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Evaluation of Antibody Response to Newcastle Disease Vaccination in Chickens in Some Commercial Farms-Ii Two Local Government Areas in Lagos State, Nigeria

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Abstract

Newcastle disease is of great economic importance causing devastating losses among both intensive, extensive and backyard poultries that provides lifeline to many poor people across the developing world. Despite the availability of ND vaccines, vaccination failure suffices in most poultry farms. This study evaluated antibody response following vaccination against Newcastle disease. A total of five hundred and thirty four sera were collected and one hundred and thirty three were negative. The sera were tested for NDV antibody using Haemagglutination-inhibition test in two Local Government Areas (LGAs): Badagry and Ojo of Lagos state, Nigeria. A total of two hundred and thirty seven sera were tested in Badagry, and seventy six were negative with mean Ab titre of 5.04 ± 3.26, mode of ≤ $3\log_2$ of 32.1 while one hundred and sixty one were positive with $4\log_2$ of 38.4. Farm visited were mostly into layers production with provisions for brooding chicks and raising growers. Risk factors identified were rodent infestation, unmanned gate, lizard infestation and lack of PPE. Ojo had a total of two hundred and ninety seven sera tested with one hundred and fourteen were negative with mean Ab titre of 4.36 ± 3.36 mode of ≤ $3\log_2$ of 38.4% while one hundred and eighty three were positive with mode of ≥ $4\log_2$ of 61.9. The immunity in different age group varies within the 2 LGAs. The risk factors identified were rodent infestation, lizard infestation, lack of PPE and risky visitors. In conclusion more than thirty percent of chickens within Badagry and Ojo LGAs in Lagos State of Nigeria vaccinated against NDV were not protected from the disease.

Keywords: Antibody; Birds; Newcastle disease virus; Badagry; Ojo

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Introduction

Newcastle disease (ND) also known as Ranikhit disease (RD) is a highly contagious viral disease that attacks many species of domestic and wild birds [1]. Several species of birds are affected by ND with chickens and turkeys being readily susceptible. It was reported that more than 250 species of free living and caged birds have been infected with NDV therefore; it is probable that most avian species, domestic and feral, can be infected with strains of NDV [2]. There can be little doubt that the highly pathogenic form of ND is a serious problem, either as an enzootic disease or as a cause of regular, frequent epizootics throughout Africa, Asia, Central America and parts of South America [3-6]. Several outbreaks have been reported in guinea fowls in some African countries such as Nigeria and Niger [7], including Morocco [8]. There was serological evidence of ND infection in pigeons in Nigeria [9].

Serology is the measurement for antigen/ antibody interaction for diagnostic purposes [10]. Newcastle disease vaccines are tested for potency and antibody response by a number of serological methods. The two methods used to measure antibody titres are: the haemagglutination inhibition (HI) test, and the enzyme-linked immunosorbent assay (ELISA). At present the HI test is the most widely used for detecting antibodies to NDV in birds although some poultry producers prefer the use of commercial ELISA kits [11]. Despite vaccination of birds, some farmers and other stakeholders are of the opinion that there is shortened protection interval between vaccinations leading to frequent outbreaks [12]. There is

therefore need to evaluate the response against ND vaccination in some chicken farms in two Local Government Areas in Lagos State.

Materials and Methods

Study area

The study was carried out in Lagos State which is located in South Western part of Nigeria and lies between Latitude 6°2' N-6°2' N and Longitude 2°45' E- 4° 20' E with a land area of 3,475 km^2 (1.341 sq mls). It has an estimated population of 9.113,603 people according to the 2006 projected census. A total of 787 km of Lagos territory is covered by water. Lagos is a highly heterogeneous state with most ethnic groups from all over the country being represented but the Yorubas are considered the main ethnic group of the state and their language is spoken by many of its inhabitants. Animal husbandry is widely practiced in which chickens plays a major role because of both population and accessibility of raw materials. The city is the nerve centre for all commercial activities in the country and chicken and other livestock production contribute to a greater measure in the economy of the state.

Study design

A cross sectional study was performed using multistage sampling method to select 2 Local Government Areas (LGA) from the 20 recognized ones based on their chicken farming activities from the 3 senatorial districts. The LGAs were Ikorodu and Ojo. Purposive sampling was done to select farmers from each Local Government. Each enrolled farmer was administered a pretested structured questionnaire based on farm identification, demographic data of the farmer, flock identification, ND vaccination programs and biosecurity. In addition, blood samples were aseptically collected from 10 chickens randomly selected from each flock in the farm. Birds of the same age housed together under the same management were regarded as a flock irrespective of the number.

