



## Seroprevalence And Detection of Newcastle Disease Virus Matrix Gene in Domestic Local Breed of Chickens from Eight Communities in Bauchi State, Nigeria.

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### SUMMARY

Indigenous breed of domestic chickens have been identified as an appropriate tool for eradication poverty and hunger because of the ease at which poor people can acquire, grow and consume or sale the meat and eggs of these animals. The production of these chickens is largely constrained by disease especially, velogenic Newcastle disease (ND) which have the potential of wiping out an entire susceptible chicken population. Foundational to the control of ND in local breed of chickens in every community is the need for baseline information. Such information is scanty in Bauchi State even though the state is one of the major producers of these chickens in Nigeria. The aim of this study is to determine the seroprevalence of ND and also detect Newcastle disease virus (NDV) for the purpose of understanding the presence and distribution of the disease in the State. This study was conducted among 1085 village chickens from nine (9) randomly selected communities in Bauchi State, Nigeria. The seroprevalence of ND by Haemagglutination inhibition test was 36.4%. Matrix gene of ND virus (NDV) was also detected from 29.9% of 281 pooled cloacal swabs of the same chickens. The result indicates that ND virus (NDV) and its antibodies were widespread among village chickens in these communities. Vaccination is suggested as an appropriate control measure to protect chickens against a possible attack by a velogenic strain of NDV in chickens from these communities.

**Key words:** Newcastle disease, seroprevalence, molecular detection, domestic breed of local chickens, Bauchi State.

### INTRODUCTION

The ownership of local breed of domestic chickens by most rural households and the ability of these chickens to produce high quality protein

from eggs and meat within a short cycle of time with low capital input are among the reasons for their consideration as an appropriate tool for attainment of the United Nation sustainable develop goals (SDGs) of poverty alleviation and

elimination of hunger (Sustainable development goals 2016; Dolberg 2003). The production of these chickens was reported to be constrained by poor nutrition, disease, predation and housing (Bell, 2009). Among diseases of chickens, ND was recognized as the principle factor limiting village chicken production in Africa (Spadbrow 1993-94) and Nigeria (Abdu 1992; Sule *et al.*, 2017) where it causes high chicken mortality. ND was first reported in Nigeria in 1953 (Hill 1953) and subsequently, in various part of the country (Nwanta *et al.*, 2008).

Even though ND has also been reported from North eastern States of Bauchi, Borno, Gombe and Yobe States (Lawal *et al* 2015; Sule *et al* 2013; Garba *et al* 2012, Nwakiti *et al.*, 2010), the report from Bauchi State was restricted to Bauchi Metropolis despite the fact that the state has over 5 million domestic chickens (Adene and Oguntade, 2006). The need to investigate the disease in domestic chickens beyond the metropolis becomes necessary for planning the control of the disease in these chickens in the State which is a major producer of domestic chickens in Nigeria (Adene and Oguntade, 2006); and for improvement in the productivity of these chickens to meet the challenges of eradication of poverty and hunger which needs urgent attention in the State and the country.

Seroprevalence study is an epidemiologic tool that could measure antibodies of ND in population of chickens; it could also be used for diagnosis of ND in unvaccinated chickens and for evaluation of flock immunity especially following vaccination. Serology is however limited when detection of the virus is needed.

Detection, characterization, inference in to pathotype, and epidemiology are important aspect of diagnosis of ND that can be achieved by isolation of the live virus which is cumbersome and time consuming and alternatively by molecular techniques (aldous and alexander 2001). Molecular detection of ND offers a quick and sensitive alternative to isolation of ND virus (NDV) and can also be used to characterize NDV as well as undertake

phylogenetic studies. Molecular assay targeting the M gene has been used with success for screening of NDV in Europe, Africa and Asia (Camenisch *et al.*, 2008; Cattoli *et al.*, 2011; Cattoli *et al.*, 2010).

