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Research Article
Sero-Prevalence of Avian Influenza in Poultry in Kogi State, Nigeria

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Abstract

A survey was carried out to determine the seroprevalence of avian influenza (AI) in poultry from 12 Local Government Areas (LGAs) of Kogi state. A total of 779 sera were analyzed for AI (H5) antibodies by haemagglutination inhibition test with overall seroprevalence of 4.2% and mean antibody titre of 8.42 ± 0.254 $\log_2$. The seroprevalence of AI (H5) among the LGAs was highest in Kabba/Bunu LGA with 12.3% while it was zero in Ajaokuta and Okene LGAs. The seroprevalence of AI (H5) in the sampling units was highest for backyard poultry with 4.5% and lowest for rural poultry with 3.8%. Using enzyme link immunosorbent assay, the same sera were analyzed for AI nucleoprotein antibodies with overall seroprevalence of 30.8%. The seroprevalence of AI nucleoprotein in the LGAs was highest for Mopamuro LGA with 41.8% and lowest for Adavi LGA with 18.6% while among the poultry species it was highest in chicken with 31.6% and zero in duck. Also, among the sampling units the seroprevalence of AI nucleoprotein was highest in rural poultry with 35.0% and lowest in backyard poultry with 26.5%. The presence of subtype specific (H5) and type A influenza antibodies in poultry in the surveyed area indicated a previous exposure to the AI viruses. The detection of AI (H5) antibodies was more in backyard poultry and might be due to vaccination against HPAI (H5N1) by poultry farmers in Kogi state. It is recommended that further surveillance for AIVs and subtype characterization of AIVs should be carried out.

Key words: Avian influenza antibodies, backyard poultry, rural poultry, haemagglutination inhibition test, ELISA

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

There had been reports of the detection of AI antibodies of H1N1 and H5N1 subtypes in pigs and poultry before the maiden outbreak of HPAI H5N1 in Nigeria on 8 February 2006 (Adeniji *et al*., 1993; Owoade *et al*., 2002). The AI infection once established in a region is difficult to eradicate especially in countries that undertake vaccination of poultry as control policy such as Egypt and China (FAO., 2011; Wakawa *et al*., 2012). Teru *et al*. (2012) carried out surveillance in wild birds from 8 states across Nigeria and identified species which could potentially act as links for spill over infection from wild bird reservoirs to poultry due to the reoccurrence of HPAI in two Nigerian states in 2008 after a long absence. Recently, there are reports of HPAI outbreaks in poultry farms and LBMs in about 18 states of Nigeria, speculated to be from Wild birds (Vetline Newsletter, 2015).

In Nigeria and may be most part of Africa, smallholder rural poultry farmers source their stock for rearing from the live bird markets (Ameji, 2010). The live bird market is an area of high risk of diseases and has been reported to play a role in AI transmission (Webster, 2004; Pagani *et al*., 2008; Aye, 2010). The high risk is associated with the mixing of poultry of different ages and from different places in the live bird market where pathogens may be shared before they are sold and carried to different places for household slaughter, sacrifice or rearing (Ameji, 2010). The LBM provides the platform for interaction among different poultry species and some wild birds before they are sold out. Hence, the live LBM can be a niche and disseminating point for contagious poultry diseases (Webster, 2004; Pagani *et al*., 2008).

Surveillance helps in effective control of AI especially in countries that had adopted eradication policy by providing information on the status of AI introduction and spread in poultry in a given area. Presently, there is no Government funded active surveillance for AI in Kogi state and the institutionalized disease reporting system is weak with no feedback disease control policy that is needed for improved poultry production. In an earlier study, the presence of AI antibodies was detected by Agar Gel Precipitin Test (AGPT) in chickens from 6 LGAs of Kogi state (Ameji *et al*., 2011). It became imperative to design the present study to further monitor and determine the seroprevalence of AI in poultry from 12 LGAs in Kogi state, Nigeria.

MATERIALS AND METHODS

Study area: The study area is Kogi state which lies between latitude 6°44'-7°36'N and Longitude 7°49'-8°27'E situated at a height of about 789 km above sea level and covering a land area of 29,833 km^2 (Fig. 1). The state is bordered by FCT and Niger state on the North, Benue and Nasarawa states on the East, Ekiti and Kwara states on the West, Edo, Anambra and Enugu states on the South.

The vegetation of Kogi state is guinea savannah on the North and a belt of rain forest on the southern fringe with rivers Niger and Benue passing through the state, which later converged at a point to form a confluence. The annual rainfall ranges from 1100-1250 mm starting from April to October (KGSADP., 2009).

