# Seroprevalence of Newcastle Disease Virus from Locally Bred Chicken in Kaduna South Local Government Area. Kaduna State, Nigeria.

*Abraham, O.J., Onwuatuegwu, J.T.C., Nwobodo, **A.H.,** Zakka, A. **W., Egbunu, Z.K., Oruma, Y.U,** Ahmed, S.f **and** Ngbo,* F. *S.*

*1. Dept of Science Laboratory Technology, Federal Polytechnic Idah, Kogi State, Nigeria.*

*2. Dept of Microbiology, Tansian University Umunya, Anambra State, Nigeria.*

*3. Dept of Medical Laboratory Sciences, Enugu State University of Science and Technology, Enugu, Nigeria.*

*4. Dept of Microbiology, Kaduna State University, Kaduna, Nigeria.*

*5. Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Plateau State, Nigeria.*

*Correspondence: E-mail Phone: 08062908906*

**Abstract:** Newcastle disease (ND) is a highly contagious viral disease of domestic poultry, caged, aviary and wild birds caused by the Newcastle disease virus (NDV). Sero prevalence of Newcastle disease virus was determined with 250 sera samples collected from unvaccinated local chickens marketed and slaughtered in Kaduna South Local Government Area, Kaduna State. Northern Nigeria using the Haemagglutination inhibition (HI) method. Of the 250 sera examined, 138 (55.5%) tested positive for NDV antibodies, with 112 negative. The result highlights the epizootic nature of the disease among local chickens in the area and concludes that the data is of economic importance since poultry local chickens are known to spread NDV to other avian species. Vaccination of local chicken is recommended when practicable.

Keywords: Newcastle disease, virus, epizootic, local chickens, haemagglutination inhibition test.

## Introduction

Newcastle disease is a highly infectious, contagious viral disease of chickens, turkeys and ducks characterized by high morbidity and high mortality in unvaccinated birds. First reported in Nigeria by Hill *et al.* (1953), the disease is now known to be enzootic in the country. Clinically, It is generally characterized by drastic reduction in egg production, laying of soft shelled and malformed eggs, partial *suppression and at times complete ceasation of* immunological response (Ameh *et al.*, 2017). Consequently, the disease constitutes a major threat to poultry industry in Nigeria. Epidemiological investigation have shown that the indigenous breed of chicken which are predominantly on free range and whose population is about 124 million accounting for over 92% of the total chickens in Nigeria are believed to act as reservoirs of this rather than fulminating viral infection (Musa *et al.*, 2016). Newcastle disease is a *highly contagious and wide spread viral disease* affecting domestic and wild avian species, causing severe economic lost in domestic poultry especially chickens. The disease is caused by an Avian Paramyxovirus 1 (APMV-1), a negative-sense, single-stranded RNA virus. NDV/APMV-1 belongs to the genus *Avulavirus* in the family *Paramyxoviridae* (Hines and Miller, 2012). Transmission occurs by exposure to faecal and other excretions from infected birds and through contact with contaminated food, water, *equipment and clothing.*

*Corresponding author:
*josephoyiguh@yahoo.com * Abraham, O.J.*



NDV strains can be categorised as velogenic (highly virulent), mesogenic (intermediate virulence) or lentogenic (nonvirulent). Velogenic strains produce severe nervous and respiratory signs which spread rapidly and cause up to 90% mortality. Lentogenic strains produce mild signs with negligible mortality (Moura *et al.*, 2015; Schwaiger *et al.*, 2017). The disease can be diagnosed in the laboratory using some *techniques such as enzyme linked Immunosorbent* assay (ELISA), polymerase chain reaction (PCR) and gene sequencing. The most convenient method of propagating new castle disease virus in the laboratory is by inoculation of the allantoic cavity of embryonated eggs with the clinical samples. This method is also important to grow the virus for other purposes such as preparation of viral antigens and vaccine production (Ezeibe *et al.*, 2015; El-Bahrawy, 2016).

Village poultry accounts for about 90% of the *total poultry population in Nigeria (Lawal et al., 2016).* It provides an important source of high quality animal protein and income for rural dwellers (Ibrahim *et al.*, 2016). Investment in the form of breeding, housing, feeding, labour and veterinary care is minimal, therefore egg production is low and mortality is high as a result of infection by contagious diseases and predators and other loses. The ND virus in locally bred chickens limit their growth, productivity and causes high economic loss to the farmers, it has no treatment *except prophylactic vaccination and vaccines failures* are common and has been observed as yearly so the prevalence rate is been studied to reduce the high risk of contracting the disease (Musa *et al.*, 2016).