Combining both molecular and serological study offers the advantage of knowing the distribution, immune status and the presence of NDV in breeds of domestic chickens in a given population. The objective of this study was to determine the seroprevalence of ND and detect matrix gene of NDV from cloacal swabs by molecular method among local breeds of domestic chickens in Bauchi State, Nigeria.

## MATERIALS AND METHODS

### Study Areas

This study was carried out in Bauchi State, Nigeria (Figure I). The State occupies a land mass of 48,382 sq km that is located within latitudes 7° 52'N and 8° 56'N and longitudes 7° 25'E and 9° 37'E. The state lies on the Bauchi plateau with dry and wet season and with a vegetation regarded as Savannah woodland. The state has river Hadejia in its northern part and River Gongola in its southern part. The state shares boundary with Kaduna, Benue, Yobe, Gombe, Plateau, Taraba, Kano and Jigawa States (INEC, 2008). The state has twenty Local Government Areas (LGAs), a domestic poultry population of about 5,832,750 (Adene and Oguntade, 2006).

### Sampling Design

Sample size was calculated using the formula outlined by Joachin (1998):

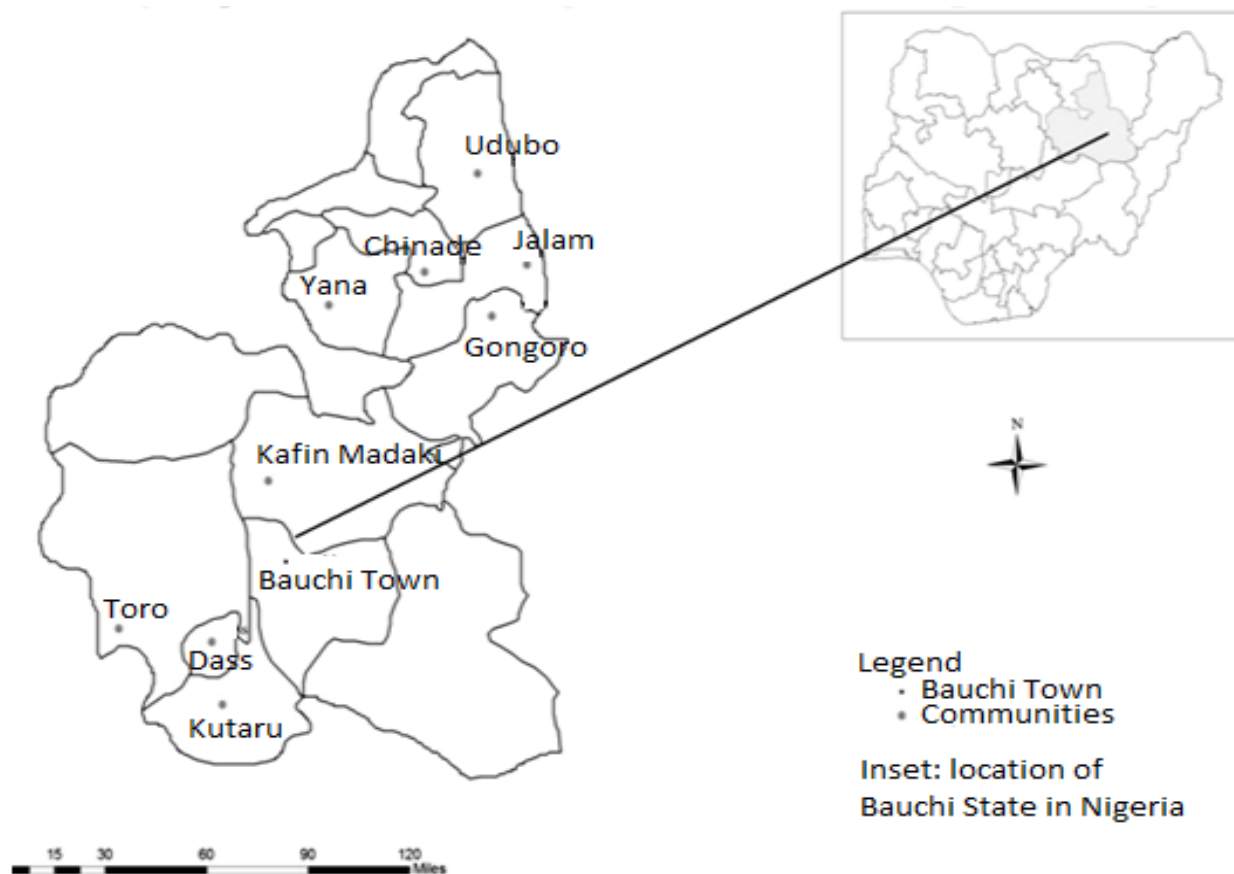
$$n = p \times (100 - p) \times z^2 / d^2$$

