample were collected at the age of three weeks before vaccination and thereafter after every two weeks five times post vaccination (Appendix 1). Using 2mls sterile syringe and needle, 1-2 mls of blood was collected from each chicken through wing vein (Brachial vein), before blood collection, feathers were removed and the site was disinfected using cotton wool soaked in 70% alcohol (Yongolo, 1996) and put in the plain vacutainer tubes and the tubes were labeled.

Blood was left to coagulate in a refrigerator at 4^{o}C overnight (Allan and Gough, 1974a) and centrifuged the following day at 1500 rpm for 2 minutes for clear serum collection. Each serum was kept in a labeled cryovial and stored in the deep freezer at -20^{o}C until HI testing (Allan and Gough, 1974b)
Wing tag number (ID number of chicken) and date of blood sample collection were marked on the vacutainer tube and corresponding cryovial.

Serum Testing Procedure

The Haemaglutination Inhibition (HI) titre of sera from experimental chickens was measured by using standard procedure of microplate HI test (Allan and Gough, 1974a). HI test was performed using four HA units (4HA) of ND virus and a 1% suspension of chicken red blood cells (Allan and Gough, 1974a) in V shaped well microtitre plates. All titres were recorded as log2 of the reciprocal of the end point dilution. In this study the HI titre ≥3 (Log2) was considered positive based on the findings of Allan and Gough (1974) and Bell *et al*. (1991a) who reported that birds with HI titre ≥3 (log2) were protective against challenge with a virulent strain of ND virus. The end point dilution forming a stream following tilting the plate was recorded as a true positive.

Preparation of Newcastle disease virus antigen for use in HI tests

Antigen was prepared by inoculating embryonated chicken eggs free from NDV (SPF) from SUA farm. The driller was used to open the allantoic cavity and 100µL of sample of NDV from I-2 vaccine was inoculated, the opening was sealed by wax and then eggs were incubated for four days before harvesting allantoic fluid. A volume of 60ml of allantoic fluid was harvested and centrifuged at 1,200 g to clarify and remove contaminating red blood cells. The working antigen was stored in a refrigerator at 4°C

and the stock antigen kept at -20°C (Allan and Gough, 1974a).

Preparation of Washed Red Blood Cell Suspension

About 6 mls of blood was collected from 3 chickens found in SUA poultry farm by using 2mls syringe and needles and then transferred to a vacutainer tubes impregnated with an anticoagulant (EDTA), then blood was gently mixed. The blood was centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded, and the tube was refilled with PBS and centrifuged again at 1500 rpm for 5 minutes, again the supernatant was discarded. This step was repeated three times. The last round of centrifugation was done without adding PBS and afterwards the supernatant was discarded. The small part of RBC was diluted to a 1% solution, by adding PBS to prepare a working RBC solution (1ml RBCs: 99 ml PBS).

The control sera

For control purposes, positive secondary laboratory standard serum from SUA VET Virology Laboratory (SUA) was used. This secondary laboratory standard serum was developed following comparative testing with the standard serum from Veterinary Laboratory Agency (Ministry of Agriculture. Weybridge, Surrey, UK). This secondary standard serum was used to confirm our prepared antigen by HI test and had HI titre of log25 and therefore used as control positives, the consistency of results when tested with 4HA units of antigen was observed (Allan and Gough, 1974a). For negative control PBS was used. The positives and negative controls were run simultaneously in the plates and acted as a golden standard for the results.