This study shall serve as a documentary source of information to future researchers pertaining ND virus affecting locally bred chickens. There is need to determine the Seroprevalence of Newcastle Disease Virus in Locally bred chicken in Kaduna South Local Government Area, Kaduna State. This study determined the presence of Newcastle Disease Virus antibodies in locally bred chicken in Kaduna South Local Government Area, Kaduna State.

## Materials and Method

### Study Area

The study was conducted using chickens blood specimens collected randomly from abattoir in Kaduna metropolis. Kaduna is in the north-western part of Nigeria and is presently the capital of Kaduna state, centrally located and share boundaries with the following states; Niger, Plateau, Abuja, Nasarawa, Kastina and Kano. The population of Kaduna was 760,084 as of the 2006 Nigeria census. Rapid urbanization over the past decade has created an increasingly large population, now estimated to be around 1.3 million. The climate is tropical, dry and rainy seasons and when compared with winter, the summers have much more rainfall. The climate here is classified as Aw by the Kopper-Geiger system. The average annual temperature in Kaduna is 25.2°C. About 1211 mm of precipitation falls annually. Kaduna metropolis comprises of two local government areas: Kaduna north local government and Kaduna south local government. The two local governments are made up of mostly civil servants, industrial workers, traders, farmers and students. Kaduna is the commercial and industrial centre of Northern Nigeria. English and Hausa are the major language in Kaduna.

### Collection of Samples

About two (2) ml of blood was collected from each of Two hundred and fifty (250) birds, between August and December 2017.Each chicken was held horizontally on its back. The assistant used one hand to hold the legs and placed the other hand under the back to support the chicken. The wings of the chicken were pulled outward and a feather plucked. The area was disinfected by swabbing with 70% alcohol. The needle was inserted under the tendon. Once the tip of the needle was in the vein, the plunger of the syringe was gently pulled to draw blood into it. After removing the needle, pressure was applied to the vein for a few seconds to stop further bleeding.

### Preparation of serum

The needle was removed and the syringe placed upside down on a level surface and left for about one hour for serum to form. The serum formed was dispensed into plain sterile sample bottles and stored in the refrigerator until used.

### Haemagglutination Inhibition (HI) test

### Reagents required

Isotonic saline buffered with phosphate (0.05M), pH 7.0-7.4; Antigen diluted with PBS to contain 4 HAU per 0.025ml; 1% suspension of chicken RBCs (c-RBC); Negative control chicken serum; Positive control chicken serum.

### Preparation of 5% chicken red blood cells

Chicken red blood cells (cRBC) collected from Specific (minimal) Pathogen free (SPF) was put in a sterile centrifuge tube and filled up with phosphate buffer saline (PBS) and centrifuged at 3000rpm for 10 minutes. The supernatant was decanted (Pour off) from the packed red blood cells. The tube was filled with PBS and shaken gently to re-suspend the red blood cells and centrifuged again at 3000rpm for about 10 minutes and the supernatant pour off. The procedures were repeated until a clear supernatant was obtained. About 0.5ml of the packed cell was picked and suspended in 95ml of PBS in a sterile tube and shaken gently to mix.

### Haemagglutination Inhibition (HI) test

About 0.025ml PBS was dispensed into all wells of a plastic V-bottomed Micro-titre plate. About 0.025ml of test serum was placed in the first wells of the plate. Two-fold dilutions of the serum were made across the plate. About 0.025ml of antigen containing 4 HAU was added to each well and mixed by tapping gently and the plate placed at 20°C for 30 minutes. About 0.025ml 1% cRBC was added to each well and mixed by gentle tapping and placed at 20°C. Plates were read after 30 minutes when control RBCs had settled. This was done by tilting and observing the presence (i.e HA inhibition by serum/positive result) or absence (i.e no HA inhibition/negative result) of tear-shaped streaming at the same rate as the control wells containing RBCs (0.025ml) and PBS (0.05ml) only.

### Interpretation of result

The HI titre is the highest dilution of antiserum causing complete inhibition of 4 units of virus (Important: an HA titration to confirm the presence of the required HAU should be included in each test). The validity of the results is dependent on obtaining a titre of less than 23 for 4 HAU or 22 for 8 HAU with a negative control serum and a titre of within one dilution of the known titre of the positive control serum.

