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Full Length Research Paper

A study on the occurrence of Newcastle disease virus antibodies in local and exotic chickens in Nigeria

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This present study was conducted to determine the sero-prevalence of Newcastle disease (ND) antibodies in both local and exotic chickens in Gwagwalada, Nigeria. Two hundred sera were randomly collected post slaughter from chickens and analysed by Haemagglutination (HI) and Haemagglutination Inhibition Test (HIT). Fifty structured questionnaires were distributed among poultry keepers, workers and other stakeholders to evaluate their level of awareness and impact of avian ND in the study area. Results revealed an overall sero-prevalence of 63.5%. Breed sero-prevalence was 54% and 73% for local and exotic chickens respectively. However, 46% local chickens and 27% of exotic chickens had no detectable antibodies. Statistical analysis of the avian ND antibody titres showed association between breeds of chickens [$P<0.05$; ($\chi^2=7.79$); df= 1)]. The questionnaire study further revealed high level of ND awareness and the adverse effects of ND virus in poultry. Local breeds were rarely vaccinated against ND virus as indicated by the respondents and low antibody titres detected. This study provides preliminary information on ND prevalence in exotic and local chickens in Gwagwalada. Hence, the need to conduct further researches on ND in the study area using molecular diagnostic techniques in order to affirm disease burden and impact especially amongst local chickens for the purpose of control.

Key words: Newcastle disease antibodies, sero-prevalence, haemagglutination, questionnaires.

INTRODUCTION

Newcastle disease (ND) is one of the most devastating poultry diseases in Nigeria. The disease has synonyms such as avian paramyxovirus infection, Pneumo encephalitis, Pseudo fowl pest, Ranikhet disease or Pseudoplague of fowl disease (CIDRAP, 2003). ND is an infectious disease of birds caused by the avian paramyxovirus serotype 1 virus (APMV-1) (OIE, 2000) which has four pathotypes namely, velogenic (highly virulent), mesogenic (moderately virulent), lentogenic (low virulence) and avirulent (Spradbrow, 1987). ND has been reported consistently from all continents worldwide (Munir et al., 2012). The epizootics of ND in poultry continue to occur in Asia, Africa, Central and South America while in Europe, sporadic epizootics occur (Naveon et al., 2013). The negative impact of the disease in both commercial and village rearing poultry production

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systems is of great significance especially in Africa (Nwanta et al., 2006; Mohamed et al., 2011; Rezaeianzadeh et al., 2011).

In Nigeria, poultry production is estimated at 137.6 million, with backyard poultry production constituting 84% (115.6 million) (FMA, 2006). This poultry production provides quality protein and financial income for rural families and the nation as a whole (Abubakar et al., 2008). ND has been associated with mild conjunctivitis and influenza-like symptoms with slight public health significance in human beings (Nelson et al., 1955). Annual economic losses in millions of dollars have been associated with the disease course (Susta et al., 2011) and heavy mortality patterns in poultry (Waheed et al., 2013). However, these continuous outbreaks have been poorly reported and demonstrated with un-robust laboratory diagnostic investigations in Gwagwalada Area Council. The paucity of information on ND status both in local and exotic chickens within this Guinea savanna zone premised this survey. This study aimed at establishing the sero-prevalence of avian ND virus antibodies in this study area with the view of quantifying the infection or disease burden and providing sustainable control and possible eradication measures.

MATERIALS AND METHODS

Study area

The study was conducted in Gwagwalada metropolis, Gwagwalada Area Council of the Federal Capital Territory, FCT Abuja. Gwagwalada is one of the six Area Councils of the Federal Capital territory, Abuja; alongside Abaji, Kuje, Bwari, Kwali and Abuja Municipal Area Council within this Guinea savanna zone. Gwagwalada covers an estimated land mass of 1043 km^2 and a population of 157,770 during the 2006 census (Anon, 2009), where the University of Abuja is located. Gwagwalada is located on geographical coordinates of 8°56′29″ North, 7°5′31″ East (3D Google Earth). The area is characterized by two seasons consisting of the raining season and the dry season. The dry season lasts between May and October with a uni-modal peak of rainfall in August. Gwagwalada Area Council is extremely hot in terms of temperature with a mean daily temperature of 31°C (Awowole and Francis, 2007). The high temperature has been suggested as a predisposing factor of Newcastle disease outbreaks (Njagi et al., 2010). This hot weather in Gwagwalada also premised the choice of this location for the survey (Figure 1).