Preparation of 4HA units of Newcastle disease virus antigen

The standard amount of Newcastle disease virus used in the haemagglutination inhibition (HI) test is 4HA units (OIE, 2012). It was necessary to prepare and test a suspension of Newcastle disease virus containing 4HA units in order to carry out the HI test. This involved a series of following steps.
The antigen prepared was tested by Haemagglutination test (HA)
Procedure (OIE, 2012).

i) 25 µL of PBS was dispensed into each well of a plastic V-bottomed microtitre plate.
ii) 25 µL of the virus suspension was placed in the first 4 wells of the first column of the microtitre plate.
iii) Two fold dilutions of 25µL a volume of the virus suspension was made across the plate iv) 25 µL of PBS was dispensed to each well.
v) 25 µL of 1% (v/v) chicken RBCs was dispensed to each well.
vi) The solution was then mixed by tapping the plate gently. The RBCs were allowed to settle for 40 minutes at room temperature.
vii) HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration should be read to the highest dilution giving complete HA (no streaming) (Figure 5); this represents 1 HA unit (HAU). The found to have end point titre at 210 (1:1024) (Figure 5).
Therefore, to get 4HA Units = Titre/4
=1024/4= 256
Therefore, 1ml of Antigen was mixed with 255ml of PBS to make an antigen working solution.

The results of the back titration of the diluted antigen and the HI titre of the laboratory standard positive (SUA VET Virology Laboratory) were both used to confirm the antigen if has been diluted to a concentration equivalent to the standard 4 HA units.

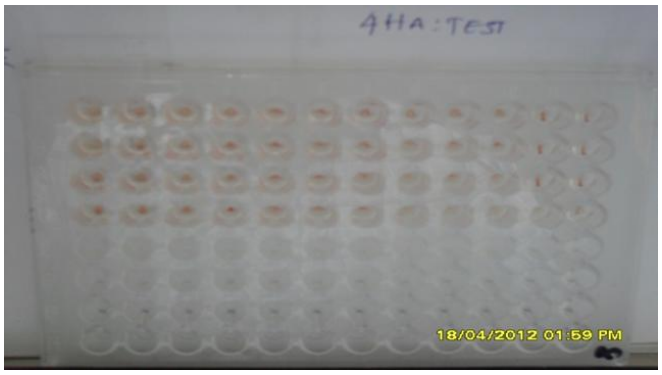

Figure 5. 4HA testing (HA titre of 210 = 1:1024) was recorded

Haemaglutination Inhibition Test Procedure according to Allan and Gough, 1974

Materials required
-Thawed serum samples in racks
-V-bottom microwell plates and covers
-Phosphate Buffered Saline (PBS)
-1 percent washed red blood cells
-V-bottom reagent trough
-25 µL single and multichannel pipettes and tips
-Microwell plate recording sheet.
-Newcastle disease virus antigen diluted to 4 HA units per 25 µL
-Standard positive and negative control (PBS)

Procedure

i) Each test serum was recorded correspondingly to the well on a microtitre plate
ii) 25µL of PBS was dispensed into each well of V botton micro well plate.
iii) After shaking the cryovial of test serum, 25 µL of each test serum was added into the first well on a column and the last well of a row.
iv) By using a multichannel pipette two fold serial dilutions was done along the row until the second last well from the end and discarding the last 25 µL from the second last well.
v) Then, 25 µL of the 4HA dilution of antigen was added into each well except the control wells in the last column.
vi) Then, the loaded microwell plate was gently shaken to allow the reagents to mix. Followed by covering the plate with a lid and allowed to stand for 30 minutes at room temperature.
vii) Then, 25 µL of 1 percent washed red blood cells was added into each well including the control wells in the last column.
viii) The plate was gently held and shaken to allow mixing of the reagents. The plate was then covered by a lid and allowed to stand at room temperature for 45 minutes before start reading.
Reading Results and interpretation

In the well where antibodies are present there will be haemagglutination inhibition, free red blood cells will settle down, tears of RBCs will be made in a well when the plate is tilted at an cute angle, it was done so because sometimes it is not easy to determine degree of haemagglutination inhibition by looking the size of the button and control wells (Allan and Gough, 1974a/b).

The end point of titration is the well that shows complete haemagglutination inhibition by forming tears when a plate is tilted at an acute angle (Figure 6).-In the well where antibodies are not present, there is agglutination and therefore no free RBCs and tears formation when the plate is tilted at an acute angle (Figure 2).