## Results

### Hi Titre of NDV Antibodies in Local Chicken In Station Market, Kaduna South

Eighty-four(84) serum samples from healthy chickens in station market, Kaduna South had HI titre value of $2^0$.5 samples. $2^1$, 2 samples, $2^2$, 6 samples, $2^3$, 8 samples, $2^4$, 9 samples, $2^5$, 9 samples, $2^6$, 11 samples, $2^7$, 8 samples, $2^8$, 10 samples, $2^9$, 8 samples, $2^{10}$, 7 samples and $2^{11}$, 1 sample (Table 1).

**Table 1: HI Titre of NDV Antibodies in Local Chicken In Station Market, Kaduna South**

| Titre Values | $2^0$ | $2^1$ | $2^2$ | $2^3$ | $2^4$ | $2^5$ | $2^6$ | $2^7$ | $2^8$ | $2^9$ | $2^{10}$ | $2^{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Samples | 5 | 2 | 6 | 8 | 9 | 9 | 11 | 8 | 10 | 8 | 7 | 1 |
| Description of Results | - | - | - | - | + | + | + | + | + | + | + | + |

**HI Titre of NDV Antibodies in Local Chicken in Monday Market, Kaduna South**

Eighty-three (83) serum samples from healthy chickens in Sabo Market, Kaduna South showed HI titre value of $2^0$ 32 samples, $2^1$, 5 samples, $2^2$, 5 samples, $2^3$, 3 samples, $2^4$, 3 samples, $2^5$, 8 samples, $2^6$, 7 samples, $2^7$, 8 samples, $2^8$, 6 samples, $2^9$, 6 samples, $2^{10}$, 0 sample and $2^{11}$, 0 sample (Table 2).

**Table 2: Hi Titre of NDV Antibodies in Local Chicken in Monday Market, Kaduna South**

| Titre Values | $2^0$ | $2^1$ | $2^2$ | $2^3$ | $2^4$ | $2^5$ | $2^6$ | $2^7$ | $2^8$ | $2^9$ | $2^{10}$ | $2^{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Samples | 32 | 5 | 5 | 3 | 3 | 8 | 7 | 8 | 6 | 6 | 0 | 0 |
| Description of Results | - | - | - | - | + | + | + | + | + | + | + | + |

**Hi Titre of NDV Antibodies in Local Chicken in Kakuri Market, Kaduna South**

Eighty-three(83) serum samples from healthy chickens in Kakuri market, Kaduna south demonstrated HI titre value of $2^0$ 33 samples, $2^1$, 3 samples, $2^2$, 5 samples, $2^3$, 5 samples, $2^4$, 9 samples, $2^5$, 7 samples, $2^6$, 8 samples, $2^7$, 5 samples, $2^8$, 4 samples, $2^9$, 3 samples, $2^{10}$, 1 sample and $2^{11}$, 0 sample (Table 3).

**Table 3: Hi Titre of NDV Antibodies in Local Chicken in Kakurimarket, Kaduna South**

| Titre Values | $2^0$ | $2^1$ | $2^2$ | $2^3$ | $2^4$ | $2^5$ | $2^6$ | $2^7$ | $2^8$ | $2^9$ | $2^{10}$ | $2^{11}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Samples | 33 | 3 | 5 | 5 | 9 | 7 | 8 | 5 | 4 | 3 | 1 | 0 |
| Description of Results | - | - | - | - | + | + | + | + | + | + | + | + |

**Seroprevalence of NDV Antibodies In Local Chicken In Station, Monday And Kakuri Markets Kaduna South**

The seroprevalence rate of the respective markets for NDV antibodies include Station markets with 63 positive samples (25.2%), Sabo market with 37 positive samples (14.8%) and Kakuri market with 38 positive samples (15.2%) each had HI mean titre of respectively (Table 4).

**Table 4: Seroprevalence of NDV Antibodies in Station, Monday and Kakuri Markets**

| Markets | no of samples | no of positive samples | %positive |
|---|---|---|---|
| Station | 84 | 63 | 25.2% |
| Kakuri | 83 | 38 | 15.2% |
| Monday | 83 | 37 | 14.8% |
| Total | 250 | 138 | 55.2% |

Discussion

The present study revealed the prevalence of haemagglutination antibodies in samples from the three markets in Kaduna South and this indicates that Newcastle disease virus infection is endemic in the area, and the markets are serving as mixing point of infected birds with susceptible ones. The sellers and buyers as well as those processing the meat are veritable vehicle of transmission of the disease. There is therefore a great threat to commercial poultry production in the Kaduna. The implication of the spread and the carrier status of the rural household chickens could be of importance considering the fact that rural chickens were reported to constitute over 90% of chicken population in Nigeria and are capable of scavenging around the environment spreading of the NDV to vaccinated and unvaccinated healthy exotic birds.