Kogi state has a total of 21 Local Government Areas (LGAs) that are grouped under three senatorial districts based on cultural and geopolitical similarities. The human population of the state is 2,099,046 with the major economic activities of the people being farming, fishing and trading (KGSADP., 2009). The poultry population is estimated to be 3,685,211 with 91.5% being rural or backyard poultry and rest being commercial poultry (Adene and Oguntade, 2006).

Sample size

Sample size for avian influenza survey in rural poultry: The sample size for the study was determined using the formula by Cohen (1988):

$$N = \frac{Z^2Pq}{L^2}$$

Where:

Z = 1.96

P = Prevalence of AI = 18.7%

q = 1-P = 1 – 0.187 = 0.813

L = Measure of the confidence interval = allowable error (5%) = 0.05

$$N = \frac{(1.96)^2 \times 0.187 \times 0.813}{(0.05^2)} = 233.6 = 234$$

Sample size for AI survey in poultry from the 3 sampling units = 234×3 = 702. Prevalence of AI (P) is reported by Ameji (2010).

Sampling method for poultry from backyard farms, live bird markets and rural households: Multistage, simple random and convenient sampling methods were used. Multistage sampling was used for sampling 4 LGAs in each of the 3 senatorial districts of Kogi state. Simple random and convenient samplings were used to sample poultry.

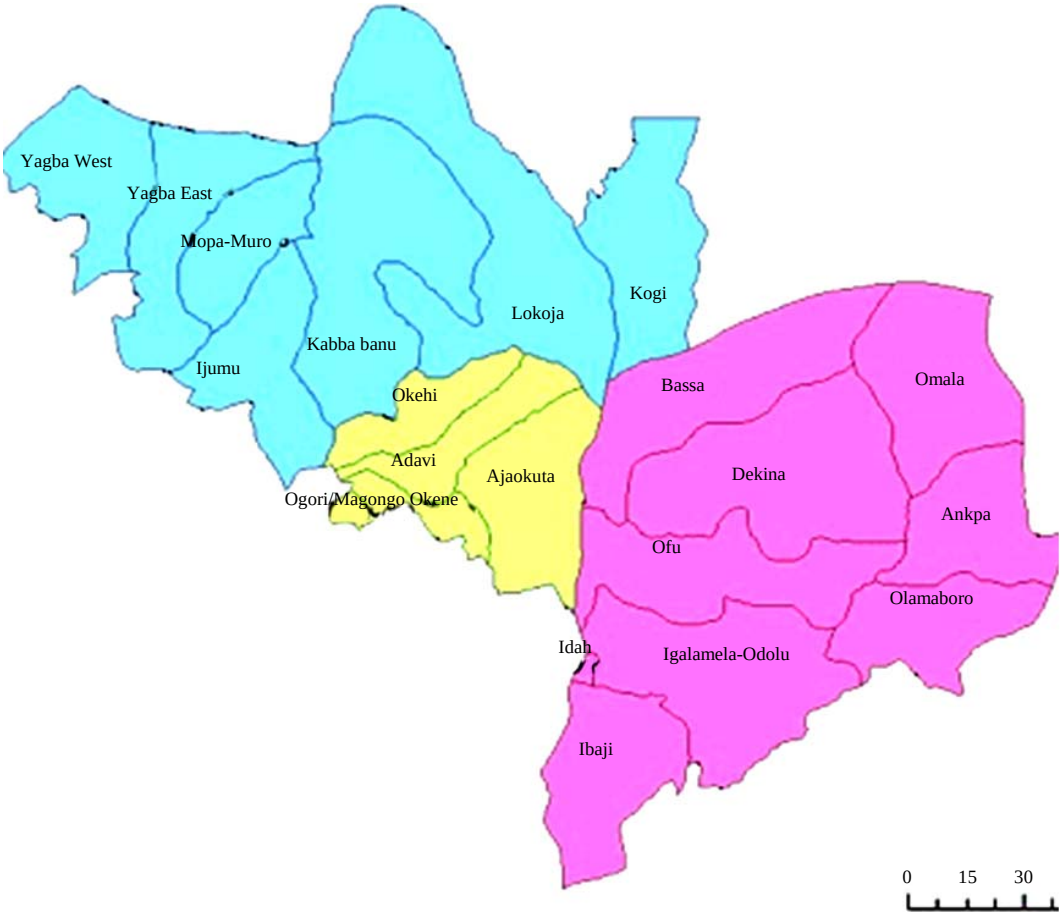

Fig. 1: Map of Kogi state showing the 21 local government areas grouped into western (blue area), central (yellow area) and eastern (pink area) senatorial districts. (Source: www.ncocusa.com, accessed September 14th, 2014, 3 pm)

Collection of blood: The blood sample was collected from a bird after proper restraint with the use of a 21 gauge needle and a 5 mL syringe, 2 mL of blood was withdrawn from the wing vein. The blood collected was allowed to stand for 2 h for clotting to occur and the serum decanted into a bijou bottle, stored under ice then transported to the laboratory within 48 h and kept at -20°C until used. A total of 779 poultry sera were collected from apparently healthy poultry during the survey from September, 2012 to February, 2013.