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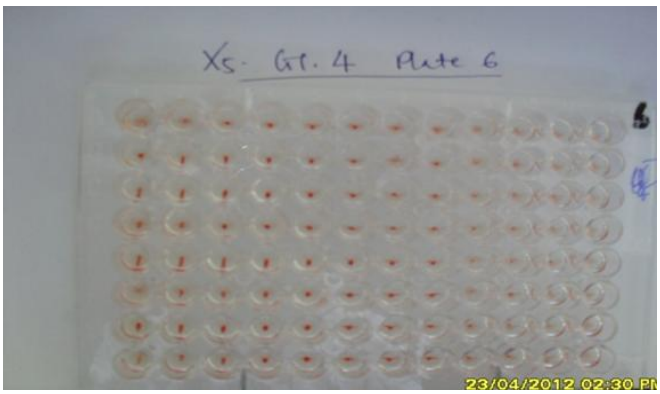

Figure 6. The HI test, Row 1 reads HI negative throughout, Row 5 read HI positive up to well 3 (Log23).

Data Analysis of the test results

The microsoft office excel 2007 spread sheet was used to enter data of end point of dilution showing haemagglutination inhibition of each chicken (vaccinated and non- vaccinated) in each vaccination group (HI titre) before and post vaccination. The excel spread sheet was used to store, summarize, analyse, design and present data. Geometric Mean titre to determine the flock immunity in each vaccination group was calculated by calculating an average of the individual chicken HI titre in a group (Appendix 2). The level of significance before and after vaccination in the same group was calculated by using Chi square test. The number of seropositive and seronegative chickens before and after vaccination was used to find out the effect of vaccination (Appendix 3).

The level of significance between the two vaccines in a similar vaccination strategy was calculated using paired student's T test by comparing their respective GMTs (Table 3). The level of significance to determine the difference in antibodies level in control positive between the two types of vaccines was calculated by using paired student's T test by comparing their respective GMTs before and after vaccination (Table 3).

The level of significance when comparing the GMT of the vaccination strategy and the respective control was calculated by using paired student's T test as well.

RESULTS

12.5% vaccination strategy

For the group vaccinated using I-2. The HI before vaccination (X0) indicated that 9 chickens (44%) were seropositive (HI positive) and 56% of the

chickens were seronegative (HI negative) before vaccination (Table 1). At the 8th (X4) and 10th week (X5) post vaccination no in contact chicken had protective antibodies level except for the two vaccinated ones. The highest Geometric Mean titre (GMT) of 2.00 was recorded at two weeks post vaccination (X1) and 1.13 at the 8thweek (X4) and 10th week (X5) at the end of the experiment (Table 1).

For the effect of vaccination, there was no significant difference in numbers of seropositve chickens before and post vaccination ($p>0.05$). Likewise, there was no significant difference ($p>0.05$) in GMT when compared to negative control group. But when compared to the positive control group (100% vaccinated), there was significant different between the two groups ($p<0.04$).

For the group vaccinated using La Sota, the HI results before vaccination (X0) indicates that 50% of the chickens were seropositive and 50% of the chickens were seronegative (Table 1). The number of seropositive and seronegative chickens keep changing, at the 8th week (X4) 63% of the chickens were seropositive and 37% of the chickens were seronegative which was maintained until the end of the experiment at the 10th week (X5) (Table 1).

The GMT of 2.75, 3.00, 2.13 and 3.19 were recorded at the 2nd week (X1), 4th week (X2), 6th week (X3) and 8th week (X4) post vaccination respectively (Table 1). The highest GMT of 3.31 was recorded 10th week (X5) post vaccination at the end of the experiment (Table 1).

For the effect of the vaccine, there was no significant difference in number seropositive and seronegative before and after vaccination ($p>0.05$). When the GMTs of this vaccination strategy were compared to the GMTs of the positive control (100%), the two groups were not significant different ($p> 0.05$) in protecting chickens. However, at this vaccination strategy (12.5%), when the effect of La Sota and I-2 vaccines were compared using their GMT, they were significant different ($p< 0.05$) in protecting chickens.