From table 1, 84 samples from healthy chickens in station market were screened, 63 local chickens were positive for HI antibodies to New Castle Disease virus in station market. A prevalence rate of 25.2 was gotten.

From table 2:83 samples from healthy chickens in Monday market were screened, 38 local chickens were positive for HI antibodies to New Castle Disease virus in station market. A prevalence rate of 15.2 was gotten.

From table 3:83 samples from healthy chickens in kakuri market were screened, 37 local chickens were positive for HI antibodies to New Castle Disease virus in station market. A prevalence rate of 14.8 was gotten.

From table 4: Which determine seroprevalence of NDV antibodies. In station market total numbers of samples collected were 84, while in Monday market total number of samples collected were 83 and in Kakuri market total number of samples collected was 83 which gave the sum total of 250 samples. The numbers of positive samples in station market are 63 samples, while in Monday market are 38 positive samples and in Kakuri market there were 37 positive samples, which gave the sum of all positive samples as 138 samples. The percentage positive for station market is 25.2%, while for Monday market is 15.2% and for Kakuri market is 14.8% which gave the sum of 55.2%. Of the 250 samples screened, 138(55.5%) were positive for antibodies to NDV while 112(44.5%) were negative as shown in table 4. the results of this study shows that most of the local chickens marketed and slaughtered in Kaduna south have antibodies to NDV, the presence of antibodies indicates a previous natural infection with ND virus since number of record of vaccination of the birds exist.

Haemagglutination Inhibition (HI) antibody titre between 0log2 and 3log2 is considered negative because they produced no antibody against the virus while HI-antibody titre between 4log2 and 11log2 is considered positive for antibodies production against the virus based on OIE recommendation of 2000 (Susta *et al.*, 2015). In all the three (3) markets studied an HI antibody titre of 4log2 and the above ones were observed and this is indicative of exposure to the virus at one time or the other and eventual production of neutralizing antibodies to protect the chicken up to the point of sale. The high HI antibody titre may be due to infection by a virulent strain of the virus such as mesogenic strains which are viruses causing clinical signs consisting of respiratory and neurological signs with low mortality and lentogenic strains which are viruses causing mild infection of the respiratory tract without visible morbidity and mortality (Mohammed *et al.*, 2016). In the US, however, the virus has been eradicated due to stringent adherence to poultry management rules and any virulent strains are of foreign origin from places where strict compliance to management regulations and good sanitary practices is lacking (Yuan *et al.*, 2015).

The prevalence of NDV in this study was 55.2%. this is lower than the reports of Ibitoye *et al.* (2013) in a retrospective study where the reported an NDV seroprevalence of 80.9% from chickens in Sokoto state .This may be due to the seasonality of NDV having high occurrence in the months of March and October (Ibitoye *et al.*, 2013) which coincide with onset of the rainy season and dry season, respectively. The high wind movement transfers infection from one poultry house or flock to the other (Musa *et al.*, 2009) Kaduna is neighbour to Plateau State where the National Veterinary Research Institute is situated and thus could be benefiting from the surveillance services of the institute, thus stemming the tide of the disease.

The seroprevalence of NDV antibody was higher in Station market (25.2%) than in Monday market (15.2%) and other markets studied. Musa *et al.* (2009) observed variation in prevalence rate of NDV from different study sites. The high rate may be due to the higher concentration of commercial poultry in Station market than the other markets studied. Higher seroprevalence rate of New castle disease virus antibodies was detected in both household (26.8%) and live bird markets (35.8%) and an overall seropositive rate is 32.5% by Jibril *et al.* (2014).The prevalence rate of 55.5% recorded in this study falls within the range of findings of various studies carried out previously in Kaduna . In Kaduna prevalence of 73.0, 74.3 and 67.3% have been reported (Nwanta, 2003; Nwankiti *et al.* 2010). The drop in prevalence as recorded now when compared to that reported earlier may point to a reduction in the occurrence of the disease in Kaduna over a period of years.

