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# Prevalence of Newcastle disease virus antibodies in sera and eggs of helmeted guinea fowls (*Numida meleagris galeata pallas*) in Borno and Yobe States, Nigeria

SU Hassan[1]*, AD El-Yuguda[2], HI Gambo[1], SS Baba[2], AG Ambali[3] & IO Igbokwe [1]

1. *Department of Veterinary Pathology, University of Maiduguri, Nigeria,*
2. *Department of Veterinary Microbiology and Parasitology, University of Maiduguri, Nigeria,*
3. *Department of Veterinary Medicine, University of Maiduguri, Nigeria*

*Correspondence: Tel.: +234 8038047109, E-mail: shehuhassandaudawa@yahoo.com

**Abstract**

The seroprevalence and maternal antibody profiles to Newcastle disease virus infection of guinea fowls were studied using haemagglutination inhibition (HI) test, in Borno and Yobe States of Nigeria. Of 822 sera and 354 egg yolk extracts tested, 327 (39.8%) and 242 (68.4%) were positive for NDV antibodies respectively. The seroprevalence was significantly higher (P< 0.05) in the dry (47%) than in the rainy (32.8%) seasons. Maternal antibodies in egg yolk extract (sampled during the rainy season), were significantly (P< 0.05) higher (68.4%) than the seroprevalence. The frequency distribution of antibody titers were skewed with 79.8% and 63.2 % of sera and egg yolk respectively having reciprocal antibody titres ≤ 20. The geometric mean antibody titre was higher (P< 0.05) in the dry season (3.7±2.0) than in the rainy season (2.5 ±1.7). The geometric mean titre was also higher (P<0.05) in egg (8.9±2.5) than in sera (3.1±1.9). These results showed that Newcastle disease virus is enzootic among guinea fowls in Borno and Yobe states, especially in the dry season. The geometric mean titer of antibodies from egg yolk (8.9) was below the protective reciprocal titres ≥35.4, suggesting the need for control measures immediately after hatching. In addition, birds with titers ≤ 35.4 are partially immune and may shed the virus without a clinical disease when infected thereby becoming a risk to in-contact birds.

*Keywords*: Egg, Guinea fowls, Maternal antibodies Newcastle disease, Seroprevalence,
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## Introduction

The helmeted guinea fowl is a semi- domestic to wild galiform indigenous to West Africa and is favoured for its high quality eggs and meat (Ayeni, 1983). About 6 million guinea fowls (13% of the national total) were estimated to be found in Borno and Yobe states, raised mostly on a free range scavenger system by nomadic herds men and villagers as income supplements (Hassan, 2007).

Newcastle disease (ND) is an important viral disease primarily of the chicken, as well as other species of domestic and wild birds. It is characterized by a high morbidity and mortality (Alexander *et al*., 2012). Outbreaks of Newcastle disease in the guinea fowl have been reported in parts of the country (Haruna *et al.*, 1993), and elsewhere (Mishra *et al*., 2001). Recently, an outbreak in guinea fowls was reported in Maiduguri, Borno state (Hassan *et al.,* 2014). Serological surveys in guinea fowls for haemagglutination inhibition (HI) antibodies against Newcastle disease virus (NDV) have indicated an increasing prevalence of the virus around Zaria in northern Nigeria (Saidu *et al*., 2004), and a similar trend was earlier reported in Borno and Yobe states (Ambali *et al*., 2000). This study is a follow up to assess changes in seroprevalence and evaluate maternal antibody levels against NDV among guinea fowls in Borno and Yobe states.

## Materials and Methods

*Study area*

The study was conducted in Maiduguri (Borno state) and Potiskum (Yobe state), in northeastern Nigeria between $9^0$ and $13^0$ N and $11^0$ and $15^0$ E. The climate is semi-arid with two seasons; a rainy season (June to October) and a dry season (November to May ). The avrage annual rainfall is

650mm (Ishaku & Majid, 2010). However some areas may have as low as 500 mm (NBS, 2012).

*Sample collection*

A total of eight hundred and twenty-two (n=822) guinea fowl sera were collected from poultry slaughter slabs in Maiduguri and Potiskum in Borno and Yobe states respectively. These urban centers represented collection points for the surrounding countryside. Blood was collected at slaughter in plain tubes, kept slanted for 3-4 hours at room temperature (35-40 $^0$C) to clot. Subsequently the serum was harvested from each tube with a suction micropipette and stored in small plastic serum bottles in a deep freezer (Thermocool®, Nigeria) at -20 $^0$ C until the time of analysis. Three hundred and fifty four (n =354) fresh guinea fowl eggs were purchased from farmers in batches of 4-5 from 71 points in the rainy seasons. Antibodies were extracted from the egg yolk extract (EYE) by the method of Piela *et al.* (1984).

*Serology*

Newcastle disease virus antibodies were detected in sera and EYE by HI test using the method of Allan *et al. (*1978) as described by Hassan (2007).

*Data analysis*

The skewed frequency distribution of reciprocal antibody titers was normalized by log transformation after which the geometric mean titer (GMT) and standard deviation (SD) were calculated (Petrie & Watson, 2006).

$GMT = \text{antilog}_{10}\{1/n\ (\sum f_1 \log_{10} X_i)\}$.