Vaccination strategy 25 %

For the group vaccinated using I-2, the HI results before vaccination (X0) indicates that 38% of the chickens were seropositive and 62% of the chickens were seronegative (Table1). At the 8th week (X4) post vaccination 56% of the chickens were seropositve and 44 % of the chickens were seronegative (Table 1). At 10th week (X5) 75% of the chickens were seropositive and 25% of the chickens were seronegative (Table 1) at the end of the experiment. The highest GMT of 3.25 was recorded at 6th weeks (X3) post vaccination followed by a subsequent falling in the GMT to 2.50 in the 10th weeks (X5) post vaccination at the end of the experiment (Table 1).

For the effect of vaccination, the number of seropositive and seronegative chickens before and after vaccination was found to be significant different ($p<0.05$). When the GMTs are compared to the GMTs of the positive control group (100% vaccinated) the two groups were not significant different ($p>0.05$).

For the group vaccinated using La Sota, the HI results before vaccination (X0) indicates that 44% of the chickens were seropositive and 56% chickens were seronegative (Table3). At the 4th week (X2) post vaccination, 69% of the chickens were seropositive and at the 10th week (X5) post vaccination, 75% were seropositive and 25% were seronegative (Table 1). The highest Geometric Mean titre of 3.25 was recorded at two weeks (X1) post vaccination and 3.06 in the 10th week (X5) post vaccination at the end of the experiment (Table 1).

For the effect of vaccine, the number of seropositive and seronegative chickens before and after vaccination were statistically significant different ($p<0.05$). When the GMTs of this strategy are compared to the GMTs of the positive control the two were not significantly different ($p> 0.05$).
However, at this vaccination strategy, when the effect of La Sota and I-2 vaccines in chickens were compared themselves using their GMTs, statistics shows that they are not significant different in protecting chickens ($p> 0.05$).

Vaccination strategy 50%

For the group vaccinated using I-2, the HI results before vaccination (X0) indicates that 63% of the chickens were seropositive and 37% of the chickens were seronegative (Table 1). At the 2nd week (X1) post vaccination 31% of the chickens were seropositive and 69% of the chickens were seronegative. The number of seropositive was increased to 37% of the chickens at 4th and at 6th week (X3) post vaccination 87% of the chickens were seropositive followed by subsequent fall in seropositive chickens to 81% in the 8th week (X4) which was maintained to the 10th week (X5) which was the end of the experiment (Table 1). The highest GMT of 3.00 was recorded at 6th weeks (X3)

post vaccination followed by a subsequent falling in the GMT to 2.75 in the 10th week (X5) post vaccination which was the end of study period (Table 1). For the effect of the vaccine, number of seropositive and seronegative chickens before and post vaccination were not significantly different ($p>0.05$). When the GMTs of this vaccination strategy were compared to those of positive control the two groups were not significantly different ($p>0.05$) in protecting chickens. For the group vaccinated using La Sota, the HI results before vaccination (X0) indicates that 31% of the chickens were seropositive and 69% of the chickens were seronegative (Table 1). At the 2nd week (X1) and 4th week (X2) post vaccination 63% of the chickens were seropositive and 37% were seronegative. The number of seropositive chickens at 6th week post vaccination (X3) was increased to 81%. The number of seropositive chickens was increased to 94% at the 10th week (X5) post vaccination at the end of the experiment (Table 1). The high Geometric Mean titre of 4.38 was recorded at 6thweeks (X3) post vaccination and followed by subsequent rising to the highest 5.56 in the 10th week (X5) post vaccination at the end of the experiment (Table 1).

For the effect of vaccine, the numbers of seropositive and seronegative chickens before and after vaccination were compared and found to be significant different ($p < 0.05$). When the GMTs of this vaccination strategy were compared to those of positive control, the two groups were not significantly different ($p>0.05$) while significant different to the control negative ($p<0.05$). However, at this vaccination strategy, when La Sota and I-2 vaccines were compared using their GMTs in this vaccination strategy (50%), they were not significant different in protecting chickens ($p>0.05$).

