

# Seroprevalence of Newcastle disease in local chickens from Southeast derived savannah zone of Nigeria

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## Key words

Chickens - Newcastle disease -  
Immunodiagnosis - Morbidity -  
Season - Nigeria.

## Summary

A serologic surveillance study was conducted in the Southeastern zone of Nigeria to document some operative epidemiologic factors which determine Newcastle disease (ND) epizootic in village chickens of the area. A high seroprevalence, indicator of ND virus activity of 63%, was recorded in the birds. Infection was widespread as no single village chicken population was free of ND. A seasonal pattern of ND virus activity is depicted by a higher prevalence and intensity of ND virus activity (HI titres) in the dry season (harmattan) than wet season. Egg-yolk hemagglutination inhibition test is proposed as a useful methodology for smaller scale survey or for commercial poultry with no constraints in egg supply. The implications of the results for a vaccinal control of ND in the area are discussed.

## ■ INTRODUCTION

Newcastle disease is a viral and often fatal disease that affects a wide range of avian hosts, irrespective of age. It has been a cause of great set back in poultry production throughout the world. Indeed in Africa and Asia, it is a major constraint to the development of the village poultry sub-sector (2, 11).

The main control method for ND is by vaccination using the conventional vaccines. Generally, routine vaccination is undertaken in the intensive and commercial poultry. For rural scavenger chickens in which vaccination is rarely done, the stocks remain highly susceptible with periodic decimation of flocks by the disease and the attendant economic losses (1, 3). In view of the enormous economic importance of ND, indications are that local poultry farmers would welcome ND vaccinations capable of protecting the local chickens (3). For vaccination to be an effective method for controlling ND, there is the need to study the epidemiology of the disease in different ecological zones.

The objective of this study was therefore to investigate the operative epidemiological factors that specifically determine ND epizootic in village poultry in Southeastern Nigeria.

## ■ MATERIALS AND METHODS

### *The study area*

The study covered five Local Government Areas (LGAs) in two states, Anambra and Enugu respectively located within the tropical, humid and derived savannah zone of Southeastern Nigeria. Southeastern Nigeria is situated between 4°21' and 7°5' N and 6° and 10° E. The temperature varies usually from 27°C to 35°C. The hottest months are February to April while the coldest period is between December and January during the harmattan. Rainfall is seasonal, being wet during April to October and dry from late October to early April (4, 7).

### *Sampling*

Blood samples for serum, and egg for egg yolk extraction were collected from village adult chickens of varying ages and sexes. Chicks blood was collected on filter paper while non-embryonated local chicken eggs used were bought randomly from households. The study covered part of rainy season (May-July, 1998) and part of dry season (harmattan, December, 1998).

### *Serum collection*

Test materials were composed of chicken sera harvested from clotted venous blood and later centrifuged at 3000 revolutions per minute (RPM) and chloroform extract from egg yolks.

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### Antigen

Newcastle disease vaccine virus *lasota* strain obtained from National Veterinary Research Institute (NVRI), Vom, was used as antigen after reconstitution of 200 dose vial in 8 ml of distilled water.

### Indicator

0.5% washed chicken red blood cells (RBC) suspension was prepared essentially as described by Wosu (1984).

### Hemagglutination (HA) test

The HA test was done by the microtest method using two-fold serial dilutions of 50 µl of reconstituted vaccinal virus (antigen), and 50 µl of the 0.5% chicken RBC was added to each well. Equivalent volume of chicken RBC suspension, added to wells containing PBS alone, served as control. The plate was gently tapped to mix the contents and after 45 min of incubation at room temperature, the end point of the HA was read. The titre was taken as the reciprocal of the highest dilution giving a 100% agglutination of the 0.5% chicken RBC. This amount of virus also represents one hemagglutination (HA) unit.

### Hemagglutination inhibition (HI) test

The HI test was performed using beta-technique (constant virus and varying serum) against 4 HA units of virus computed from the results of the HA titration. Doubling dilutions (50 µl) of the different chicken sera or egg yolk extract were reacted with 50 µl of 4 HA units of the antigen suspension per well. The mixture was tapped gently to mix and allowed to stand for 30 min at room temperature for antigen-antibody reactions to take place. Antigen control wells were also included. 50 µl of 0.5% washed chicken RBC was added to all the wells and tapped to mix, incubated and

read after 45 min. The titres were taken as the reciprocal of serum or egg yolk dilutions giving 100% inhibition of the chicken RBC.

### Statistical analysis

The data collected were statistically analyzed using completely randomized ANOVA to determine significant differences. The least significant difference (LSD) procedure was used to detect means causing any significant "F" values (10, 12).