Where: n = sample size, p = expected prevalence (56.3%) (Nwakiti, *et al.*, 2010), d= desired precision (.05) and z = appropriate value from the normal for the desired confidence (1.96).

A total of 378 serum samples were computed from an expected prevalence of 56.3% (Nwakiti, *et al.*, 2010). The sample size was increased fourfold from 378 to1512 to obtain a result comparable to that of random sampling (Martins

et al., 1987). The sample size was divided by 9 (which is the number of communities included in the study) and further subdivided by 20 [which is the maximum number of chickens to be sampled per household (HH)] following the recommendation of CEC (1992) resulting in 8.4

HHs to be sampled. Accordingly, 20 chickens were to be sampled from eight HHs in nine (9) communities giving 160 chickens to be sampled per village and a total of 1,440 samples for the whole study.



**Figure I: Map of Bauchi State Nigeria showing communities in which the study was carried out.**

**Collection of Blood**

Two (2) mls of blood was obtained from the brachial vein of chickens using a 2ml syringe and a 23-gauge needle. The syringe containing the blood was kept in a slanted position until the serum has separated. Serum was extracted from the syringe using another 2ml syringe with a 23-gauge needle and transferred to cryovials which is stored in cool box for onward transportation to laboratory where samples were frozen

**Detection of Antibodies to ND Antigen**

Newcastle disease positive and negative sera were obtained from the National Veterinary Research institute (NVRI), Vom, and used for HI-test as described by OIE, (2000). Four Haemagglutination unit (HAU) of the virus antigen titre was calculated and diluted accordingly for use in Haemagglutination Inhibition test (HI-test).

**Test Procedure**

HI-test was performed against 4HAU of the virus antigen following the procedure described by

OIE, (2012). The titres were expressed as  $\log_2$  of the reciprocal of the highest dilution of serum giving 100% inhibition of the 4HAU. Titres equals or higher than  $4\log_2$  was considered positive.

### Molecular Detection of Newcastle Disease Virus

Cloacal swabs were obtained from domestic local breeds of chickens with swab sticks and used for detection of NDV. From 1,440 chickens calculated above for serological studies 288 samples of cloacal swabs was calculated by pooling 5 swabs which resulted in four pooled cloacal swabs from each HH.

One step PCR Kit was obtained from Qiagen and used following the manufacturers instruction. Briefly, a 25  $\mu$ l was used as the final mix for the master mix (x40 $\mu$ l) of the following stock reagent: 11.5  $\mu$ l of Nuclease free water, 5.0  $\mu$ l of 5x PCR buffer (Tris.cl, Kcl,  $(\text{NH}_4)_2\text{SO}_4$ , 12.5 mM  $\text{MgCl}_2$  DTT PH 8.7), 0.5  $\mu$ l dNTPs, 1.0  $\mu$ l APM-1 M4079 (10 uM), 1.0  $\mu$ l APMV-1 M4337 (10  $\mu$ M), 0.5  $\mu$ l Enzyme mix, 0.5  $\mu$ l Rnase inhibitor giving a total of 20  $\mu$ l to which RNA template of 5  $\mu$ l was added to each individual PCR tube and stored at -20 °C. Amplification was carried out in a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems). The cycling conditions were 45°C for 60 minutes to enable reverse transcription of RNA to complimentary DNA; and at 94°C to inactivate reverse transcriptase enzyme and activate Taq polymerase enzyme. A 3-step amplification of 40 cycles of denaturation at 94°C for 45 seconds, Annealing at 60°C for 45 seconds and extension at 72°C for 45 seconds and final extension at 72°C for 5 minutes. Positive control was obtained from NVRI, VOM Diagnostic Virology Laboratory while RNASE free water was used as negative control.