Sample collection and processing

A total of 200 blood samples were collected post slaughter from both local chickens (N=100) and exotic chickens (N=100) (consists mainly of broilers) at the Gwagwalada market for ND virus antibody detection. Blood samples were collected in plane sample bottles that contain no anti-coagulant during slaughtering on a weekly basis between February and March, 2015. The samples were preserved at 45°C slants for sera separation at room temperature for two hours (OIE, 2012). The sera were then transferred into cryovials, labelled and stored at 4°C.The sera were later transported in ice parked flask for analysis at the Avian Influenza laboratory, Virology Unit, National Veterinary Research Institute (NVRI), Vom, Nigeria.

Laboratory analysis

Haemagglutination test

This test was conducted in accordance with the procedures outlined by OIE (2012). 0.025 ml of Phosphate buffered saline (PBS) was dispensed into each well of a plastic V-bottomed microtitre plate. 0.025 ml of the virus suspension (infective or inactivated allantoic fluid) was placed in the first well. For accurate determination of the Haemagglutination Assay (HA) content, this was done from a close range of an initial series of dilutions, 1/3, 1/5, 1/7…..1/20. Two fold dilutions of 0.025 ml volumes of the virus suspension were made across the plate. A further 0.025 ml of PBS was dispensed to each well. 0.025 ml of 1% (v/v) chicken Red Blood Cells (RBCs) was dispensed to each well. The solution was mixed by tapping the plate gently. The RBCs were allowed to settle to a distinct button for about 40 min at room temperature (20°C). Plate titling and observation for the presence or absence of tear shaped RBCs, streaming was used for determination of HA. The titration was read against the highest dilution giving complete HA (no streaming) which represents one HA unit (HAU) and was calculated accurately from the initial range of dilutions.

Haemagglutination inhibition test

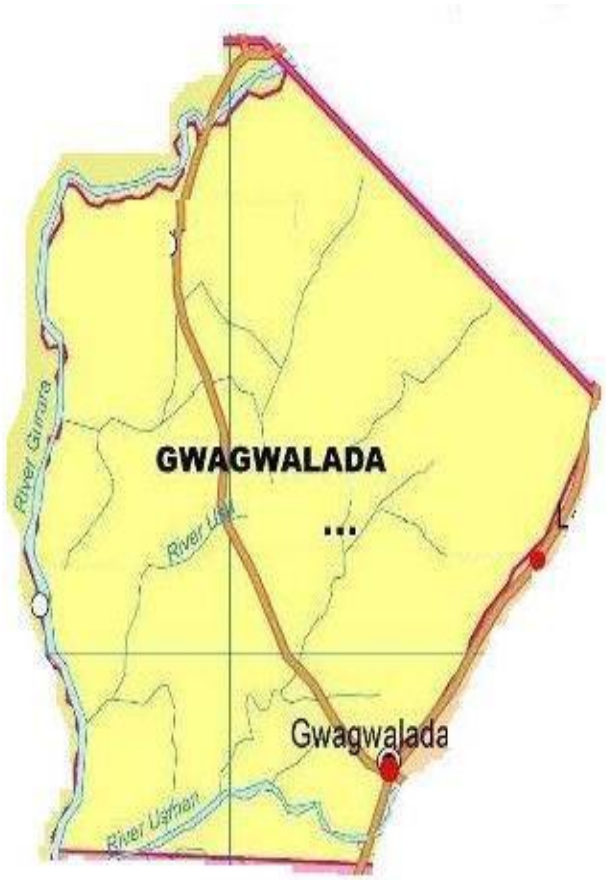
This was also conducted based on the OIE Terrestrial Manual (2012). About 0.025 ml PBS was dispensed into each well of a plastic V-bottomed microtitre plate and 0.025 ml of serum was later placed into the first well of the plate. Two fold dilutions of 0.025 ml volumes of the sera were made across the plate. Four HAU virus/antigen in 0.025 ml was added to each well and the plate was left for a minimum of 30 min at room temperature (20°C). Later, 0.025 ml of 1% (v/v) chicken RBCs was added to each well and mixed gently, the RBCs were allowed to settle to a distinct button for about 40 min at room temperature (20°C). The Haemagglutination Inhibition (HI) titre was read from the highest dilution of serum causing complete inhibition of four HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as the control wells (positive serum, virus/antigen and PBS controls) were considered to show inhibition. The validity of this result was assessed against a negative control. Serum titre greater than or equal to 1/4 (2^2 or $\log_2 2$) when expressed as reciprocal was considered as positive.

