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Prevalence of Newcastle Disease Virus and Infectious Bursal Disease Virus in Local Adult Chickens in Aba, Abia State, Nigeria

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ABSTRACT

Infectious bursa disease (IBD) also known as Gumboro, is a highly contagious disease of young chickens caused by the infectious bursa disease virus (IBDV), characterized by immunosuppresion and mortality generally at 3 to 6 weeks of age.The disease is economically important to the poultry industry worldwide due to the increased susceptibility to other diseases and negative interference with effective vaccination. Newcastle disease virus (NDV) is so virulent that birds die without showing any clinical signs of Newcastle disease. A death rate of almost 100 percent can occur in unvaccinated poultry flocks. NDV can infect and cause death even in vaccinated poultry. This study determined the prevalence of infectious Bursa Disease Virus (IBDV) and Newcastle Disease Virus in local adult chickens in Aba, Abia State. Two milliliter blood samples were collected from each of two hundred and fifty (250) local chickens by exsanguination and the prevalence of IBDV and NDV was determined using Agar gel immunodifussion test, Haemagglutination test and Haemagglutination Inhibition test. Results showed IBDV prevalence of 42% for Ariara, Eziukwu market had 34%, Ekeoha shopping centre had 58%s, Nkwo-Ngwa market had 126% and New market had 34%, with an average of 38.8% seroprevalence for Aba. NDV had antibody Geometric mean titer of 18.7. for Aba. The prevalence rate of 38.8% (97/250) obtained for IBDV and 26.4% (66/250) as well as GMT of 18.7 for NDV are significantly ($p<0.05$) high.Good bio-security such as minimizing travel on and off the facility and disinfecting vehicles and equipments that enter the farmshould be observed.

Keywords: Infectious bursa disease, Gumboro, chickens, infectious bursa disease virus, Newcastle disease virus, Newcastle disease

INTRODUCTION

Infectious bursa disease (IBD) also known as Gumboro, infectious bursitis and infectious avian nephrosis is a highly contagious disease of young chickens caused by the infectious bursa disease virus (IBDV) [1]. It is characterized by immunosuppresion and mortality generally at 3 to 6 weeks of age. The disease is economically important to the poultry industry worldwide due to the increased susceptibility to other diseases and negative interference with effective vaccination [1]. In recent years, very virulent strains of IBDV (vvIBDV), causing severe mortality in chicken have emerged in Europe, Latin America, south East Asia, Africa and the Middle East. Infection is via the oro-fecal route, with affected bird excreting high levels of the virus for approximately 2 weeks after infection [1].

IBDV is a double-stranded RNA virus that has a bi-segmented genome and belongs to the genus

Avibirnavirus of family *Birnaviridae*. There are two distinct serotypes of the virus, but only serotype 1 viruses causes disease in poultry [1]. Young birds at around 2 to 8 weeks of age that have high active bursa of *fabricius* are more susceptible to disease. Birds over eight weeks are resistant to challenge and will not show clinical signs unless infected by highly virulent strains [1]. After ingestion, the virus destroys the lymphoid follicles in the bursa of *fabricius* as well as the circulating B-Cells in the secondary lymphoid tissues such as GALT (gut associated lymphoid tissues), CALT (conjunctiva), BALT (bronchial) caecal tonsils, Harderiar gland, etc [1]. Acute disease and death is due to the necrotizing effect of these viruses on the host tissue. If the bird survives and recovers from this phase of the disease, it remains immuno-compromised which means it is more susceptible to other disease [1]. In the acute form, birds are depressed, debilitated and dehydrated. They produced watering diarrhea and have swollen, blood-stained vent. It is common for the birds to be recumbent and show a ruffling of the feathers. Mortality rates vary with virulence of the strain involved, the challenge dose as well as the flock's ability to mount an effective immune response [1]. Infection with less virulent strains may not show event clinical signs but the birds may have fibrotic or cystic bursa of *fabricius* that has atrophied prematurely (before 6 months of age) and may die of infections by agent that would not usually cause disease in immunocompetent birds [1]. Newcastle disease virus (NDV) infection exhibits Clinical signs which are extremely variable depending on the strains of virus, species and age of bird, concurrent disease, and pre-existing immunity [2]. Four broad clinical syndromes are recognized by scientists. They are Viscerotropic and Lentogenic. NDV is so virulent that many birds die without showing any clinical signs. A death rate of almost 100 percent can occur in unvaccinated poultry flocks. NDV can infect and cause death even in vaccinated poultry [3].