Apparently healthy birds are carriers of Newcastle disease virus and is a threat to commercial poultry production in the study area (Salihu *et al.* 2012), and reveals the epizootic nature of the virus in the study area and is suggestive of an inter-epidemic phase or early phase of infection pointing a finger to possible economic losses in the event of an outbreak, alongside the role of

local chickens in the transmission cycle of NDV to other avian species (Nwankiti *et al.* (2010).

**Conclusion**

The results of this study shows that most of the local chickens marketed and slaughtered in Kaduna south had antibodies to NDV, the presence of antibodies indicates a previous natural infection with ND virus since number of record of vaccination of the birds exist. Management practices such as disease monitoring programme, appropriate prevention, and control measures should be put in place. Local poultry farmers should ensure that they vaccinate their flocks. Awareness programme among the farmers about the disease and routine survey to assess the degree of Newcastle disease distribution will help in planning appropriate intervention strategy.

**References**

Ameh, J.A., Mailafia, S., Olabode, O.H., Adah, B.J., Okoh, G.R., Ogbole, M.E. and Alalade, D.I. (2017). Sero-prevalence of Newcastle disease virus antibodies in local and exotic chickens in Gwagwalada, Nigeria. *Journal of Veterinary Medicine and Animal Health*, 8(11), 2141-2529.

Hill, O.H., Olive, S.O. and Wlde, J.K.H. (1953). Newcastle Disease in Nigeria. *British Veerinary Journal*, 9(1), 11-13.

Schwaiger, T., Knittler,M.R., Grund, C., Roemer-Oberdoerfer, A., Kapp, J., Lerch, M.M. Mettenleiter,T.M., Mayerle, J. and Blohm, U. (2017). Newcastle disease virus mediates pancreatic tumor rejection via NK cell activation and prevents cancer relapse by prompting adaptive immunity. *International Journal of Cancer*, 141(12) 2505-2516.

El-Bahrawy, A., Zaid, A., Sunden, Y., Sakurai, M., Ito, H., Ito, T. and Morita, T. (2016).Pathogenesis of Renal Lesions in Chickens After Experimental Infection With 9a5b Newcastle Disease Virus Mutant Isolate. *Veterinary Pathology*, 54(1) 113-180.

Hines, N.L. and Miller, C.L. (2012): Avian Paramyxovirus Serotype-1: A Review of Disease Distribution, Clinical Symptoms, and Laboratory Diagnostics. Veterinary Medicine International. 2012, 17 pages. http://dx.doi.org/10.1155/2012/708216

Lawal,J.R., El-Yuguda, A.D. and Ibrahim, U.I. (2016). Survey on Prevalence of Newcastle Disease in Village Poultry at Live Birds Markets in Gombe, Nigeria. *Journal of Animal Sciences and Livestock Production*, 1(1): 112-176.

Ibrahim, U.I., Ibrahim, J.R. and El-Yuguda, A.D. (2016).Level of Newcastle disease vaccination awareness and its effects on village chicken production in Gombe State, Nigeria. *Direct Research Journals*, 4(3): 2354-4321.

Musa.W.I., Lawal, S., Bello, M. and Abdu, P.A. (2016). Evaluation of the Relative Livestock Populations and the Assessment of the Status and Impacts of Newcastle Disease in Rural Communities of Two Northeastern States. Nigeria. *Open Journal of Animal Sciences*, 6(1). 49-58.

Jibril, A.H., Umoh,J.U., Kabir, J., Saidu, L., Magaji, A.A., Bello, M.B. and Raji, A. A. (2014). Newcastle Disease in Local Chickens of Live Bird Markets and Households in Zamfara State, Nigeria. *Nigerian Veterinary Journal*, 4(1), 12-19.

Jibril, A.H., Umoh, J.U., Kabir, J., Gashua, M.M. and Bello, M.B. (2016). Application of participatory epidemiology techniques to investigate Newcastle disease among rural farmers in Zamfara state, Nigeria. *The Journal of Applied Poultry Research*, 24(1), 233–239.

