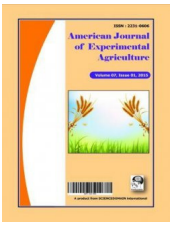

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Assessment of Antibody Response to Newcastle Disease Vaccination in Chickens in Some Commercial Farms in Three Local Government Areas in Lagos State, Nigeria

H. M. Ambali[1], R. I. O. Nwoha[2*] and P. A. Abdu[3]

[1]Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Veterinary Medicine, Micheal Okpara University of Agriculture, Umudike, P.O.Box 824, Nigeria.
[3]Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author PAA designed the study, wrote the protocol and wrote the first draft of the manuscript. Author RION wrote and reviewed the manuscript. Authors HMA and PAA managed the analyses of the study. Authors HMA and PAA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Despite the rigorous vaccination programs, outbreaks of Newcastle disease (ND) are often reported in vaccinated as in unvaccinated flocks. This study evaluated antibody (Ab) response following vaccination against Newcastle disease in three Local Government Areas (LGAs) of Lagos State. A total of five hundred and twenty eight sera were collected and tested for NDV antibody using Haemagglutination- inhibition test. The three Local Government Areas (LGAs) were: Epe, Etiosa and Ojo of Lagos state, Nigeria. One hundred and three samples were negative and the mean Ab titre ranged from 2.86±2.92 to 5.19±3.00. Etiosa had a total of two hundred and forty six

**Corresponding author: E-mail: rosemarynwoha@yahoo.com;*

sera tested with forty one negative samples and a mean Ab titre of 5.19±3.00 and mode of 6 log_2. It had 25.1% of Ab titre of unprotective level at ≤3 log_2 and 74.9% of protective level at ≥4log_2. Antibody titre at protective level across the age distribution was recorded at 80.0%, 60.0% and 78.9% in Chicks, Growers and Layers respectively. The risk factors identified in Etiosa were rodent infestation 1.75, lizard infestation 2.00 and carcass disposal 1.82. A total of one hundred and twenty nine sera were tested for ND Ab in Epe with fifty seven negative samples and mean Ab titre of 5.63±2.15 and mode of 5 log_2. The number of samples with antibody titre at ≤ 3 log_2 was 15.0% and 85% sera at ≥4 log_2. The age distribution of Ab titre at protective level was recorded at 76.7% in growers and 74.4% in layers while about 25.6 % in layers and 23.3% in Growers were unprotected. The risk factors identified were unmanned gate 2.10, Feed Spillage 2.10, Fly infestation 3.00, Carcass disposal 2.00 and backyard poultry 2.10. A total of one hundred and twenty two sera were tested in Ikorodu and fifty samples were negative with a mean antibody titre of 2.86±2.92. It had 54.3% antibody titre at ≥3 log2 and 45.7% at ≥4 log_2. It recorded percentage age distribution of unprotected antibody titre of 65.0 % in grower and 52.3% in layers. While 35.0% in growers and 47.7% in layers had protective antibody titre. Risk factors identified were unmanned gate 2.30, rodent infestation 2.30, lack of personal protective equipment (PPE) 2.40 and risky visitors 1.80.

Conclusion, birds in Epe were better immune from Newcastle disease compared to Etiosa and Ikorodu in that order. Ikorodu recorded highest number of farms with risk factors and at risk of outbreak of Newcastle disease compared to Epe and Etiosa.

Keywords: Antibody; Newcastle disease virus; layers; chicks; growers.

1. INTRODUCTION

Newcastle disease virus (NDV) is one of avian diseases of great economic importance causing devastating losses amongst both intensive, extensive and traditional village poultry practices that provides lifeline to many poor people across the developing world [1]. Newcastle disease is reported as the most important viral disease of poultry in the world including developing countries [2,3]. The use of viable non-pathogenic isolates of NDV to immunize poultry against pathogenic strains of the virus has been a common practice since the B1 strain was first described in 1948. Numerous NDVs with different levels of pathogenicity have been used to achieve desired immunologic response [4] using different vaccination regimes [5]. Vaccines containing inactivated NDV in oil emulsion adjuvant induce long term protection against viscerotropic velogenic NDV and live vaccines such as Lasota, Hitchner B1 and Komarov strains have gained acceptance by poultry producers in several countries [6]. To some extent, the degree of resistance induced by any vaccine is subject to the level of challenge present and in tough challenges, vaccines are unlikely to give protection [7]. Despite the availability of ND vaccines such as B1, F, Clone 30, Lasota, Mukteswer or other lentogenic/ mesogenic strains of NDV, vaccination failure is common due to non maintenance of cold chain, poor selection of vaccine strain, insufficient dose, presence of maternal antibody and faulty vaccination schedules [8]. These challenges gave impetus to the evaluation of antibody response in Chickens vaccinated against Newcastle disease in some poultry farms in 3 LGA of Lagos State, Nigeria.