Questionnaire survey

Fifty questionnaires were distributed amongst poultry farmers, farm workers and undergraduate students in Faculties of Veterinary Medicine and Agriculture in Gwagwalada, F.C.T-Abuja. This was to establish the level of awareness of ND amongst stakeholders as well as the attitude and practice of poultry farmers during ND management. The indices assessed include ability to suspect the disease, clinical signs usually noticed, age mostly affected, season of outbreaks, mortality patterns, and farmers' attitude towards ethno-veterinary measures, routine treatment attempts employed by farmers, other veterinary interventions and vaccination protocols employed.

Statistical analysis

Statistical Package for the Social Science (SPSS) was used for the



...Gwagwalada

Figure 1. Map of Gwagwalada showing the location of sample collection at the Chicken Slaughter Market.

Source: Anon, 2009

Table 1. Sero-prevalence of Newcastle Disease antibodies in local and exotic chickens inGwagwalada.

Category of chickens	Local chicken	(%)	Exotic chicken	(%)
Number of positive	54	54	73	73
Number of negative	46	46	27	27
Total number tested	100	100	100	100

data analysis as adopted by Njagi et al. (2010). The numbers of positive antibody titers were expressed using descriptive statistics including frequencies and percentages. Data was also extrapolated as bar charts while Chi-square (χ^2) was used to compare the level of association between sero-prevalence of ND antibodies and the breeds of chickens. $P<0.05$ was significant.

RESULTS

Antibody titer

Out of 200 sera samples collected from 100 local and100 exotic chickens and analyzed using haemagglutination test and haemagglutination inhibition test, one hundred and twenty seven sera showed detectable antibody. The overall sero-prevalence of ND antibody revealed in this study was 63.5%. Breed distribution, showed sero-prevalence of 54% in local and 73% in exotic chickens. The results also revealed that 46% local chickens and 27% of exotic chickens showed no detectable antibodies for ND as indicated in Table 1. Statistical analysis of the avian ND antibody titres showed association between breeds of chickens [$P<0.05$; ($\chi^2=7.79$); df= 1)]. Figure 2 showed HI titer of 1.2 ($\log_2$ 2) as the most frequently found titer in the two breeds of chickens examined.