Ethical approval

The care of the animals was in conformity with the guideline for animals' experimentation of Council for International Organization of Medical Sciences (CIOMS) for biomedical research involving animals.

Blood collection and handling

Blood collection: Blood samples were aseptically collected through the wing vein. About 2.0 mL of blood was collected from each selected chicken from the same flock using sterile hypodermic needle and 2 mL syringe and delivered into 5 mL plain sample bottle. The blood samples were placed in a slanting position on the bench for an hour to clot and the sera obtained by decanting into new well labeled sample bottle with sample number consisting of Local Government, farm number, and flock number. The sera were kept in a cooler with ice packs during transportation from the farm to the house and stored at -20°C in a freezer. Sera were later transported to the Avian Laboratory of Ahmadu Bello University Zaria in a Coleman box with ice packs and stored at-20°C before being tested for NDV antibodies.

Newcastle disease virus antigen preparation

The antigen was prepared from NDV-LaSota vaccine obtained from the National Veterinary Research Institute, Vom**.** The 200 dose vial of the NDV-La sota vaccine was reconstituted in 2 mL of phosphate buffer solution (PBS) (pH 7.4).

Preparation of 1% red blood cells

Five milliliter of blood was aseptically collected from an apparently healthy bird using sterile hypodermic needle and syringe into EDTA test tube and then washed by centrifuging at 2,500 rpm for 5 minutes. The supernatant was discarded and replaced with fresh PBS and centrifuged. The process was repeated thrice. Forty milliliters of PBS was measured into sterile container and 0.05 µL of the washed RBC was added 8 times to get the 1% (v/v) washed RBC.

Determination of antibodies to Newcastle disease antigen titre test

The haemagglutination (HA) titre was determined as described by OIE (2012) [11] and diluted to contain 8HA units for use in the HI test as described by Allan WH and Gough [12].

1. First 25 µL of PBS were dispensed in the wells of the first (A) and second (B) rows of a clean and dry V- bottom microtitre plate.

2. Some 25 µL of prepared antigen were dispensed into the first wells of A and B rows and serial double dilutions were made down to the last wells of each row (representing a 2 fold serial dilution).

3. Some 25 µL of 1% (v/v) chicken RBC were dispensed in each of the wells of rows A and B.

4. The solutions were gently mixed by tapping the plate and left for 15 to 20 minutes for reaction.

5. The result was recorded as the reciprocal of the well with the highest titre value.

6. The end point was determined by converting one haemagglutinine unit to 8HA units obtained by dividing the titre value of HA by 8.

Determination of antibodies to Newcastle disease test

The haemagglutination inhibition (HI) test was used for detection of the presence of antibodies against ND according to the Office International Epizootics (OIE, 2012) [11].

1. First 25 µL of PBS was dispensed into each well of the first row of a clean and dry V-bottom microtitre plate starting from the 2nd well down to the 12th.
2. A 25 µL of the test serum was dispensed into the first and 2nd wells only.
3. A two fold serial dilution was made starting from the 2nd well tot the 11th.
4. A 25 µL of antigen (obtained by diluting 1 mL of antigen in 127 mL of PBS) was dispensed into the first well except the 12th.
5. The set up was allowed for 20 to 30 minutes for antigen/ antibody reaction.

6. A 25 µL of 1% (v/v) chicken RBCs was dispensed into each of the wells (1 to 12) and gently tapped to mix.
7. The setup was allowed for additional 30 minutes before reading.
8. The first and 12th wells served as the positive and negative controls respectively.
9. Haemagglutination was determined by tilting the plate to observe for presence or absence of tear shaped streaming of the RBCs.
10. Haemagglutination was determined by tilting the plate to observe for presence or absence of tear shaped streaming of the RBCs.

Data analyses

Data was analyzed with Statistical Package for Social Science (SPSS) Version 16. The student T test was used to determine the means of the results. Odd ratio (OR) and 95% confidence interval was used on variables to test the strength of association.

Results

Distribution of Newcastle disease antibody titre of chickens from two local government areas of Lagos state, Nigeria

A total of 534 sera were tested with 130 being negative and 404 positive. The mean antibody titre from the 2 Local Governments of the state ranged from 5.04 ± 3.26 in Badagry to 4.36 ± 3.36 in Ojo **(Table 1)**.

