Research Article

# Evidence of Antibodies to H5 Subtype Avian Influenza Virus in Commercial Layer Farms in Jos, Nigeria

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**Abstract** | Highly pathogenic avian influenza (HPAI) devastated the poultry industry in Jos north and south local government area (LGAs) of Plateau State, Nigeria during the 2006 epidemic. A combination of modified stamping out and improved biosecurity was used to eradicate the disease. This study was conducted in 2011 with the aim of determining the presence of antibodies to $H_5$ subtype avian influenza (AI) virus in layers in previously affected and non-affected farms in two local government areas (LGA) of Plateau State. Records of the number of farms that were depopulated, and laboratory confirmation of HPAI in farms that had birds' positive for AI $H_5N_1$ virus during the 2006 outbreaks was use for this study. Twenty farms that had birds that suffered from the 2006 epidemic of HPAI were randomly selected from the list of confirmed HPAI positive farms and referred to as affected farms. Another 20 farms that were located close to the affected farms and had bird during the 2006 AI epidemic but did not suffer from the disease were selected and referred to as non-affected farms. Blood samples were collected from 10 layers in each of the previously HPAI affected and non-affected farms and serological assay was done using haemagglutination inhibition (HI) test and enzyme linked immunosorbent assay (ELISA). An overall sero prevalence of 31.6% (ELISA) and 69% (HI) and an overall mean HI titre of 9.86±0.035 $log_2$ was recorded for the affected and non-affected farms. The non-affected farms had a higher prevalence (38.3%) than the affected farms (24.9%), while Jos South LGA had a higher prevalence (34%) than Jos North LGA (30%) using ELISA. Antibodies to $H_5$ using both ELISA and HI test were present and in high concentration in layers in both affected and non-affected farms.

**Keywords** | Avian influenza, Prevalence, H5 subtypes antibodies, Jos, Nigeria

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## INTRODUCTION

Avian influenza (AI) commonly referred to as 'bird flu' is an infectious disease of birds caused by influenza virus 'A' genus of the *Orthomyxoviridae* family (Kuiken et al., 2006). It is a highly contagious acute viral disease primarily of domestic and wild free-flying birds (Abdu, 2007). The *Orthomyxoviridae* family of RNA viruses consist of three types, influenza virus A, B, and C (Fouchier et al., 2005; Gottfried, 2005; Wong and Yuen, 2006). Only influenza A virus is known to infect birds (Bello et al.,

2008). An important feature of AI epidemic and virus is the wide range of transmission among species of birds (Sa'idu et al., 2008). The disease also affects mammals (man, pigs, horses, cats, seals and whales) (Alexander, 2000). Avian influenza has a worldwide distribution which includes Africa (NADIS, 2006). In Nigeria, the first reported outbreak of HPAI was in a commercial poultry farm near Kaduna town, Kaduna State; Nigeria in February, 2006 (Adene et al., 2006; Bello et al., 2008). Prior to this outbreak, there has been no evidence suggesting the presence of HPAI in the country (Adene et al., 2006). As at April 2006, HPAI had spread to poultry in 12 of 36 states and the Federal Capital Territory of Nigeria. As at May, 2007, 24 of the 36 states in Nigeria had documented HPAI $H_5N_1$ outbreak in poultry (Monne et al., 2008). Since the successful control of HPAI in Nigeria in 2008, there has not been any serological survey to determine if there are antibodies to HPAI virus in farms in Plateau State that reported the disease. Serology was used for this study due to its simplicity and availability of research kits. Therefore, the aim of this study was to conduct a serological survey using ELISA and HI for AI $H_5$ antibodies in commercial layer farms in two LGAs of Plateau State where outbreaks were recorded in 2006.

## MATERIALS AND METHODS

### Study Area

Jos city is located between Lat. 9° 56'N and 8° 53'E. It has a population of about 900,000 residents and a poultry population of 400,689 commercial birds (NADIS, 2006; NPC, 2006). Jos has a near temperate climate with an average temperature of between 18°C and 22°C. The people of Plateau State are predominantly farmers. Jos is divided into three LGAs of Jos North, Jos South, and Jos East and the study was carried out in Jos North and Jos South LGA (Plateau State Government, 2003).