## RESULTS

A total of 763 samples comprising 317 hens, 119 cocks, 175 growers, 38 chicks blood on filter paper and 114 local chicken eggs were tested. Newcastle disease seroprevalences in the different locations and categories of samples tested is shown in table I. Of the 649 birds (hens, cocks, growers and chicks) and 114 eggs tested, seroprevalences of 63% and 91.2% respectively were recorded. Among the birds, the prevalences were 73.3% in the cocks, 65.3% in the hens, 60% in the growers and 34.2% in the chicks which were significantly different ( $p < 0.01$ ). Significant differences also existed between the LGAs ( $p < 0.01$ ) with ND seroprevalences ranging from 52.4 to 79.9%. No individual village or batch of chickens was ND free; seroprevalence in village batches ranged from 42.2 to 93.2%. Peak prevalence occurred in Akpo (93.2%) in Anambra State, and Obollo-Afor (78.2%) in Enugu State.

Table II shows the seasonal (harmattan and wet season) distribution of ND seroprevalence. There was a significant seasonal effect ( $p < 0.01$ ) between the wet season (58%) and harmattan (90%).

The analysis of HI titres is presented in table IIIa and IIIb. The titres ranged from 1 log<sub>2</sub> to 11 log<sub>2</sub>. The percentage distribution of the titres showed that greater percentage of titres above 6 log<sub>2</sub> were recorded in harmattan (35%) than wet season (14.2%).

Table I

Total sero-prevalence of ND in local chickens in the Eastern zone, Nigeria

| State   | Local Govt Area | Villages    | Serum samples (No. tested) |                        |                       |                      | Egg Yolk No. tested   | Total No. tested |                          |
|---------|-----------------|-------------|----------------------------|------------------------|-----------------------|----------------------|-----------------------|------------------|--------------------------|
|         |                 |             | Hens                       | Cocks                  | Growers               | CBFP*                |                       | Village          | LGA                      |
| Anambra | Aguata          | Akpo        | 33 (97)                    | 14 (93)                | 24 (100)              | 4 (25)               | 13 (92)               | 88 (93)          | 139 (79.9) <sup>f</sup>  |
|         |                 | Nkpologwu   | 19 (68)                    | 4 (75)                 | 20 (45)               | 5 (40)               | 3 (67)                | 51 (57)          |                          |
|         | Dunukofia       | Nagbana     | 20 (30)                    | 5 (0)                  | 10 (80)               | 5 (20)               | 5 (80)                | 45 (42)          | 98 (52) <sup>e</sup>     |
|         |                 | Mbuke       | 21 (71)                    | 4 (100)                | 19 (42)               | 5 (40)               | 4 (75)                | 53 (60)          |                          |
| Enugu   | Nsukka          | Amofie      | 18 (56)                    | 2 (50)                 | 20 (65)               | 5 (40)               | 5 (100)               | 50 (62)          | 138 (65.9) <sup>ef</sup> |
|         |                 | Amora       | 25 (76)                    | 13 (85)                | 28 (54)               | 6 (33)               | 16 (18)               | 88 (68)          |                          |
|         | Igbo-eze        | Ovoko       | 35 (60)                    | 18 (67)                | 21 (38)               | 5 (40)               | 16 (94)               | 95 (61)          | 200 (57.5) <sup>ef</sup> |
|         |                 | Ibagwa      | 47 (43)                    | 18 (67)                | 22 (50)               | 3 (33)               | 15 (87)               | 105 (54)         |                          |
|         | Udenu           | Obollo-afor | 99 (72)                    | 41 (73)                | 11 (82)               | -                    | 37 (100)              | 188 (78)         | 188 (78.2) <sup>f</sup>  |
| Total   |                 |             | 317 (65) <sup>b</sup>      | 119 (72) <sup>ab</sup> | 175 (60) <sup>b</sup> | 38 (34) <sup>c</sup> | 114 (91) <sup>a</sup> | 763 (68)         | 763 (68)                 |

\* CBFP : Chichen blood on filter paper

Numbers in parenthesis indicate proportion of seropositives; a, b, c: proportion of seropositives (row totals) with different superscript are highly significant ( $p < 0.01$ ); e, f: proportion of seropositives (column total) with different superscript are significant ( $p < 0.05$ )