Detection of avian influenza antibodies

Haemagglutination inhibition test: The inactivated AI (H5) antigen (Batch 20060212) used was produced from China and the antiserum (H5N2 Batch 1/10) used was produced by OIE/FAO reference laboratory for AI and ND, delle Venezie, Italy. A 1% suspension of chicken Red Blood Cells (RBC) was prepared and used as indicator in the haemagglutination (HA) and Haemagglutination Inhibition (HI) tests. Haemagglutination (HA) test was carried out according to the WOAH (2009) protocol to determine the antigen titre, diluted to 4HAU that was used for the HI tests.

The alpha HI test was also carried out as described by WOAH (2009) and the results were expressed in $\log_2$.

Enzyme link immunosorbent assay: The AI ELISA antibody test kit used was manufactured by Affinitech Ltd, Bentonville, AR 72712, USA. The reagents supplied included antigen coated microtitre plates, sample diluent, wash solution, conjugate (IgG alkaline phosphate), substrate (p-Nitrophenyl phosphate), stop solution (3.0 M NaOH), positive and negative controls which were prepared and used according to manufacturer's instructions. The ELISA plates were read using dual wavelength microtitre plate ELISA reader with 405 nm primary filter and 630 nm reference filter blanked on air. Serum with 15 ELISA Units (EU) or more were taken as positive.

RESULTS

Avian influenza antibodies detection by haemagglutination inhibition test and enzyme link immunosorbent assay: A total of 779 poultry sera collected from live bird markets, rural households and backyard farms in 12 LGAs of Kogi state were analyzed for AI (H5) antibodies using HI test. Thirty three (4.2%) of the poultry sera were positive for AI (H5) antibodies and none of the poultry species other than chicken was positive (Table 1). The seroprevalence of AI by HI test in the 12 LGAs surveyed were 12.3% for Kabba/Bunu, 8.3% for Ofu, 7.9% for Mopamuro, 6.4% for Ijumu, 3.3% for Ogori-Mangogo, 3.2% for Dekina, 2.5% for Lokoja, 2.4% for Ankpa, 1.4% for Adavi and 1.2% for Olamaboro while Ajaokuta and Okene had 0.0% (Table 2). The seroprevalence of AI (H5) in the different sampling units was 4.5% for backyard poultry, 4.3% for poultry from live bird market and 3.8% for rural poultry (Table 3). The overall AI antibody mean titre by HI test was 8.42±0.254 log_2 with 95.8% of the sera having antibody titre ≤3log_2 and 3.7% having antibody titre ≥7log_2 (Table 4). Using the ELISA AI antibody test kit, the same poultry sera from live bird markets, rural households and backyard farms in 12 LGAs of Kogi state were tested. One hundred and thirty nine (30.8%) poultry sera were positive for AI nucleoprotein antibodies with species seroprevalence being 31.6% for chicken, 26.9% for turkey and 0.0% for ducks (Table 5). The seroprevalence of AI in the surveyed 12 LGAs were 41.8% for Mopamuro, 40.0% for Olamaboro, 39.0% for Ijumu, 33.3% for Kabba/Bunu, 32.6% for Ofu, 28.6% for Dekina and Ogori-Mangogo, 26.9% for Lokoja, 26.7% for Okene, 21.7% for Ajaokuta, 20.0% for Ankpa while Adavi had 18.6%, respectively (Table 5). The overall ELISA mean antibody titre is 16.588±0.882 EU while the seroprevalence for sampling units were 26.5% for backyard farms, 32.9% for live bird markets and 35.0% for rural poultry (Table 6).