Table 1. HI titre and GMT results for each vaccination strategy and controls

Vaccination Strategy	Vaccine Type	HI TITRE RESULTS												Comparison of GMTs (Means) (p=0.05)
		X0		2nd (X1)	Week	4th (X2)	week	6th (X3)	week	8th week (X4)		10th week (X5)		
		+	-	+	-	+	-	+	-	+	-	+	-	
	I-2	44%	56%	44%	56%	6%	94%	13%	87%	13%	87%	13%	87%	
12.5%	GMT±1.01	2.6		2.00		0.94		0.93		1.13		1.13		
	La Sota	50%	50%	56%	44%	50%	50%	31%	69%	63%	37%	63%	37%	
	GMT±1.16	2.19		2.75		3.00		2.13		3.19		3.31		
														P<0.05
	I-2	38%	62%	38%	62%	38%	62%	50%	50%	56%	44%	75%	25%	
25%	GMT±2.03	1.81		2.25		2.56		3.25		2.31		2.50		
	La Sota	44%	56%	62%	38%	69%	31%	25%	75%	31%	69%	75%	25%	
	GMT±1.9	2.25		3.25		3.00		1.44		1.75		3.06		p>0.05
	I-2	63%	37%	31%	69%	63%	37%	88%	12%	81%	19%	81%	19%	
50%	GMT±1.44	2.63		2.00		2.75		3.00		2.73		2.79		
	La Sota	31%	69%	63%	37%	63%	37%	81%	19%	69%	31%	94%	6%	
	GMT±2.29	2.00		3.38		2.94		4.38		3.50		5.56		p>0.05
	I-2	56%	44%	12%	88%	88%	12%	81%	19%	81%	19%	81%	19%	
100%	GMT±1.46	2.63		1.53		3.00		3.25		3.06		3.00		
	La Sota	25%	75%	100%	0%	69%	31%	88%	12%	94%	6%	100%	0%	
	GMT±1.78	1.69		3.94		2.50		5.00		4.56		4.13		p>0.05
	I-2	69%	31%	19%	81%	12%	88%	6%	94%	0%	100%	0%	100%	
0%	GMT±1.11	2.50		1.69		0.69		0.88		1.38		1.69		
	La Sota	44%	56%	44%	56%	19%	81%	6%	94%	0%	100%	0%	100%	
	GMT±1.30	2.06		2.31		1.88		1.19		1.50		1.58		p>0.05

+ = ≥3Log base 2, - = <3Log base 2 GMT= Geometric Mean Titre, GMT± SD

The control positive (100% vaccinated)

For the group vaccinated using I-2, the HI results before vaccination (X0) indicates that 56% of the chickens were seropositive and 44% were seronegative (Table 1). At the 2nd week (X1) post vaccination 13% of the chickens were seropositive and 87% were seronegative. At the 4th week post vaccination (X2) 88% of the chickens were seropositive and 12% were seronegative. Seropositive chickens were reduced to 81% at 8th week (X4) and maintained until the end of the experiment at 10th week (X5) (Table 1). The highest Geometric Mean titre of 3.25 was recorded at 6th weeks (X3) post vaccination and followed by fall in the Geometric Mean titre to 3.00 in the 10th week (X5) post vaccination at the end of the experiment (Table 1).

For the effect of the vaccine, when the number of seropositive and seronegative chickens before vaccination were compared to the number of seropositive and seronegative chickens post vaccination and found to be significant different ($p < 0.05$).

For the group vaccinated using La Sota, the HI results before vaccination (X0) indicates that 25% of the chickens were seropositive and 69% were seronegative (Table 1). At the 2nd week (X1) post vaccination all 100% of the chickens were seropositive. The percentage of chickens who were seropositive at the 2nd week gradually reduced in the 4th week (X2), 6th week (X3) and 8th week (X4) to 69%, 88% and 94% respectively before peaking up again to (100%) in the 10th week (X5) at the end of the experiment (Table 1). The Geometric Mean Titre recorded before vaccination (X0) was 1.69 and 3.94 two weeks (X1) post vaccination and peaked up in the 6th week (X3) post vaccination to 5.00 before drop down to 4.56 and 4.13 in the 8th week (X4) and 10th week (X5) respectively. For the effect of vaccine, the number of seropositive and seronegative chickens before vaccination when compared to the number of seropositive and seronegative chickens post vaccination was

significant different ($p < 0.05$). When the two vaccines I-2 and La Sota were compared at this positive control vaccination by using their GMTs, the two vaccines were not significantly different ($p>0.05$) in protecting chickens.