NDV is spread primarily through direct contact between healthy birds and the bodily discharged of infected birds. The disease is transmitted through infected bird's dropping and secretions from the nose, month and eye. [4]. NDV spreads rapidly among birds kept in confinement, such as commercially raised chicken. NDV effects are most notable in domestic poultry due to their high susceptibility and the potential for severe impacts of an epizootic on the poultry industries [4].

NDV strains can be categorized as velogenic (highly virulent), mesogenic (intermediate virulence) or lectogenic strains produce severe nervous and respiratory signs, spread rapidly and cause up to 90% mortality [4].The incubation period for the disease ranges from 2-15days. An infected bird may exhibit respiratory signs (gasping, coughing) nervous signs (depression, inappetence, muscular tremors, dropping wings, twisting of head and neck, circulating, complete paralysis), swelling of the tissues around the eyes and neck, greenish, watery diarrhea, misshapen, rough or thin- shelled eggs and reduced egg production. In acute case, the death is very sudden and in the beginning of the outbreak, the remaining birds do not seem to be sick [5]. Therefore, this project determined the prevalence of infectious Bursa Disease Virus (IBDV) and Newcastle Disease Virus in local adult chickens in Aba, Abia State.

MATERIAL AND METHODS

Study Area

Samples were collected from apparently healthy-local birds in by official service in slaughter house located in Aba, a big trading center in Abia State, Southern Nigeria.

Period of Study

The prevalence of NDV and IBDV, viral disease of birds in commercial birds reared in Southern Nigeria was studied for a period of ten (10) months. Five veterinary establishment located within the area of Aba were used in the study.

Sample Size

The calculation of the number of sample for the study was based on the total population of birds, 250 samples were collected in the area from 250 local chickens.

BLOOD COLLECTION

- Hold the chicken firmly, supporting the animal body with the assistant hand.
- Hold back the wings to expose the brachial vein of the chicken.
- Remove a few feathers if necessary to aid visibility.
- Disinfect the exposed skin area to be punctured using 70% alcohol.
- Insert a sterile needle fixed on a 5/10ml syringe with 1ml ACD (or other anticoagulants) into the muscle around the vein and gently manocure into the brachial vein. Pool blood from at least three specific antibodies negative chickens.
- Collect 4ml of blood into the ACD, making a total of 5ml.
- Gently removes the needle and syringe and clean up the punctured area.

WASHING AND PREPARATION OF c-RBC

1. Put the blood mix with ACD in a graduated centrifuge tube.
2. Add PBS to make a suitable volume and spin at 1000rpm.
3. Decant supernatant and repeat the above process (2) for two more times.
4. Measure the packed red blood cells and add 9 pants of PBS to make a 10% c-RBC. For example, if the packed RBC is 2.5ml, add 22. 5ml of PBS to make a total of 25ml of 10% c-RBC.
5. Store in a standing fridge (+4^{0}c) for use

HEAMAGGLUTINATION (HA) TEST

Reagents

1. Isotonic saline buffered with phosphate (0.05ml) to PH 7.0-7.4
2. Red blood cells (RBC) taken and pooled from a minimum of three specific pathogen, free chickens into an equal volume of Alsever's solution (anticoagulant solution). Cells should be washed three times in PBS before use. A 1% suspension (packed cell V/V) in PBS should be used.