2. MATERIALS AND METHODS

2.1 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 (1.341sqm). 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 ethnic groups from all over the country represented; however the Yorubas are considered the main ethnic group of the state and their language spoken by most inhabitants. Animal husbandry is widely practiced in which poultry 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 poultry and other livestock production contribute to a greater measure in the economy of the state.

2.2 Study Design

A cross sectional study was performed using multistage sampling method to select 3 Local Government Areas (LGA) from the 20 recognized ones based on their poultry farming activities from the 3 senatorial districts. The LGAs were Ikorodu, Etiosa, and Epe. Purposive sampling was done to select farmers from each Local Government Area.

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 birds 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.

2.3 Blood Collection and Handling

2.3.1 Blood collection

Blood samples were aseptically collected through the wing vein. About 2.0 mL of blood was collected from each selected birds 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℃ 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℃ before being tested for NDV antibodies.

2.3.2 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).

2.3.3 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.

2.3.4 Determination of antibodies to Newcastle disease antigen titre test

The haemagglutination (HA) titre was determined as described by [9] and diluted to contain 8HA units for use in the HI test as described by [10].

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.

2.3.5 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 [9].

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 2^{nd} well down to the 12^{th}.
2. A 25 µL of the test serum was dispensed into the first and 2^{nd} wells only.
3. A two fold serial dilution was made starting from the 2^{nd} well tot the 11^{th}.
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 12^{th}.
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.

2.4 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.

3. RESULTS

3.1 Distribution of Newcastle Disease Antibody Titre of Chickens from Three Local Government Areas Lagos State, Nigeria

A total of 528 sera were tested with 103 being negative and 425 positive (Table 1). The mean antibody titre from the 3 Local Government Areas of the state ranged from 2.86± 2.92 in Ikorodu to 5.63±2.15 in Epe. Etiosa had the least no of negative sera 41 compared to Epe 57 and Ikorodu 50. Epe had the highest no of negative sera at 57 compared to Ikorodu 50 and Etiosa 41.

3.2 Distribution of Chickens with Newcastle Disease Antibody Titre $\geq$ 4 Log_2 from the Three Local Government Areas, Lagos State, Nigeria

The overall Ab titre of ≥ 4log_2 in all the three LGAs was represented on (Table 2). Epe with a total of 129 sera had the highest number (85.0%) of samples with antibody titre at ≥ 4 log_2 followed by Etiosa with 246 sera and 74.90% of the samples at ≥ 4 log_2. Ikorodu having 122 sera had the least (45.7%) number of samples with antibody titres at ≥4log_2.

3.3 Distribution of Unprotected Chickens from Newcastle Disease from the Three Local Government Area, Lagos State, Nigeria

The overall percentage distribution of unprotected chickens in all the 3 LGAs with ND Ab titre less than equal to 3log_2 was represented on (Table 2). The highest number of unprotected chickens was found in Ikorodu with percentage distribution of 70 (54.3%), Etiosa had 65(25.1%) while Epe had the least at 21 (15.0%).

3.4 Distribution of Newcastle Disease Antibody Titre by Age group in Chickens from Three Local Government Areas, Lagos State, Nigeria

There was significant association between age and antibody titre of Chickens. The birds were grouped into 3 (1-6 weeks for Chicks, 7-8 weeks for Growers and greater than 19 weeks for Layers). The mean Ab titre of birds in Etiosa varies from 3.11±0.01 in Chicks, 5.77±0.02 in Growers and 6.39 ±0.01in Layers. In Epe the Ab titre among the age groups ranges from 3.91±0.11 in Growers and 5.34 ±0.01in Layers. Ikorodu had 3.3 ± 0.00 in Growers and 2.9 ± 2.20 in Layers. The mode for Chicks and Layers was 6 log_2 and 3 log_2 in Growers. Etiosa had 80.0% of the Chicks, 60.0% of Growers and 78.9% of Layers with protective immunity (Table 4). Epe had 76.7% Growers and 74.4% Layers with protective immunity while only 35.0% of Growers and 47.7% of Layers had immunity in Ikorodu (Table 9).