The control negative (0% vaccination)

For the control negative group stayed in the I-2 vaccinated chickens, 69% of the chickens were tested seropositive and 31% tested seronegative before vaccination (X0) (Table 1). The seronegative chickens were sharply increased to 81% two weeks later (X1) and there was slight increase to 88% at 4th week later (X2). Seronegative chickens were then increased to 94% and 100% at the 6th week (X3) and 8th week (X4) respectively and maintained until the end of the experiment at 10th week (X5) (Table 1). The highest GMT of 2.5 was recorded during the first sampling (X0) and decreased to 1.69 and 0.69 at the 2nd week (X1) and 4th week (X2) respectively before starts to increase to 0.88, 1.38 and 1.69 at the 6th week (X3), 8th week (X4) and 10th week (X5) respectively at the end of the experiment (Table 1). Statistically there was significant difference ($p<0.05$) in the number of seropositive and seronegative chickens at the beginning and at the end of the experiment.

For the negative control group in the La Sota vaccinated group, 44% of the chickens were seropositive and 56% were seronegative at first sampling (X0) and second sampling two weeks later (X1) (Table 1). Then there was a steady increase in seronegative chickens to 81% and 94% at the 4th week (X2) and 6th week(X3) respectively. Seronegative chickens were then increased to 100% at the 8th week (X4) and maintained at the 10th week (X5) the end of the experiment (Table 1). The GMT of 2.06 was observed during the first sampling (X0) which was then increased to 2.31 at the 2nd week sampling (X1). During the third sampling at 4th week (X2) the GMT was decreased to 1.88 then decreased to 1.19 at the 6th week (X3). Then there was gradual increase in GMT to 1.50 and 1.58 at the 8th week (X4) and 10th week (X5) at the end of the experiment (Table 1). There was significant difference in number of seropositive and seronegative chickens at the beginning when compared to the number of seropositive and seronegative chickens at the end of the experiment ($p < 0.05$). When the negative control groups stayed in the I-2 and La Sota vaccination were compared by using their GMTs, they were not significant different ($p> 0.05$).

DISCUSSION

This experiment shows that chickens primed vaccinated using I-2 and Lasota were protected against Newcastle disease just like many research findings (Mazija, 1990, Tu, 1997, Dias, 2001). For each type of vaccine results shows that, vaccine viruses have the potential of horizontal spread ability from one vaccinated chicken to other in contact chicken(Dias 2001, Nazeri 2011). The usefulness of vaccine in protecting chickens has been described by Darrel *et al.* (Darrel *et al* 2013) when they challenged vaccinated chickens against ND and found no immune depressive elements. This study used day old broiler chicks from parents with a history of being vaccinated against ND because it was not easy to acquire large number of chicks from non-vaccinated parents. This led to the use of chickens with different immune status at the beginning of the experiment due to maternal immunity. OIE recommends that to avoid interference of maternal antibody in chicks, vaccination should be done until the chickens are at the age of 2-4 weeks when most of them would have been susceptible. In this experiment vaccination was done when chickens were 3 weeks of age (OIE, 2012). Al Zubeedy (Al Zubeedy, 2009) recommended early vaccination to enhance not only maternal derived immunity but also cell mediated immunity. The major factors affecting seroconversion and seroreversion in this study were vaccines and individual chicken's response to vaccines. Different strains of NDV used to prepare these vaccines can have effect in the immune response in vaccinated chickens and so was the objective of this study. The individual chicken's response to vaccines was taken care by using the geometric mean titre to find out effect of vaccine in immune response of the vaccinated chickens and non- vaccinated in contact chickens. For the evaluation of the effect of I-2 and La Sota vaccines, GMT obtained from each vaccination strategy were compared amongst the vaccines themselves and to the controls.