Procedure

1. Dispense 0.025ml PBS into each well of a plastic microtitre plate (V-bottomed wells).
2. Place 0.025ml at virus suspension (allantoic fluid or the antigen to be titrated) in the first well.
3 Make two-fold dilutions (from 1:2 to 1.4096) of virus across the plate.
4. Dispense a further 0.025ml of PBS to each well.
5 Add 0.25ml of 1% red blood cells prepared as above to each well.
6 Mix by tapping gently and place at 20^{0}c for 30 minutes
From H2 to the H6 and discard the last 0.025ml in order to obtain 4, 2, 1, 0.5 0.25, 0125. HAU. This antigen control (back titration) must be include in each micro plate.
7 Add 0.025ml of PBS + albumin in all wells of the row H
8 Mix by tapping gently and place plates of 20^{0}c for 30 minutes or 4^{0}c for 40 minutes.
9 Add 0.025ml of 1% washed c-RBC to each well.
10 Mix by gentle taping and place at 20^{0}c for 30 minutes or 4^{0}c for 40 minutes.
11 Plates are read after 30-40 minutes where control RBCs has settled. This is done by tilting and observing the presence (i.e, HA inhibition by serum/position result) or absence at tear-shaped streaming at the same rate as the control wells containing RBCs (0.025ml) and PBS (0.05ml) only.
12 The HI title is the highest dilute of antiserum causing complete haemagglutination inhibition of 4 units of virus (important: an HA titration to confirm the presence of the required HAU should be included in each test)
13 The validity of the results is observed.
14 Read the plate after 30 minutes when the RBC control has settled. Reading is done by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBC. Wells with no HA (negative results) should flow at the same rate as the control cells with no virus.
15 The HA title is the highest dilution that causes agglutination of the RBC. The dilution may be regarded as containing one HA unit (HAU)

HAEMAGGLUTINATION INHIBITION (HI) TEST

1 Isotonic saline buffered with phosphate (PBS) 0.05ml) to ph 7.0-7.4
2 PBS and albumin (PBS/albumin 0.05%).
3 Antigen is diluted with sterile distilled water to contain 4 HAU per 0.025ml.
4 1% suspensions of chicken RBC (c-RBC)
5 Negative control chicken serum
6 Positive control chicken serum
7 Reconstituted inference sera must be stored at -20^{0}c and reconstituted antigens at -80^{0}c

Procedure

1. Dispense 0.025ml PBS into all wells of a plastic microtitre plate with (V-bottomed wells)
2. Place 0.025ml of serum into first wells of microplate. Add 0.025mlthe positive control serum with (known HI titre) in the FI well and 0.025ml of the negative control sera row for at least every 10 microtitre plates.
3. Using a multichannnel micropipette, make two field dilution of serum across plate (AI-A12) discard the last 0.025ml.
4. Add 0.025ml of antigen containing 4 HAU to all the wells except row H.
5. Add 0.025ml of antigen suspension containing 4 HAU into each of the first two wells of row H (4 HAU control from HI-H6), make-two fold dilution dependent on obtaining a title of less than 2^3

for 4 HAU or 2^2 for 8 HAU with a with negative control serum and a title of within an dilution of the know tile at the positive control serum

AGAR GEL IMMUNODIFUSION AGID TEST

Preparation of Agar (100ml)

1. Weigh Ig of agarose and 8g of sodium chloride
2. Pour the salt into sterile Durham bottle (500ml)
3. Add 100ml of PBS (ph adjusted to 7.2
4. Heat the mixture until it completely dissolves
5. Dispense 15ml of the melted agar into 100x15mm sterile Petri dishes placed on a leveled surface
6. Leave the Petri dishes open until the agar is set
7. Cover the plates and store at $+4^0$c unit use

Test Procedure

1. Cut wells in set agar using a tubular cutter
2. Remove the agar from the wells using sunction pump
3. Dispense the test sera into the wells marked T1-T4
4. Dispense standard antigen into the control well
5. Dispense standard positive antiserum in the peripheral well opposite to the standard antigen
6. Dispense standard negative antiserum in the well opposite the standard positive antiserum
7. Incubate the plates at between 37^0C for 24-48 hour in a humid chamber to avoid drying the agar.
8. Examine the plates against a dark background with an oblique light source
9. Line of identify is observed between the positive antiserum and standard antigen.
10. Any test sera showing the same line of identify as the control indicate a positive result.
11. Document your results

RESULTS

HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN ARIARIA MARKET

Of the 50 local chickens sampled from Ariaria international market Aba, 8 samples had 2^0 titre value, 13 samples had 2^1 titre value, 11 samples had 2^2 titre value, 4 samples had 2^3 titre value, 4 samples had 2^4 titre value, 3 samples had 2^5 titre value, 5 samples had 2^2 titre value and 2 samples had 2^8 titre value. (Table 1).