3.5 Biosecurity Risk Factors Identified from the Three Local Governments Areas, Lagos State, Nigeria

Biosecurity risk factors identified in the three Local Government Areas were ten in descending order: Rodent infestation with an average of 1.91, Personal protective Equipment 1.86, Allowing risky visitors 1.78, Unmanned gate 1.65,Carcass Disposal 1.44, Lizard infestation 1.41, Fly infestation 1.29, Backyard poultry 1.29, Feed Spillage 1.15 and Poor handling of sick poultry. Worse risk level was observed in Ikorodu with an average of 2.3, Epe with 2.10, and Etiosa has the lowest risk level of 0.75. Ikorodu ranked highest in most risk factors like rodent infestation, lack of PPE and allowing risky visitors into the pens (Table 9).

Table 1. Distribution of Newcastle Disease Antibody Titre of Chickens from Three Local Government Area of Lagos State, Nigeria

Local Government Area	No. of sera tested	No. of sera negative	ND Ab Titre in Log_2										Mean titre ± SD
			1	2	3	4	5	6	7	8	9	10	11	
			Number of chickens with the titre											
Etiosa	259	41	2	6	16	34	26	39	33	23	24	15	0	5.19± 3.00
Epe	140	5	0	4	12	15	36	16	23	15	13	1	0	5.63± 2.15
Ikorodu	129	57	1	6	6	14	18	9	10	4	4	0	0	2.86± 2.92
Total	528	103												

Table 2. Distribution of Newcastle disease antibody Titre of chickens from three local government area of Lagos State, Nigeria

Local Government Area	No. of sera tested	No. (%) of sera with titre $\leq$ 3 Log_2	No. (%) of sera with titre $\geq$ 4 Log_2
Etiosa	259	65 (25.1)	194 (74.9)
Epe	140	21 (15.0)	119 (85.0)
Ikorodu	129	70 (54.3)	59 (45.7)

Key: %-Percentage, ≤ - Less than equal to, ≥ - Greater than equal to

Table 3. Distribution of Newcastle disease antibody Titre by age group in chickens from Etiosa Local Government Area, Lagos State

Age (weeks)	No of sera tested	No of sera negative	1	2	3	4	5	6	7	8	9	10	Mean titre ± SD
Chicks (1-6)	10	1	0	0	0	1	1	2	1	0	2	1	3.11±0.01
Grower (7-18)	70	14	0	3	11	4	8	7	8	5	10	0	5.77±0.02
Layers (>19)	166	26	2	3	4	25	14	24	24	18	12	14	6.39±0.01
Total	246	41	2	6	16	30	23	33	33	23	24	15	6.22±0.01

Key: SD- Standard deviation

Table 4. Distribution of Newcastle Disease Antibody Titre by age group expressed in percentage from Etiosa Local Government Area, Lagos State

Age (weeks)	Percentage –ve (%)	Percentage $_+$ve (%)
Chicks (1-6)	20.00	80.00
Grower (7-18)	40.00	60.00
Layer (>19)	21.10	78.90

Key: %- Percentage, –ve: Negative, $_+$ve: Positive

Table 5. Distribution of Newcastle disease antibody Titre by age group in chickens from Epe 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
Chick (1-6)	-	-	-	-	-	-	-	-	-	-	-	-	-
Grower (7-18)	20	9	1	1	2	3	2	2	0	0	0	0	3.91± 0.11
Layer(>19)	109	48	0	5	4	11	16	7	10	4	4	0	5.34±0.01
Total	129	57	1	6	6	14	18	9	10	4	4	0	8.38±0.10

Key: –ve: Negative

Table 6. Distribution of Newcastle disease antibody Titre by age group expressed in percentage from Epe Local Government Area, Lagos State

Age (weeks)	Percentage –ve (%)	Percentage ₊ve (%)
Chicks (1-6)	-	-
Grower (7-18)	23.30	76.70
Layers >19	25.60	74.40

Key: –ve: Negative, ₊ve: Positive

Table 7. Distribution of Newcastle disease antibody Titre by age group in chickens from Ikorodu 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)	20	9	1	1	2	3	2	2	0	0	0	0	3.3± 0.00
Layers (>19)	102	41	0	5	4	11	16	7	10	4	4	0	2.9± 2.20
Total	122	50	1	6	6	14	18	9	10	4	4	0	2.86± 2.62