Questionnaire survey response

Questionnaire survey revealed the knowledge, attitude

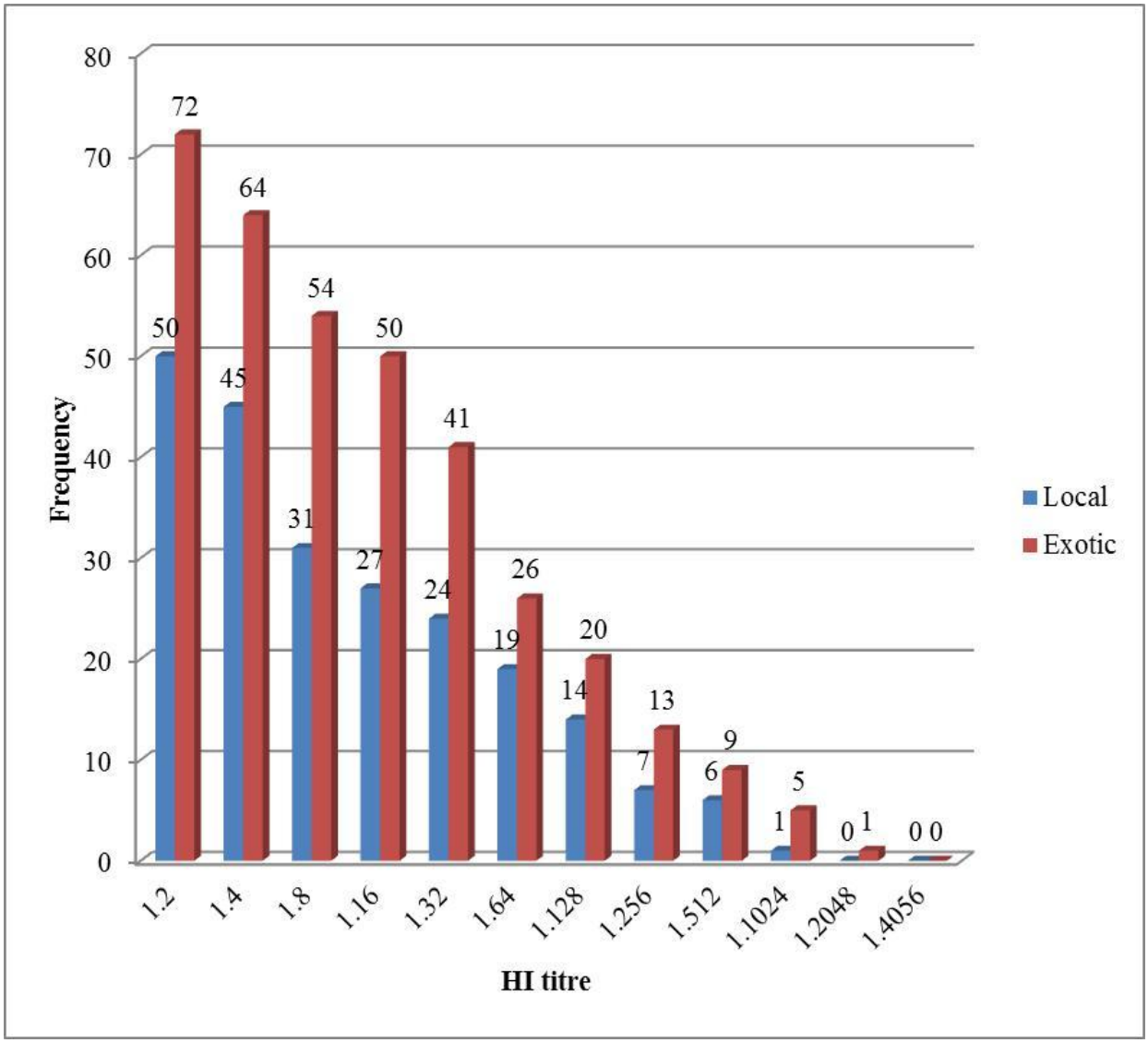


Figure 2. A bar chart showing ND antibody titres of both local and exotic chickens assayed in Gwagwalada.

and practice of farmers in the phase of ND outbreaks as well as the general awareness amongst Veterinary Medical Students. The questionnaire responses obtained is as shown in Table 2. This indicated 84% of respondents can suspect ND, and 68% of birds within the ages of 2-4 months were mostly affected. It also showed that exotic chickens (70%) are more susceptible to ND than local chickens (30%). Respondents (70%) reported ND outbreaks occur more during dry season than in rainy seasons. Most respondents (94%) had no idea on possible veterinary interventions for ND during outbreaks while 6% of respondents acknowledged that they used antibiotics as attempts for treatment of secondary bacterial infection. This questionnaire survey also revealed that 100% of the exotic breeds were vaccinated against ND while only 4% of the local chickens were exposed to ND vaccines. However, the knowledge on ND zoonotic importance in humans indicated low awareness response of 14%.