### Detection of Amplicons

That only 36.4% of 1085 chickens examined were positive at  $4\log_2$  (TABLE I) implies that 63.6% have no protective immunity to with stand challenges from ND virus. The lack of protective immunity in these

Polymerase chain reaction products (amplicons) and dyes (blue/green, Promega<sup>R</sup>) were mixed in ratio of 2:8. The gel was placed in an electrophoresis tank and set at a voltage of 120 V for 35 minutes. RNase free water was used as negative control while positive NDV obtained from Diagnostic Virology Laboratory, NVRI, Vom, was used as positive control. Amplicons generated were visualized with BIO-RAD Molecular Imager<sup>R</sup> U.S.A. Expected band of ND was considered positive at 280bp.

### Data analysis

The antibody prevalence for ND was calculated using the formula using the formula outlined by Bennette *et al.* (1991):

Where: prevalence = Number of serum samples positive x 100/ Total sera examined

## RESULTS AND DISCUSSION

The overall seroprevalence of ND of 36.4% found in this study (Table I) supports the fact that NDV is widespread in Nigeria. The seroprevalence of 55.7%, 54.9%, 52.7% (TABLE I) may not be unconnected with the experience of high chicken mortality experienced by farmers about two months ago in Yana, Dass and Jalam (Personal communication). The disperse nature of settlements in some parts of Udubo may likely be the reason for the lowest prevalence of 5.7%. That the prevalence was lower than 56.3% obtained in a previous study conducted within Bauchi Metropolis may be related to the many areas and large number of chickens sampled in this study.

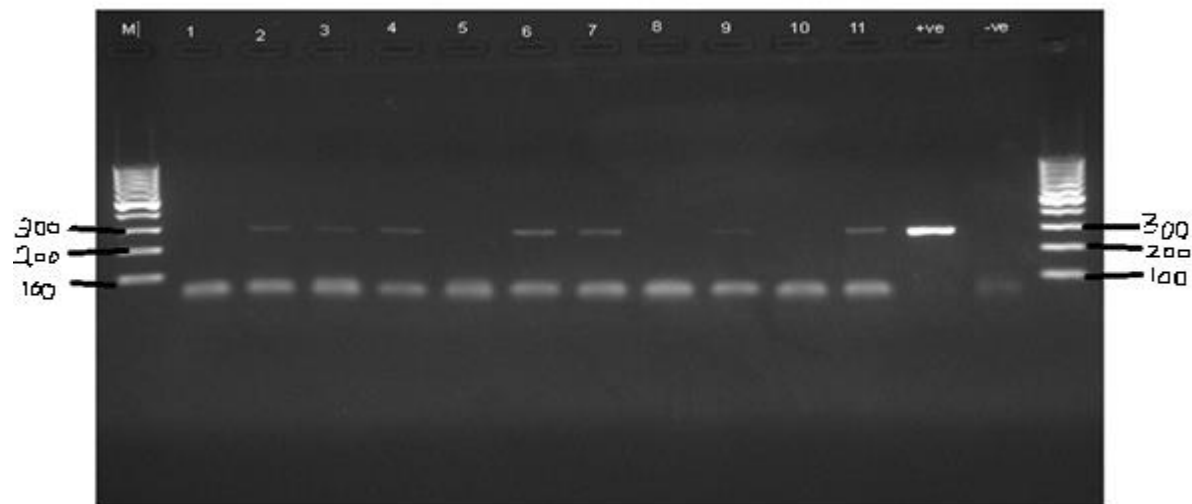
The prevalence of 36.4% obtained in this study is comparatively higher than 34.5% experienced in Yobe state (Sule *et al.* 2013) but lower than 38.8% obtained among local breed of domestic chickens in the neighbouring Jigawa state (Wakawa, *et al.* 2009) and 51.9% among local breed of domestic chickens in Plateau state (Musa *et al.* 2009).