Table 1: HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN ARIARIA MARKET ABA.

Titre Value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
No of samples	8	13	11	4	4	3	0	5	2	0

HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN EZIUKWU MARKET ABA.

Out of 50 local chickens sampled from Eziukwu market Aba, 7 samples, had 2^0 titre value, 14 samples had 2^1 titre value, 4 samples had 2^2 titre value, 6 samples had 2^5 titre value, 1 sample had 2^6 titre value, 1 sample had 2^7 titre value and 1 sample had 2^9 titre value. (Table 2).

TABLE 2: HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN EZIUKWU MARKET ABA.

Titre Value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
No of samples	7	14	4	6	10	6	1	1	0	1

HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN EKEOHA SHOPPING CENTRE ABA.

Of the 50 local chickens sampled from Ekeoha shopping centre, 9 samples had 2^0 titre value, 12 samples had 2^1 titre value, 7 samples had 2^2 titre values, 5 samples and 2^3 titre value, 9 samples had 2^4 titre value, 2 sampled had 2^5 titre,1 sample had 2^6 titre value, 4 samples had 2^7 titre value and 1 sample had 2^8 titre value. (Table 3).

Table 3: HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN EKEOHA SHOPPING CENTRE ABA.

Titre Value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
Noof samples	9	12	7	5	9	2	1	4	1	0

HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN NKWO-NGWA MARKET ABA.

Of the 50 local chickens sampled from Nkwo-Ngwa market Aba, 13 samples had 2^0 titre value, 10 samples had 2^1 titre value, 12 samples had 2^2 titre value, 6 samples had 2^3 titre value, 6 samples had 2^4 titre values, 1 sample had 2^6 titre value, 1 sample had 2^7 titre value, and 1 sample had 2^8 titre value. (Table 4).

Table 4: HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN NKWO-NGWA MARKET ABA.

Titre Value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
No of samples	13	10	12	6	6	0	1	1	1	0

HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN NEW MARKET ABA

Of the 50 local chickens sampled from New market Aba, 9 samples had 2^0 titre values, 14 samples had 2^1 titre values, 11 samples had 2^2 titre value, 9 samples had 2^3 titre value, 2 samples had 2^4 titre value, 2 samples had 2^5 titre value, 1 sample had 2^6 titre value and 2 samples had 2^7 titre value. (Table 5)

Table 5: HI TITRE OF NDV ANTIBODIES IN ADULT CHICKENS IN NEW MARKET ABA.

Titre Value	2^0	2^1	2^2	2^3	2^4	2^5	2^6	2^7	2^8	2^9
No of samples	9	14	11	9	2	2	1	2	0	0

Geometric mean titre $= \sqrt[n]{A \times B \times C \times \ldots\ldots\ldots\ldots\ldots\ldots\ldots n}$

$= \sqrt[5]{21 \times 17 \times 29 \times 13 \times 17}$

$\sqrt[5]{2288013} = 18.7$ GMT

SEROPREVALENCE OF NDV IN ADULT LOCAL CHICKENS IN 5 DIFFERENT MARKETS IN ABA.

50 samples were collected from the five (5) different markets in Aba respectively. Ariaria int'l market had 14 (28%) positive samples with mean H1 titre 5.8, Eziukwu market had 19 (38%) positive samples with 4.8 mean H1 titre, Ekeoha shopping centre had 17 (34%) positive samples with 4.8mean H1 titre, Nkwo-Ngwa market and 9 (18%) positive samples with 4.1 mean HI and new market had 7 (14%) positive samples with 5.4 mean HI titre. (Table 6).

TABLE 6: SEROPREVALENCE OF NDV IN ADULT LOCAL CHICKENS IN 5 DIFFERENT MARKETS IN ABA.