Where n= number tested, $X_i$ = the reciprocal dilution and $f_i$ = frequency.

$SD = \text{Antlog}_{10} \sqrt{\frac{1}{n-1}\sum\{f(log_{10}X - \log_{10}X)^2\}}$.

The GMT values were compared for seasonal differences and titer differences between sera and EYE by a 2-tailed student's t- test, while seasonal differences were compared by chi-square analysis using computer software (GraphPad Instat Inc. San Diego, California 1998 version, www.GraphPadInStat.com).

**Results**

The prevalence of antibodies to NDV in sera and EYE of helmeted guinea fowls in Borno and Yobe states are presented in table 1. Three hundred and twenty seven (39.8%) sera were positive for HI antibodies against Newcastle disease out of the 822 tested. The seasonal distribution of positive samples showed significantly ($p< 0.05$) higher prevalence in the dry season (45.7%) than the rainy season (32.8%). Also, the prevalence of antibodies in EYE (68.4%) was significantly higher ($p< 0.05$) than that of the sera.

The GMT values for sera and eggs are presented in table 2. The frequency distribution of antibody titers were skewed to lower titers with 79.8% and

**Table 1:** Prevalence of antibodies against Newcastle disease virus in sera and egg yolks of helmeted guinea fowls in Borno and Yobe States, Nigeria

| | Seasonal distribution of serum antibody titer | | | EYE antibody titer (Rainy season) |
|---|---|---|---|---|
| | Rainy | Dry | Total | |
| Number tested | 378 | 444 | 822 | 354 |
| Number (%) positive | 124 (32.8)[a] | 203 (45.7)[a] | 327 (39.8)[b] | 242(68.4)[b] |

Matched superscripts are significantly ($p < 0.05$) different by chi-square statistics

**Table 2**: Frequencies of antibody titers in sera and egg yolks of helmeted guinea fowls and their geometric mean titers (GMT) with standard deviations (SD)

| Reciprocal of antibody titer | Frequency of antibody titer | | | |
|---|---|---|---|---|
| | Seasonal distribution in serum | | | |
| | Rainy | Dry | Total | Egg yolk |
| 10 | 61 | 100 | 161 | 83 |
| 20 | 48 | 52 | 100 | 70 |
| 40 | 12 | 39 | 51 | 44 |
| 80 | 3 | 9 | 12 | 25 |
| 160 | 0 | 3 | 3 | 17 |
| 320 | 0 | 0 | 0 | 2 |
| 640 | O | 0 | 0 | 1 |
| GMT ± SD | 2.5 ± 1.7[a] | 3.7 ± 2.0[b] | 3.1 ± 1.9[c] | 8.9 ± 2.5[d] |

Unmatched superscripts are significantly ($p < 0.05$) different by Student's t-test

63.2% having reciprocal antibody titres ≤ 20 in sera and EYE respectively. Serum GMT was significantly higher in the dry (3.7 ± 2.0) than the rainy season (2.5 ± 1.7). The GMT was also significantly higher in EYE (8.9 ± 2.5) than in sera (3.1 ±1.9).

**Discussion**

The results of the study showed serological evidence of NDV infections in guinea fowls in Borno and Yobe states of Nigeria. The 39.8 % seroprevalence of NDV infection among guinea fowls was higher than the 23.9% reported earlier by Ambali & Aliyuda (1992). This increase supports report of similar trends of increasing seroprevalence in Zaria (Saidu *et al*., 2004). The enzootic nature of NDV in Nigeria, the high prevalence reported in the study area among free-range scavenger and commercial chicken flocks (Ambali *et al*., 2000) and the lack of vaccination and other control measures for free-range poultry including guinea fowls may explain the increasing prevalence. Guinea fowls in the study area were probably infected with NDV during their regular contact with chickens in the extensive free-range scavenger system they share. The significantly higher prevalence observed during the dry season has also been reported for free range scavenger chicken (El Yuguda *et al*., 2009). The spread of the disease was more extensive in the dry season possibly due to windy conditions that prevail during the hamatan, in which the virus may be transported by aerosol and dry faecal dust to distant places (Hassan, 2007).

A reciprocal titre of ≥ 35.4 was reported to be protective against NDV infection (Allan *et al.*, 1978). Most of the sera (78.9%) had reciprocal titres ≤ 20. This class of birds is partially immune and can succumb to a velogenic NDV infection or maintain a subclinical disease during which active excretion of the virus may occur making them a possible source of infection for in-contact birds (Parede & Young, 1990; Wambura, 2010; Kapezynski *et al*., 2013).

Majority of the guinea fowl eggs had maternal antibodies against NDV in the EYE, with a GMT that was significantly higher than that of the sera, but was not sufficient (≥ 35.4) to protect the keets when they hatch. Outbreaks of Newcastle disease could therefore occur within the first week of life.

In conclusion, there was serological evidence of increasing, NDV infection in guinea fowls from Borno and Yobe states. The antibody titers in both sera and eggs were not protective, but could mask a subclinical infection, which may lead to virus shedding with considerable risk to in-contact birds. It is therefore recommended that guinea fowls should be protected by vaccination in the first week of life. Biosecurity should be improved for guinea fowls and other poultry on free-range management

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