chickens could result in great economic loss in the event of an ND outbreak. An immediate vaccination of such susceptible chickens is therefore advocated to prevent the likelihood of ND outbreak.

**TABLE I: prevalence of ND among village chickens in Bauchi state**

Villages	Number		Prevalence %
	Examined	Number positive	
Chinade	92	30	32.9
Dass	131	72	54.9
Jalam	93	49	52.7
Gongoro	135	32	23.7
Kafin- Madaki	132	42	31.8
Kutaru	127	53	41.7
Toro	110	41	37.3
Udubo	143	8	5.6
Yana	122	68	55.7
Total	1085	395	36.5

Detection of M gene in the present study by RT-PCR (Plate I) is a demonstration of infection by the virus in these chickens. The occurrence of positive result supports the use of the technique to identify NDV. Positive samples obtained in this study can further be subjected to pathotyping and phylogenetic studies which is an advantage that goes beyond the previous serological study.



**Plate I: RT-PCR product of M-gene, an expected product size of 280 bp were detected at lane 2(JC 21), 3 (JC 22), 4 (JC 23), 6 (JC 24), 7 (JC 26), 9 (JC27) 11(JC 29) from Jalam in Bauchi State, Nigeria.**

The Detection of NDV at 29.9% obtained in this study (TABLE II) supports the view that ND is

endemic in Nigeria. The presence of this virus among chickens represents a continual threat to

newly hatched susceptible chicks and to commercial poultry. The prevalence of 29.9% was lower than the 36.4% obtained from serological test perhaps due to our inability to detect any virus in Dass and Gongoro. The detection of the virus in the neighbouring State of Yobe State and other parts of the country suggests a wide distribution of NDV (Garba *et al.*, 2012; Echeonwu *et al.*, 1993; Snoeck *et al.*, 2009). The detection of NDVs and antibodies to

ND in domestic chickens in Bauchi State and many parts of Nigeria coupled with the need to improve the productivity of these chickens seem to hint at a reason to have a National programme of immunisation against this killer chicken disease. This could bring a lot of benefit to both the Veterinarians in terms of employment and to owners of these chickens who have need for more income and food security.

**TABLE II: Prevalence of Newcastle disease virus in nine communities in Bauchi State, Nigeria**

Community	Total cloacal swabs examined for Newcastle disease virus	Number of cloacal swabs positive for Newcastle disease virus by RT-PCR	Prevalence of Newcastle disease detection by (%)
Chinade	28	12	42.9
Dass	32	0	0.0
Gongoro	32	0	0.0
Jalam	30	18	60.0
Kafin Madaki	32	7	21.9
Kutaru	32	13	40.6
Toro	32	20	62.5
Udubo	31	6	19.4
Yana	32	8	25.0
Overall prevalence	281	84	29.9

That no virus was detected in Dass and Sade either signify no recent infection in such house holds or the primers used are not suitable for

viruses from those communities. Such anomalies are worthy of further investigation.

Combining seroprevalence and molecular detection of NDV in chickens could increase our knowledge of the distribution and level of protective immunity; and diagnosis of NDVs.

## CONCLUSION AND RECOMMENDATION

The seroprevalence of ND among village chickens in Bauchi State was 36.4%; NDVs were also detected from pooled Cloacal swab of the same chickens by molecular method was 29.9%. The study recommends the immediate vaccination of chickens in order to raise the immune status of village chickens to prevent the likelihood of ND outbreaks within these communities.

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