DISCUSSION

The results of this study conducted in Gwagwalada area council reveals the occurrence of ND antibodies in both local and exotic chickens. The finding in this study is in-line with previous report (Alders and Spradbrow, 2001). The overall ND sero-prevalence of 63.5% encountered in this study is lower than 97% recorded by Saidu et al. (2004) in Zaria, Nigeria. This variation may be attributed to physical factors including vaccination, vaccine failure or infection rates (Spradbrow, 1994; Anebo et al., 2014). Although, comprehensive history taking and laboratory analysis to differentiate ND antibodies associated with vaccination, vaccine failure or infection was not conducted in this study. The high prevalence of ND antibodies detected may therefore suggest the burden and endemicity of ND in the study area.

Sero-prevalence of 73% of ND antibodies recorded for exotic chickens in this study is higher than 22% ND

Table 2. Response of Newcastle Disease questionnaire survey administered to farmers andveterinary students in Gwagwalada.

Parameter	Response	Number of respondent	(%)
Ability to suspect ND	yes	42	84
	No	8	16
Age mostly affected	0-2months	12	24
	2-4months	34	68
	4-6months	4	8
Breed susceptibility	Exotic	35	70
	Local	15	30
Season of Outbreaks	Dry season	35	70
	Rainy season	15	30
Veterinary intervention	No idea	47	94
	Antibiotic therapy	3	6
Routine vaccination			
Local breed	Yes	2	4
	No	48	96
Exotic	Yes	50	100
	No	0	0
Zoonotic effects	Conjunctivitis	7	14
	No idea	43	86

antibody titres demonstrated in unvaccinated exotic birds by Oranusi and Onyekaba (1986) suggesting that the study birds were either previously vaccinated or were exposed to recent ND booster vaccinations prior to slaughter (Chandrasekar et al., 1988; Aldous and Alexander, 2001). This may have accounted for the high levels of protective antibody titres observed in these exotic chickens. Consequently, this observed prevalence may not reflect the true indication of ND in the study area. This would require further evaluation using other diagnostic methods.

The seroprevalence of 54% recorded in the local birds is in conformity with the findings of Adu et al. (1986) in Ibadan but higher than the 23.6% reported by Abraham et al. (2014) in Udu, Delta state using Haemagglution Inhibition Test. However, it was lower than the 73.3% prevalence reported by Ohore et al. (2002) using Enzyme Linked Immunosorbent Assay (ELISA) in Ibadan. The lower ND seroprevalence recorded in this study can be attributed to the low sensitivity of haemagglution test as compared with the more sensitive ELISA (Solano et al., 1986; Snyder, 1986). In addition, low ND antibody detection observed in some local birds could be attributed to absence of antibodies despite ND virus infection or presence of low un-detectable serum antibodies by haemagglutination test as previously reported by Adu et al. (1986). Although previous reports indicates uncommon vaccination practice in free ranged management system of local birds in Nigeria (Abdu et al., 1987), the sero-prevalence of 54% recorded amongst local birds in this study suggests exposure to ND infection in unvaccinated chickens as previously reported (Alexander, 1991). This none vaccination practice amongst local bird keepers could further enhance ND carrier status and possibility of re-infection (Adene et al., 1985) as well as disease spread to other in contact susceptible poultry especially the exotic chickens, ducks, geese (Ibrahim et al., 2005), domestic and wild scavenging birds such as pigeons, bats, doves and canaries (Bisalla et al., 2005). The questionnaire result indicates that most respondents were aware and could suspect ND to affect younger birds within the ages of 2 – 4 months. Most respondent also indicates ND outbreaks occur more frequently during dry season than in the rainy season. Vaccination history further reveals total and effective compliance with vaccination practice for exotic chickens as against the occasional use of vaccines in local chickens. However, ND zoonotic importance is not known as indicated by the responses.

In conclusion, this finding provides baseline data on the occurrence of Newcastle disease antibodies using HI and HIT tests in both local and exotic chickens in Gwagwalada, Abuja, Nigeria. The research also presents the low level of ND awareness in this study area.

Therefore, there is a need for continuous public awareness campaign programs especially amongst poultry farmers on effective ND control measures. Periodic antibody monitoring and disease evaluation in poultry flocks within the study area using molecular techniques is thus recommended.

Conflict of Interests

All authors re-affirm no conflict of interest during and after the conduct of this study.

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