Key: Ab- Antibody, –ve: Negative

Table 8. Distribution of Newcastle disease antibody Titre by age group expressed in percentage in Chickens from Ikorodu Local Government Area, Lagos State

Age (weeks)	Percentage –ve (%)	Percentage ₊ve (%)
Chicks (1-6)	-	-
Growers (7-18)	65.00	35.00
Layers >19	52.30	47.70

Key: –ve: Negative, ₊ve: Positive

Table 9. Risk factors identified from three 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						
2	EPE	2.10	2.10	0.29	0.57	3.00	2.00	1.30	2.10	1.60	0.71
3	ETI	0.75	1.00	1.75	2.00	0.80	1.82	2.10	1.30	1.40	1.30
4	IK	2.30	1.20	2.30	0.89	0.44	1.00	2.40	0.67	1.80	1.70
	Overall	1.65	1.15	1.91	1.41	1.29	1.44	1.84	1.29	1.78	1.07

Key: LGA-Local Government Area, PPE-Personal Protective Equipment, EPE-Epe, ETI- Etiosa, IK - Ikorodu

Photo 1. Indiscriminate dumping of litter within the farm with overgrown grasses

Photo 2. Poorly constructed pen house with over one thousand pullets with overgrown grasses

4. DISCUSSION

From the three LGAs of Lagos State, about 59 (45.7%), 194 (74.9%) and 119 (85.0%) of birds were reared with antibody titre ≥4 log ₂ hence are immuned to NDV. Haemagglutination inhibition (HI) Ab titre of 2_4 or higher in Newcastle disease vaccinated bird was considered protective [10], [11]. This study is in agreement with the findings of [12] who recorded 52.5% to 83.4% prevalence in Nasarawa State. [13] reported a prevalence of 46.0% in village chickens in Borno State and [14] reported 51.9% prevalence. The percentage of birds protected from ND through vaccination was more in Epe compared to Etiosa whereas birds in Ikorodu were at greater risk of outbreak of the disease.

This was deduced from the percentage of birds reared in the three LGAs with Ab titre at $\leq 3\log_2$ considered to be unprotective. Ikorodu recorded the highest number 70(54.3%) of birds at great risk of exposure to outbreaks of NDV compared to Etiosa 65 (25.1%) and Epe which had the least number at 21 (15.0%). The percentage of vaccinated birds within the 3 LGAs at risk of exposure to ND could be compared with the earlier report of [15] who recorded mean titre of 32.3%.

There was significant ($p<0.031$) association between age and Ab titres in birds within the 3 LGAs. In Etiosa LGA, immunity to ND was highest 80.0% in Chicks compared to Growers and Layers. This could be related to the findings of [16] who reported high levels of ND Abs in newly hatched village Chicken which may last up to 5week during which Chicks may become fully capable of mounting immunity. The variations in immune development within the age groups in the 3 LGAs could be attributed to various managemental practices viz à viz vaccination in different age groups of birds. Most farmers' indulge in arbitrary vaccination of certain age group of birds especially young birds at greater risk of infection probably to improve disease resistance. Such practice reflects on the immunity developed in the age group compared to others.

Bio-security and hygienic measures are very essential tools in prevention of introduction or spread of diseases in a farm establishment. Shortfalls in measures such as absence of quarantine programs and isolation of sick birds, non-restriction of movements and proper vaccination may be contributory to low levels of immunity recorded in vaccinated birds in the 3 LGAs [17,1].

Ikorodu ranked highest in most risk factors considered in this study such as rodent infestation, lack of PPE and invasion of unauthorized visitors into the pens hence could be the cause of low immunity in vaccinated birds within the LGA. It also ranked highest in other risk factors such as poor handling of sick birds, overgrown bushes and poor disposal of poultry dung all considered significant in adequate immune response.

5. CONCLUSION

Conclusions the study recorded 45.7%, 74.9% and 85.0% of birds with immunity against ND within Ikorodu, Etiosa and Epe respectively. Ikorodu had the highest 54.3% percentage of birds at risk of outbreaks of ND compared to Etiosa 25.1% and Epe 15.0%. Negligence on certain biosecurity measures could be contributory to the low immune response in vaccinated birds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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