	MARKETS	NO OF SAMPLES	NO OF POSITIVE SAMPLES	PERCENTAGE POSITIVE SAMPLES (%)	MEAN H1 TITRE
1	Ariria int'l market	50	14	28.0	5.8
2	Eziukwu market	50	19	38.0	4.8
3	Ekeoha shopping centre	50	17	34.0	4.8
4	Nkwo-Ngwa market	50	9	18.0	4.1
5	New market Aba	5	7	14.0	5.4
	Total	**250**	**66**	**26.4**	

SEROPREVALENCE OF IBDV IN ADULT CHICKENS IN THE FIVE (5) MARKETS IN ABA, ABIA STATE

Out of 50 samples from Ariaria int'l market, the number of positive samples were 21 (42%), Eziukwu market had 17(34%) positive samples, Ekeoha shopping centre had 29 (58%) positive samples, Nkwo-Ngwa market had 13 (26%) positive samples and New market had 17 (34%) positive samples (Table 7).

TABLE 7: SEROPREVALENCE OF IBDV IN ADULT CHICKENS IN THE FIVE (5) DIFFERENT MARKETS IN ABA.

	MARKETS	NO OF SAMPLES	NO OF POSITIVE SAMPLES	PERCENTAGE POSITIVE SAMPLES (%)
1	Ariria int'l market	50	21	42
2	Eziukwu market	50	17	34
3	Ekeoha shopping centre	50	29	58
4	Nkwo-Ngwa market	50	13	26
5	New market Aba	50	17	34
	Total	50	17	34

DISCUSSION

The prevalence rate of 28% (14/50) obtained for NDV in chickens from Ariaria market is very significant (P<0.05) and therefore high. A similar findings was reported by [6], [7]. Their result was 76% (19/25) samples. Chickens are susceptible to NDV due to local husbandry practices where different variations of chickens are raised together in the same cage which may encourage cross contamination of the chickens.

The prevalence rate of 38% (19/50) obtained for NDV in chickens from Eziukwu market is significant (P<0.05) and therefore high. This result could serve as a source of infection for both local and exotic chickens. Similar research was demonstrated by the 20ND outbreaks in Britain Alexander *et al.* (1984), where 68% (17/25) tested positive. The prevalence rate of 34% (14/50) obtained for NDV in chickens from Ekeoha shopping centre is very significant (P>0.05). This work is comparable to the result obtained is a similar research by [8] whose result was 24% (11/25) positive samples.

The prevalence rate of 18% (9/50) obtained for NDV in chicken from Nkwo-Ngwa market is very significant (P<0.05) and therefore high. A similar findings was reported by [8] whose result was 20% (5/25) positive samples. Fewer samples tested positive in this study and it can also serve as a source of infection for both local and foreign chickens. The prevalence rate of 14% (7/50) obtained for NDV in chickens from New market is very significant (P<0.05) and therefore also high. This result is comparable to the result obtained by [9] in a similar research. Government should show more interest in these two markets (Eziukwu market and Ekeoha shopping centre) and should also request certificate from suppliers that chickens are quarantined before they are distributed to various locations [10]. Movement of personnel between new and old chickens should be restricted [11]. Infected chickens should be isolated from the healthy ones hence the diseases are contagious.

Since the treatment of NDV and IBDV does not exist for now, poultry farmers should use prophylactic vaccines and sanitary measures to reduce the likelihood of the outbreak [10]. Good bio-security can help to prevent the diseases in poultry chickens for instance, bird-proofing houses, feed and water supplies, minimizing travel on and off the facility and disinfecting vehicles and equipments that enter the farm. Chickens should not be allowed to contact domesticated poultry of unknown health status pests such as insects and mice should also be controlled. Outbreaks are eradicated with quarantined and movement controls, depopulation of all infected and exposed chickens and thorough cleaning and disinfection of the premises. Finally, from the statistical analysis conducted on these viruses (NDV and IBDV), seroprevalence of IBDV in Aba is significant while in NDV, it is not significant, therefore, Government should show more interest in IBDV than NDV in Aba Abia State.

CONCLUSION

From the findings of this study, it can be concluded that the prevalence rate of 38.8% (97/250) obtained from infectious bursal disease virus IBDV in chickens from 5 different markets in Aba is higher than the prevalence rate of 26.4% (66/25) obtained for Newcastle disease virus NDV. From the result obtained in this research work, seroprevalence of NDV is higher in Eziukwu market and also seroprevalence of IBDV is higher in Ekeoha shopping centre.

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