### Sample Size

The sample size was determined using the formula by Thrustfield (1995):

$$N = \frac{Z^2pq}{d^2}$$

Where

N = Sample
Z = The appropriate value from the desired confidence (1.96)
p = Expected Prevalence
q = 1- prevalence
d = Allowable error

Therefore, using the prevalence 18.5% earlier determined in a previous study by Durosinlorin (2008).

$$N = 1.96^2 \times \frac{0.185 \times (1 - 0.185)}{0.05^2}$$

$$= \frac{3.842 \times 0.185 \times 0.815}{0.0025}$$

$$= 231.71 =\sim 232 \times 2 = 464$$

The sample was increase to 400 in order to increase the level of precision and minimize errors in the process of handling samples.

### Farm Selection

Record of HPAI outbreaks and the control activities implemented in 2006 in Plateau state, was obtained from the AI Control Desk Officer in Plateau State. Data containing the confirmatory diagnosis of the outbreaks was also obtained from the Head of Diagnostic Laboratory, National Veterinary Research Institute, Vom. Forty seven farms were depopulated with twenty two (47%) of the farms testing positive for $H_5N_1$ virus in Jos North and South LGA of Plateau State. Twenty farms that had bird that suffered from HPAI during the 2006 HPAI epidemic and depopulated were randomly selected from list of farms that reported the outbreak of HPAI in 2006 and were referred to as affected farms. Another set of 20 farms that were located close to the affected farms and had bird during the 2006 HPAI epidemic but do not report HPAI were referred to as non-affected farms. Approval to undertake the survey on each farm were consented verbally by the farm owners.

### Collection and Processing of Samples

The study was carried out in 40 commercial layer farms between August and September 2011. Blood samples were collected from 10 birds through the wing vein in each of the AI affected and non-affected farms using simple random sampling method. The blood was kept at 4°C in a slanting position to allow for clotting. Sterile sample bottle was labelled with an acronym number, place, and date of collection after which serum was separated by centrifugation at 447.2xg for 5

minutes and were kept in a separate vial and stored at -20°C until tested for H5 subtype antibody.

### Avian Influenza Antigen and Antiserum

Avian influenza type A H5 antigen and AI type A H5 positive serum; batch N°85 (made by the NVRI, Vom, Nigeria) was obtained from the NVRI, Vom, Nigeria and was used for the detection of antibodies to $H_5$ subtype.

### Serological for Avian Influenza H5 Antibodies

One percent Red Blood Cells (RBCs) was first prepared from blood collected from a 5 day old chick according to the standard protocol described by OIE (2004) and used as indicator. The titre of the antigen was first determined by haemagglutination test (HA) as previously described (OIE, 2004). Antibodies to AI were detected by the haemagglutination inhibition (HI) test as previously described (OIE, 2004). The HI titre considered was the highest dilution of serum causing complete inhibition of 4 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 were considered to show HI.

### Enzyme Linked Immunosorbent Assay for Avian Influenza Antibodies

The enzyme linked immunosorbent assay (ELISA) technique was carried according to the methods described by AniGen AIV Ab ELISA test kit for the detection of AI antibody. To perform the assay, the test kit contains antigen coated micro-assay plate, adhesive plate sealer, positive and negative controls, washing solution, enzyme conjugate, conjugate diluents, stopping solution and substrate A and B. Two strip wells were prepared for each sample, positive and negative controls and 50 µl of each of the positive and negative control solutions were added to each of the two strip wells for each control. Also, 50 µl of each sample were added to their respective strip wells. To each strip wells, 50 µl of anti AIV antibody-HRP (1:100 dilutions in the conjugate diluents) was added; the micro plates were covered with adhesive plate sealer and well content properly mixed using a vibrating mixer. The micro plate content was incubated for 30 minutes at 37 ± 1°C after which the wells were washed six times with 350 µl of washing solution and all liquid in the wells were aspirated. The micro plates were further incubated for 10 minutes at room temperature after dilution of 100 µl of substrate solution into each well. After 10 minutes incubation, 100 µl of stopping solution was added into each well and absorbance of each well was measured using a bichromatic spectrophotometer at 450 nm with a reference wavelength of 630 nm. All readings were taken within 1 hour at the end of the assay. The percentage inhibition (PI) value was calculated for each sample using the formula: PI value = {1- (OD sample/mean OD negative control)} x 100.