Table II

Analysis of seroprevalence of ND in local chickens in the Southeastern zone, Nigeria, according to season

| Local Govt<br>Area | Number of samples tested* |         |          |          | Total                 |
|--------------------|---------------------------|---------|----------|----------|-----------------------|
|                    | Hens                      | Cocks   | Growers  | Egg Yolk | No tested             |
| <b>Harmattan</b>   |                           |         |          |          |                       |
| Aguata             | 16 (100)                  | 4 (100) | 7 (100)  | 10 (100) | 37 (97)               |
| Nsukka             | 15 (100)                  | 8 (100) | 3 (100)  | 11 (82)  | 37 (95)               |
| Igbo-eze South     | 33 (91)                   | 24 (88) | 13 (92)  | 21 (91)  | 91 (90)               |
| Udenu              | 45 (80)                   | 21 (81) | 9 (100)  | 20 (100) | 95 (86)               |
| Total              | 109 (89)                  | 57 (88) | 32 (97)  | 62 (92)  | 260 (90) <sup>a</sup> |
| <b>Wet season</b>  |                           |         |          |          |                       |
| Aguata             | 36 (81)                   | 14 (86) | 37 (70)  | 6 (83)   | 93 (77)               |
| Nsukka             | 28 (50)                   | 7 (52)  | 45 (56)  | 10 (90)  | 90 (58)               |
| Igbo-eze South     | 49 (23)                   | 12 (25) | 30 (23)  | 10 (90)  | 101 (33)              |
| Udenu              | 54 (65)                   | 20 (65) | 2 (0)    | 17 (100) | 93 (70)               |
| Total              | 167 (53)                  | 53 (62) | 114 (51) | 43 (93)  | 377 (58) <sup>b</sup> |

\* Samples from Dunukofia LGA (98) and chicks (38) excluded from analysis because collection was done only for wet season; a, b: highly significantly different ( $p < 0.01$ )

Table IIIa

Distribution of ND antibody titres ( $\log_2$ ) of samples tested

| Titre $\log_2$ | Hens | Cocks | Growers | Egg Yolk | Blood on filter paper | Total |
|----------------|------|-------|---------|----------|-----------------------|-------|
| 1              | 110  | 33    | 70      | 10       | 25                    | 248   |
| 2              | 16   | 6     | 15      | 8        | 12                    | 57    |
| 3              | 15   | 17    | 10      | 12       | 1                     | 55    |
| 4              | 35   | 14    | 23      | 10       | -                     | 82    |
| 5              | 28   | 12    | 18      | 20       | -                     | 78    |
| 6              | 34   | 11    | 19      | 22       | -                     | 86    |
| 7              | 28   | 10    | 7       | 12       | -                     | 57    |
| 8              | 27   | 8     | 10      | 13       | -                     | 58    |
| 9              | 12   | 4     | -       | 4        | -                     | 20    |
| 10             | 10   | 4     | 2       | 2        | -                     | 18    |
| 11             | 2    | -     | 1       | 1        | -                     | 4     |
| Total          | 317  | 119   | 175     | 114      | 38                    | 763   |

Total seropositive = 515 (67.5%)

Table IIIb

Distribution of ND antibody titres ( $\log_2$ ) of samples tested in local chickens, according to season

|                   | Ranges of $\log_2$ titres |          |         |        | Total sampled |
|-------------------|---------------------------|----------|---------|--------|---------------|
|                   | 1-3                       | 4-6      | 7-9     | 10-11  |               |
| <b>Harmattan</b>  |                           |          |         |        |               |
| Hen               | 6                         | 50       | 34      | 7      | 109 (89)      |
| Cock              | 17                        | 17       | 12      | 4      | 57 (88)       |
| Grower            | 3                         | 19       | 6       | 3      | 32 (97)       |
| Egg Yolk          | 9                         | 23       | 23      | 2      | 62 (92)       |
| Total             | 35 (15)                   | 109 (46) | 75 (32) | 16 (7) | 260 (90)      |
| <b>Wet season</b> |                           |          |         |        |               |
| Hen               | 25                        | 47       | 33      | 5      | 208 (53)      |
| Cock              | 6                         | 20       | 10      | -      | 62 (58)       |
| Grower            | 22                        | 41       | 11      | -      | 143 (52)      |
| Egg Yolk          | 11                        | 29       | 6       | 1      | 52 (90)       |
| Total             | 64 (24)                   | 137 (51) | 60 (23) | 6 (2)  | 465 (57)      |

Numbers in parenthesis indicate percentage positive  
13 seropositive chicks blood on filter paper not taken into account in table III