Moura, V. M. B. D., Susta, L., Cardenas-Garcia, S., Stanton, J. B., Miller, P. J., Afonso, C. L. and Brown, C. C. (2015): Neuropathogenic Capacity of Lentogenic, Mesogenic, and Velogenic Newcastle Disease Virus Strains in Day-Old Chickens. Veterinary Pathology Online. 1-12. DOI: 10.1177/0300985815600504

OIE World Organization for Animal Health. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 6th ed. Paris, France: OIE; 2008:576–589

Musa, U., Abdu, P.A., Dafwang, I.I., Umoh, J.U., Sa'idu, L., Mera, U.M. and Edache, J.A. (2009): Seroprevalence, seasonal occurrence and clinical manifestation of Newcastle disease in rural household chickens in Plateau State, Nigeria. *Int. J. Poult. Sci.*, 8: 200-204.

Nwanta, J.A. (2003): Field vaccination trial with Newcastle Disease Vaccine (NDV4HR) in Local Chicken in Kaduna State, Nigeria. Ph.D. Thesis, Ahmadu Bello University, Zaria, Nigeria.

Nwankiti, O.O., Ejekwolu, A.J., Ibrahim, I., Ndako, J.A. and Echeonwu, G.O.N. (2010): Detection of Serum Antibody Levels against Newcastle Disease in Local Chickens in Bauchi Metropolis, Bauchi State, Nigeria. *Afr J ClinExper Microbiol*. 11: 95-101.

Ezeibe, M.C.O., Okoye, J.A.O., Ezeala, I.E., Mbuko, I.J. and Ngene, A.A. (2015): Strains of Newcastle disease Virus, Circulating among Sick Poultry Flocks in Nsukka-Nigeria. *British Microbiology Research Journal*. 5(4): 384-388.

Schwaiger, T., Knittler, M. R., Grund, C., Oberdoerfer, A.R., Kapp, J-F., Lerch, M.M., Mettenleiter, T.C., Mayerle, J. and Blohm, U. (2017 ): Newcastle disease virus mediates pancreatic tumor rejection via NK cell activation and prevents cancer relapse by

prompting adaptive immunity: NDV prompts immunity against pancreatic cancer · International Journal of Cancer 141(12)DOI: 10.1002/ijc.31026

El-Bahrawy, A., Zaid, A., Sunden, Y., Sakurai, M., Ito, H., Ito, T. and Morita, T. (2017): Pathogenesis of Renal Lesions in Chickens After Experimental Infection With 9a5b Newcastle Disease Virus Mutant Isolate. *Veterinary Pathology*. 54(1): 94-98. **DOI:** 10.1177/0300985816655852

Ibitoye, E.B. ,Jimoh, A.A. and Mungadi, H.U. (2013). A Retrospective (2007-2011) Analysis of Newcastle Disease Diagnosed at Avian Clinic of Veterinary Teaching Hospital, Usmanu Danfodiyo University Sokoto, Nigeria. *Current Research in Poultry Science*, 3: 12-17. **DOI:**10.3923/crpsaj.2013.12.17

Yuan, P., Swanson, K. A., Leser, G. P., Paterson, R. G., Lamb, R. A. and Jardetzky, T. S. (2015). Structure of the Newcastle disease virus hemagglutinin-neuraminidase (HN) ectodomain reveals a four-helix bundle stalk. *Proc. Natl. Acad. Sci. U.S.A.* 108. 14920–14925. doi: 10.1073/pnas.1111691108

Salihu, A.E.,Chukwuedo, A.A., Echeonwu, G.O.N., Ibu, J.O., Chukwuekezie, J.O., Ndako, J., Junaid, S.A., Onovoh, E.M., Paul- Abu, L.G., Ujah, A.E., Dalyop, A.K., Tende, M.D., Shittu, I., Chindo, H.Z. and Umahi N.F. (2012): Seroprevalence of Newcastle Disease Virus Infection in Rural Household Birds in Lafia, Akwanga and Keffi Metropolis, Nasarawa State, Nigeria.

Susta, L., Jones, M.E., Cattoli, G.,Cardenas-Garcia, S., Miller, P.J., Brown, C.C. and Afonso, C.L. (2015): Pathologic characterization of genotypes XIV and XVII Newcastle disease viruses and efficacy of classical vaccination on specific pathogen-free birds. Vet. Pathol. 52: 120 – 131. doi:http://dx.doi.org/10.1177/0300985814521247

Mohamed, M.H.A., Abdelaziz, A.M., Kumar, S., Al-Habib, M.A. and Megahed, M.M. (2016) Effect of phylogenetic diversity of velogenic Newcastle disease virus challenge on virus shedding post homologous and heterologous DNA vaccination in chickens, Avian Pathology, 45(2): 228-234, DOI: 10.1080/03079457.2016.1144870.