### Data Analyses

The data obtained from the serology were analysed by descriptive statistic using the Statistical Package for Social Sciences (SPSS) software package, version 20.0 (SPSS Inc. Chicago, IL, USA). Data generated on antibodies were expressed as mean ± standard error of the mean (x ± S.E.) and reduced into tables. The frequency, mean, standard error of mean and chi square values were calculated. Values of $p<0.05$ were considered significant.

## RESULTS

A total of 377/400 (94.3%) blood samples were tested for AI $H_5$ antibody from apparently healthy birds in 40 commercial layer farms in Jos North and South LGAs of Plateau State. Of the farms sampled, 25 (62.5%) are located within Jos North LGA, while 15 (37.5%) were located in Jos South LGA. The overall sero prevalence for AI antibodies (AIAbs) in both the affected and non-affected layer farms in the two LGAs was 31.6% by ELISA and 69% using HI test. Sero prevalence of 24.9% and 38.3% were determined for affected and non-affected farms respectively using ELISA (Table 1). Jos South LGA recorded higher (34%) sero prevalence than Jos North LGA (30%) using ELISA (Table 2), with an overall HI mean titre of 9.86 ±0.035 $\log_2$. However, 1.8% had an antibody titre of ≤ 7$\log_2$. Among the birds that had antibody against AI virus, 97% had an antibody titre ≥7$\log_2$. The overall mean HI antibody titre in the affected farms in the two LGAs was 4.30±0.49 and 97.4% of the samples had an HI antibody of ≥7$\log_2$. The mean HI antibody titre in non-affected farm was 5.56 ±0.53, and 97.5% had an HI antibody of ≥7$\log_2$ (Table 3). There was no significant difference ($p>0.039$) in the overall mean HI antibody titre between the flocks in Jos North and Jos South LGA which were 9.86 ± 0.044 $\log_2$ and 9.86 ± 0.059 $\log_2$ respectively (Table 4 and 5).

**Table 1**: Prevalence of avian influenza H5 antibody of chickens using ELISA in affected and non-affected commercial poultry farms inarea of Jos North and South Local Government.

| Farm category | No. of Farms | Total no. of samples obtained | No. of positive samples | Prevalence (%) |
|---|---|---|---|---|
| Affected | 20 | 189 | 47 | 24.9 |
| Non affected | 20 | 188 | 72 | 38.3 |

X2 = 7.87, df = 1, p≤ 0.005

**Table 2**: Prevalence of avian influenza H5 antibody of chickens using ELISA in commercial poultry farms in Jos North and South local government areas of Plateau state

| S/N | LGA | Total no. of samples obtained | No. of positive samples | Prevalence (%) |
|---|---|---|---|---|
| 1 | Jos North | 233 | 70 | 30 |
| 2 | Jos South | 144 | 49 | 34 |

## DISCUSSION

The overall sero prevalence of 31.6% AI H5 antiboies in commercial layer farms in Jos North and South LGAs of Plateau State is higher than 12.9% previously reported by Wakawa et al. (2012) and 18.1% by Durosinlorun et al. (2010) in similar studies conducted in Kano and Kaduna States, Nigeria respectively. Also, the overall sero prevalence recorded in this study is higher than that (28.7%) reported by Tombari et al. (2013) in a study conducted in commercial poultry farms in Tunisia. However, the sero prevalence recorded on LGA bases; Jos North (30%), and Jos South (34%) Nigeria was lower, than that recorded in same study by Tombari et al. (2013) in Tunisia where they reported a prevalence of 47.7% in Tunis, 45.7% in Nebeul, and 41.3% for Sfax.

The results of the serological test conducted on the affected and non-affected layer farms in the two LGAs showed a high prevalence rate and mean titre for AI antibodies. Antibody titre of ≥7 $log_2$ is indicative of either a recent booster vaccination or natural infection when compared with the minimum protective antibody titre of 4.0 $log_2$ recommended by OIE (2004). During the 2006 HPAI epidemic, affected farms reported incidence of the disease to AI desk office and blood samples were taken from the farms to test for AI virus.