Distribution of chickens with ≤ 3 log_2 and ≥ 4 log_2 antibody titre against Newcastle disease from two local government areas, Lagos state, Nigeria

Out of 237 sera tested in Badagry, 76 (32.1%) had antibody titre ≤ 3log_2 **(Table 2)** and 161 (67.9%) sera within ≥ 4log_2. Similarly in Ojo, out of a total of 297 sera sampled, 114 (38.4%) had antibody titre ≥ 3 log_2 and 183 (61.6%) sera within ≥ 4 log_2.

Distribution of Newcastle disease antibody titre by age group in chickens from two local government areas, Lagos state, Nigeria

The chickens were grouped into 3 (1-6 weeks for chicks, 7-8 weeks for Growers and greater than 19 weeks for Layers). In Badagary, the total mean antibody titre of chickens was 5.29 ± 1.02. Growers had 5.68 ± 2.01 titre **(Table 3)** with 67.9% negative and 22.1% positive titre **(Table 4)**. Layers had 5.31 ± 1.00 antibody titre with 61.6% negative and 29.4% positive titre. In Ojo, a total of 4.01 ± 3.44 mean antibody titre was recorded **(Table 5)**. Growers had 3.90 ± 3.47 titre and 27.3% negative and 72.7% positive titres **(Table 6)**. Layers had 3.90 ± 3.47 mean antibody titre with 45.7% negative and 54.3% positive titres.

Biosecurity risk factors identified from two local government areas, Lagos state, Nigeria

Biosecurity risk factors identified in the two Local Government Areas were ten in descending order: In Badagry the rodent infestation had risk factor of 2.20; Personal protective Equipment 2.00; Allowing risky visitors 2.30; unmanned gate 1.70; Carcass Disposal 1.00; Lizard infestation 1.70; Fly infestation 0.70; Keeping chickens at home 1.40; Feed Spillage 0.74 and Poor handling of sick poultry 0.89. Ojo recorded 3.00 risk factor in rodent infestation; Personal protective Equipment 1.50; Allowing risky visitors 1.80; unmanned gate 1.30; Carcass Disposal 1.40; Lizard infestation 1.90; Fly infestation 1.50; Keeping chickens at home 0.96; Feed Spillage 0.73 and Poor handling of sick poultry 0.77. The risk levels were high in Badagry compared to Ojo **(Table 7).**

Discussion

Lagos State being one of the biggest commercial cities in Nigeria has several well established chicken businesses which contribute in diverse ways to the economy of the state. Newcastle disease as one of the major threats facing the poultry industry in the State is presently prevented only through vaccination as obtained in various parts of the world. The Protective antibody titre against NDV in vaccinated chickens was accepted at titre 1:16 or log_2 4 or 2^4 according to OIE (2012). The study shows that only about 2/3rd of the total vaccinated chickens had protective antibody against NDV within Badagry and Ojo while the remaining 1/3rd of the population were unprotected and at risk of the disease. This implies that most of the vaccinated chickens were still vulnerable to NDV. The vaccination failure may not be unconnected with wrong vaccination protocols and nature of vaccine used by different farmers in the chicken business including wrong practice of purchase of vaccines from unauthorized dealers who invariably store vaccines under inappropriate conditions. Wrong vaccination protocols could affect vaccine response in chickens as observed by Ref. [13]. Usually the duration of immunity produced post vaccination varies with the type of vaccine and age of chicken vaccinated. Chickens vaccinated with tissue culture vaccines of NDV may still be vulnerable to velogenic strains of NDV. Such chickens are better protected by repeated ND vaccination [13]. Notwithstanding, the degree of resistance induced by any vaccine is subject to the level of challenge present and in very severe challenges, vaccines are unlikely to give protection.

Ojo Local Government had a higher number of unprotected chickens with percentage distribution of 38.4% compared to Badagry 32.1%. The result showed that more than 20 percent of chickens within the Local Governments had low immunity against NDV **(Table 2)**. As recorded, the percentage distribution of chickens with protective antibodies against NDV was higher in Badargry at 67.9% compared to that in Ojo 61.6%. This indicates immunity against NDV in more than half of chicken populations in the 2 Local Government Areas. There was significant association between age and antibody titre in the vaccinated chickens. The differences in immunity in various age groups observed in the chickens within the 2 LGAs; 22.1% in growers, 29.4% in layers in Badagry and 72.7% in growers, 54.3% in layers in Ojo could be attributed to various practices and farmers preferences in chickens' vaccination. Some farmers consider certain age bracket as most important and as such give more priority in the group vaccinations as against others. Repeated vaccinations enhance the level of immunity developed in a group [14,15].