In this research, at 12.5% vaccination strategy for I-2 vaccine, none of the in contact unvaccinated chickens was seropositive except for the two vaccinated ones until the end of the study. This finding is similar to the findings of Rahman*et al.* (Rahman*et al*, 2004) when they found only vaccinated chickens where protective. The GMTs of this vaccination strategy was not significantly different ($p>0.05$) to the GMTs of the negative control ($p>0.05$) and significantly different to the positive control ($p<0.05$). The results of having seropositive chickens following vaccination agrees to the findings of Aini*et al*. (1990) and contrast the findings of Bell *et al*. (1991) about significant increase of positive reactors after vaccination. For La Sota vaccine 62.5% of the chickens were HI tested positive at the end of the study and the GMT of 3.31 was recorded which was not significantly different ($p<0.05$) to the control positive group (100% vaccinated) and significant different ($p>0.05$) to the negative control (0% vaccinated) group. When I-2 and Lasota were compared using their GMTs at this vaccination strategy, the two vaccines were significant different ($p<0.05$) in ability for vaccine virus spread and therefore induce antibody production and confer protection to in contact chickens and La Sota has done better than I-2. This finding agrees to the findings of Feizi and Nazeri in 2011 (2011) when they compared the HI titres of Avinew and La Sota vaccines.

In the vaccination strategy 25%, I-2 vaccine showed that, 75% of the chicken were seropositive (protected) until the end of the study and the GMT of 2.5 was recorded and found to be statistically significant different ($p<0.05$) to the negative control group and not significantly different ($p>0.05$) with the positive control group. This suggests that the vaccination has done better similar to as when all chickens were vaccinated. La Sota on the other hand, 75% of the chicken were tested positive and their GMT recorded was 3.06 which was significantly different ($p<0.05$) to the GMT of the negative control group and not significantly different ($p>0.05$) with the GMT of the positive control group. When the GMTs of both vaccines were compared, they were not significant difference ($p>0.05$) in the spread of vaccine and induction of antibody production to protective levels in the chickens.

For the vaccination strategy 50%, for I-2 vaccine 81% of the chickens were tested positive and the GMT of 2.75 was recorded at the end of the study period. When this GMT is compared to the GMT of the control negative the two are significantly different ($p<0.05$) and not significantly different with the GMT of the positive control group ($p>0.05$). This means that this strategy is as good as vaccinating all chickens. The same results was obtained in Mozambique by Dias *et al*. (2001), they found that 6 chickens were protected against ND when stayed in contact with 10 vaccinated chickens (62%). La Sota vaccines on the other hand, 94% of chickens were tested positive and the GMT of 5.56 was recorded at the end of the study period. When this GMT is compared to the GMT of negative control group, the two are significantly different ($p<0.05$) but when compared to the positive control group, the two are not significantly different ($p>0.05$).When both vaccines at this strategy are compared using their GMT, statistics shows that they were not significantly different in stimulating antibody production in vaccinated and in contact none vaccinated chickens at this vaccination strategy.

This experimental study found that, for both vaccines, vaccine viruses have the potential of spreading from one chicken to another, as found in unvaccinated in contact chickens. For the vaccination strategies employed in this study, 50% vaccination strategy for both vaccines has shown to provide better protection when compared to the rest strategies as it gives higher proportion of immunized individual 81% and 94% for I-2 and La Sota vaccines respectively. Furthermore, this strategy induced highest level of GMT of 2.75 and 5.56 for I-2 and La Sota vaccine respectively the levels not attained by other vaccination strategies. This level of GMT attained were not significantly different to their respective positive controls. Therefore, instead of vaccinating the whole flock 50% vaccination is sufficient to provide flock/herd immunity similar to as vaccinating all, but field trials need to be done to comprehend this finding.

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