Blood samples that were positive to AI virus where traced to the corresponding farm and depopulation were done thereafter and farmers were advised not to stock their farms until after 6 months. But because

**Table 3**: Avian influenza prevalence and mean antibody titre in commercial poultry farms in Jos North and South local government areas of Plateau State.

| Farm category | Mean avian influenza antibody titre ± S.E ($log_2$) | Avian influenza antibody titre < 7$log_2$ (%) | Avian influenza antibody titre ≥7$log_2$ (%) | Prevalence (%) |
|---|---|---|---|---|
| Affected | 4.30 ± 0.49 | 6.5 | 97.4 | 97.4 |
| Non affected | 5.56 ± 0.53 | 0.5 | 97.5 | 99.5 |
| Over all | 9.86 ± 0.035 | 7 | 64 | 68 |

**Table 4**: Avian influenza prevalence and mean antibody titre in commercial poultry farms in Jos North local government area of Plateau State.

| Farm category | Mean avian influenza antibody titre ± S.E ($log_2$) | Avian influenza antibody titre < 7$log_2$ (%) | Avian influenza antibody titre ≥7$log_2$ (%) | Prevalence (%) |
|---|---|---|---|---|
| Affected | 4.30 ± 0.49 | 6.5 | 97.4 | 97.4 |
| Non affected | 5.56 ± 0.53 | 0.5 | 97.5 | 99.5 |
| Over all | 9.86 ± 0.035 | 7 | 64 | 68 |

**Table 5**: Avian influenza prevalence and mean antibody titre in commercial poultry farms in Jos South local government area of Plateau State.

| Farm | Mean avian influenza antibody titre ± S.E ($log_2$) | Avian influenza antibody titre < $7log_2$ (%) | Avian influenza antibody titre ≥$7log_2$ (%) | Prevalence (%) |
|---|---|---|---|---|
| Affected | 9.83 ± 0.116 | 1.2 | 42.2 | 43.4 |
| Non affected | 9.87 ± 0.067 | 2.8 | 95.8 | 95.8 |
| Over all | 9.86 ± 0.059 | 4 | 51.5 | 53 |

there was no supervision to ensure that the farm is properly disinfected and remained closed for the stated period, most of the farmers might have restocked their farms. These could be the reason for detecting antibodies in the affected farms 5 years after the epidemic, because subsequent batches of the birds in the farm must have develop antibodies to AI virus from the preceding batches of the bird in farm. Similarly, in other to avoid their farms being depopulated with little or no compensation, there were reports of sick birds being sold from the affected and non-affected farms during the 2006 HPAI epidemic. This action could equally be responsible for the high AI antibody titre reported from the non-affected farms, because the birds in the non-affected farms might have had AI virus with or without the farmers knowledge and thus; do not report to the appropriate authorities.

There is also an unconfirmed report of illegal vaccination of poultry against AI in the two LGAs despite government ban on vaccination against AI in Nigeria. The high probability of vaccination could be an attempt by the farmers to protect their flocks from the AI epidemic that arose from the experience of 2006 AI outbreak in the state. Indication that inactivated oil emulsion AI vaccines were used in commercial layer farms in Plateau State could have a negative implication due to the fact that some scientists suggested that vaccinating flocks might pose a risk of transmitting AI virus to other flocks (Cardona et al., 2006). Long-term circulation of AI virus in vaccinated population may result in both antigenic and genetic changes in the virus as was reported to have occurred in Mexico (Escorcia et al., 2008). The presence of antibody against AIV could pose serious consequences as LPAI can easily mutate to HPAI as reported by Capua and Marangon (2000); Capua and Alexander (2004) in some parts of the world. Vaccination against AI may result in shedding the virus to the environment because vaccines may not completely prevent infection and shedding of the virus by some birds which may lead to virus reassortment with unpredictable consequences (Beard, 1998). In fact, vaccines are often and continuously rendered obsolete as the virus undergoes antigenic drift and shift, however inactivated oil emulsion vaccines have been reported to be effective in reducing mortality and preventing disease both in chickens and turkeys (Beard, 1998).

The high prevalence of AIAbs will increase the cost of surveillance for Nigeria and at the same time bring a setback for the country considering her effort in trying to be certified AI free country. It was concluded that antibodies to AI $H_5$ subtype were present in layer farms in both affected and non-affected commercial farms in Jos North and South LGAs. As a follow up, the AI virus should be isolated from the layers and determine the neuraminidase subtype in other to ascertain the actual virus type. It is also important that nationwide active surveillance of AI be conducted previously affected AI farms to know the true status of the disease in Nigeria.

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