Table 1 Distribution of Newcastle Disease Antibody titre of Chickens from Two Local Government Areas of Lagos State, Nigeria.

Local Government Area	Number of sera tested	No. of sera negative	ND Ab Titre in Log_2											Mean titre ± SD	Total
			1	2	3	4	5	6	7	8	9	10	11		
			Number of chickens with antibody titre												
Badagry	237	43	11	9	13	13	24	30	30	26	24	14	0	5.04 ± 3.26	534
Ojo	297	87	3	9	15	24	25	44	28	24	26	12	0	4.36 ± 3.36	130

Table 2 Distribution of Newcastle Disease Antibody titre of Chickens from Two Local Government Areas of Lagos State, Nigeria.

Local Government Area	No. of sera tested	No. (%) of sera with titre ≤ 3 Log_2	No. (%) of sera with titre ≥ 4 Log_2
Badagry	237	76 (32.1)	161 (67.9)
Ojo	297	114 (38.4)	183 (61.6)

Table 3 Distribution of Newcastle Disease Antibody titre by Age group in Chickens from Badagry Local Government Area, Lagos State.

Age (weeks)	No tested	No of sera-Ve	1	2	3	4	5	6	7	8	9	10	Mean Ab titre
Chicks (1-6)	-	-	-	-	-	-	-	-	-	-	-	-	-
Growers (7-8)	30	2	0	1	4	4	0	2	1	4	3	0	5.68 + 2.01
Layers (>19)	129	3	3	8	11	27	14	22	11	11	10	1	5.31 + 1.00
Total	159	5	3	9	15	31	14	24	12	15	13	1	5.29 + 1.02

Table 4 Distribution of Newcastle Disease Antibody titre by Age group expressed in percentage from Badagry Local Government Area, Lagos State.

Age (wks)	Percentage -ve (%)	Percentage +ve (%)
Chicks (1-6)	-	-
Grower (7-18)	67.9	22.1
Layer (>19)	61.6	29.4

Table 5 Distribution of Newcastle Disease Antibody titre by Age group in Chickens from OJo Local Government Area, Lagos State.

Age (weeks)	No tested	No of sera -ve	1	2	3	4	5	6	7	8	9	10	Mean Ab titre
Chicks (1-6)	-	-	-	-	-	-	-	-	-	-	-	-	-
Growers (7-18)	44	9	1	2	0	7	3	8	4	5	3	2	4.8 ± 6.70
Layers (>19)	322	120	2	7	18	20	24	40	31	24	24	12	3.90 ± 3.47
Total	366	129	3	9	18	27	27	48	35	29	27	14	4.01 ± 3.44

Table 6 Distribution of Newcastle Disease Antibody titre by Age group expressed in percentage in Chickens from OJo Local Government Area, Lagos State.

Age (wks)	Percentage -ve (%)	Percentage +ve (%)
Chicks (1-6)	-	-
Growers (7-18)	27.3	72.7
Layers >19	45.7	54.3
Overall	43.4	56.6

Table 7 Risk Factors identified from Two Local Government Area of Lagos State, Nigeria.

S/NO	LGA	Gate not manned	Feed Spillage	Rodent infestation	Lizard infestation	Fly Infestation	Carcass Disposal	Lack of PPE	Backyard poultry	Visitors	Poor sick poultry handling
		Risk Level									
1	BD	1.7	0.74	2.2	1.7	0.7	1	2	1.4	2.3	0.89
2	OJO	1.3	0.73	3	1.9	1.5	1.4	1.5	0.96	1.8	0.77

Most essentially, vaccination failure or low vaccination response may be associated with the general sanitary practices in various farms. Biosecurity risk factors **(Figures 1 and 2)** observed such as poor handling of sick chickens and indiscriminate disposal of carcasses could serve as major means of managemental failures vis à vis vaccination failure [13]. In conclusion, the high percentage of unprotected chickens against NDV recorded

Figure 1 Dumping of litter within the farm close to crates of Egg.

Figure 2 Birds on overdue wet litter.

within Badagry and Ojo would affect the production system and viability of the chicken industry within the LGAs. Farmers should in addition to observing good biosecurity and sanitary practices, consult Veterinarians for expertise and advice on the right source of vaccine and procurement and also preparation and adoption of a well-structured vaccination protocol.

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