## DISCUSSION

Newcastle disease seroprevalence (ND virus activity) in village chickens has been determined in a larger geopolitical scale than hitherto. The results indicate that in all the LGAs covered by the survey, ND prevalence was 63% and 91% in the chicken and egg yolk samples respectively. However, some LGAs like Aguata in Anambra State and Udenu in Enugu State recorded significantly higher ND seroprevalence rates. The successful development and adaptation of the methodology for egg-yolk HI serology (EYS) in this study is noteworthy. The comparison of the EYS with serum HI titres showed considerable similarity. It could thus serve as a substitute for sera in HI serology especially for smaller scale survey or for commercial poultry with no constraint in egg supply. The ND seroprevalence of 63% recorded in local fowls in this study is lower than 73% reported in Zaria Northern zone (5) and much higher than seroprevalences of 38% and below recorded

around Ibadan, Southwestern zone, (6, 8, 9). These regional differences in ND seroprevalence connote ecological variations in ND virus activity and may perhaps be a reflection of the impact of environment on the viability of ND virus, spread and epizootiology of ND.

The observation that no individual village or batch of chickens was ND free indicates that ND is widespread in the area studied. Similarly, the occurrence of high ND antibody titres (7-11  $\log_2$ ) in some samples implies high ND virus activities in such foci. This would seem to suggest that the application of foci targeted ND control strategy in such circumstances might be a useful supplementary control method.

Data also portrayed a seasonal pattern of ND outbreaks with higher prevalence and greater proportion of high ND antibody titres in the harmattan period than the wet season. This revelation suggests a more intense ND virus activity at this time of the year

and provides impetus for a pre-harmattan target for annual and cost effective ND vaccination. This proposal could benefit from additional investigation in the future. This is especially noteworthy because conventional ND vaccination strategies with repeat flock boosters are hardly achievable in normal scavenger poultry.

In conclusion, this present study has confirmed the endemicity of ND in the area studied. It also reveals some epizootiologic basis for the formulation of ND control strategy in the future.

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### Résumé

**Orajaka L.J.E., Adene D.F., Anene B.M., Onuoha E.A.** Sero-prevalence de la maladie de Newcastle chez des poulets d'élevage fermier dans la zone de savanes au sud-est du Nigeria

Une étude de surveillance sérologique a été conduite dans la zone sud-est du Nigeria pour obtenir des informations sur certains facteurs épidémiologiques responsables des épizooties de la maladie de Newcastle (Newcastle Disease, ND) chez les poulets d'élevages fermiers de la région. Un taux élevé de séroprévalence — une indication de la présence du virus —, 63%, a été observé chez les volailles. L'infection était largement répandue car aucune des populations de volailles n'était indemne de la maladie de Newcastle. Une activité saisonnière du virus se manifeste par une prévalence plus élevée et une activité virale plus intense (titres d'hémagglutination) durant la saison sèche et froide (harmattan) que durant la saison des pluies. La technique d'inhibition de l'hémagglutination à partir du jaune d'œuf est proposée comme une technique utile pour une petite enquête ou pour des élevages commerciaux de volailles sans contraintes d'approvisionnement en œufs. Les conséquences de ces résultats sur le contrôle de la maladie de Newcastle par la vaccination dans la région sont discutées.

**Mots-clés** : Poulet - Maladie de Newcastle - Immuno-diagnostic - Morbidité - Saison - Nigeria.

### Resumen

**Orajaka L.J.E., Adene D.F., Anene B.M., Onuoha E.A.** Prevalencia serológica de la enfermedad de Newcastle en pollos locales de la zona derivada de la sabana sudeste en Nigeria

Se llevó a cabo un estudio de observación serológica en la zona sudeste de Nigeria, con el fin de documentar algunos factores epidemiológicos operativos, determinantes de la enfermedad de Newcastle (ND), epizootica en los pollos de los pueblos de la zona. Se detectó una alta seroprevalencia en las aves, indicador de un 63% de actividad del virus de ND. La infección se encontró ampliamente distribuida, debido a que ninguna población avícola de los pueblos de la zona estuvo libre de ND. Se observa un patrón estacional de la actividad del virus de ND, representado por una mayor intensidad y una mayor prevalencia de la actividad del virus de ND (títulos HI) en la estación seca (harmattan) que en la estación húmeda. Se propone el test de inhibición de la hemagglutinación de la yema de huevo como un método útil en estudios de menor escala o para aves comerciales sin restricciones en el suplemento de huevos. Se discuten las implicaciones de los resultados de un control mediante vacunación de ND en el área.

**Palabras claves**: Pollo - Enfermedad de Newcastle - Inmuno-diagnostico - Morbosidad - Estaciones del año - Nigeria.