Table 1: Seroprevalence of avian influenza detected by haemagglutination inhibition test in different poultry species surveyed in Kogi state

Poultry species	No. of tested	Positive		Mean antibody titre±SEM log_2
		No.	%	
Chicken	734	33	4.5	8.42±0.254
Duck	12	0	-	
Turkey	33	0	-	
Overall	779	33	4.2	8.42±0.254

DISCUSSION

The overall seroprevalence of AI (H5) of 4.2% detected by HI test in the surveyed 12 LGAs of Kogi state from this study was lower than the seroprevalence of 22.4% reported earlier in 2010 in chickens from the same study area (Ameji *et al*., 2011). The difference may be attributed to the wider area covered in the present study because large sampling population from a wide area of the study population will give a true picture of a disease in the population. Also time variation would have played a role as well, as antibodies are known to decay over a period of time in the absence of active infection. The diagnostic test used in this study was specific for AI (H5) subtype and different from that of the previous study. The test employed in the previous study was Agar Gel Immunodiffusion Test (AGID) that targeted the nucleoprotein or matrix protein of AIVs and not a subtype specific test like HI test.

Table 2: Seroprevalence of avian influenza in poultry sera detected by haemagglutination inhibition test in 12 local government areas of Kogi state

Local government areas	No. of tested	Positive		Antibody titre ≤3 log_2		Antibody titre ≥7 log_2		Mean antibody±SEM log_2
		No.	%	No.	%	No.	%	
Adavi	70	1	1.4	69	98.6	1	1.4	10.00±0.00
Ajaokuta	48	0	0.0	48	100.0	0	0.0	-
Ankpa	42	1	2.4	41	97.6	1	2.4	10.00±0.00
Dekina	62	2	3.2	60	96.8	2	3.2	8.00±1.00
Ijumu	78	5	6.4	73	93.6	3	3.8	7.60±0.927
Kabba/Bunu	73	9	12.3	64	87.7	8	11.0	8.78±0.572
Lokoja	80	2	2.5	78	97.5	2	2.5	8.50±0.500
Mopamuro	63	5	7.9	58	92.1	5	7.9	8.60±0.400
Ofu	60	5	8.3	53	88.3	5	8.3	8.20±0.374
Ogori-mangogo	60	2	3.3	57	95.0	2	3.3	8.00±2.000
Okene	61	0	0.0	61	100.0	0	0.0	-
Olamaboro	82	1	1.2	79	96.3	1	1.2	8.00±0.000
Overall	779	33	4.2	734	94.2	30	3.9	8.42±0.254

Table 3: Seroprevalence of avian influenza in poultry detected by haemagglutination inhibition test in the surveyed sampling units in Kogi state, Nigeria

		Positive		
Sampling units	No. of tested	No.	%	Mean antibody titre±SEM log_2
Backyard farm	313	14	4.5	8.64±0.401
Live bird market	232	10	4.3	8.90±0.348
Rural poultry	234	9	3.8	7.56±0.503
Overall	779	33	4.2	8.42±0.254

Table 4: Seroprevalence of avian influenza in different poultry species surveyed detected by enzyme link immunosorbent assay in Kogi state

		Positive		
Species	No. of tested	No.	%	Mean antibody titre±SEM log_2
Chicken	418	132	31.6	16.427±0.888
Duck	8	0	0.0	-
Turkey	26	7	26.9	22.081±5.490
Overall	452	139	30.8	16.588±0.882

Table 5: Seroprevalence of avian influenza in poultry sera detected by enzyme link immunosorbent assay in the surveyed twelve local government areas of Kogi state

		Positive		
Local Government areas	No. of tested	No.	%	Mean antibody±SEM EU
Adavi	43	8	18.6	17.477±3.281
Ajaokuta	23	5	21.7	12.752±2.318
Ankpa	20	4	20.0	10.610±2.959
Dekina	35	10	28.6	15.471±3.081
Ijumu	41	16	39.0	18.476±3.227
Kabba/Bunu	30	10	33.3	14.810±2.824
Lokoja	52	14	26.9	16.348±2.820
Mopamuro	55	23	41.8	21.009±3.250
Ofu	46	15	32.6	18.057±2.992
Ogori-Mangogo	35	10	28.6	12.503±1.554
Okene	30	8	26.7	15.520±2.754
Olamaboro	40	16	40.0	18.310±2.970
Overall	450	139	30.9	16.588±0.882

EU: Ezyme link immunosorbent assay unit

Table 6: Seroprevalence of avian influenza in poultry detected by enzyme link immunosorbent assay in the surveyed sampling units from Kogi state

		Positive		
Sampling unit	No. of tested	No.	%	Mean antibody titre±SEM EU
Backyard farm	189	50	26.5	14.480±1.167
Live bird market	143	47	32.9	17.360±1.662
Rural poultry	120	42	35.0	18.987±1.921
Overall	452	139	30.9	16.588±0.882

EU: Ezyme link immunosorbent assay unit

However, using ELISA, a seroprevalence of 30.8% of AI nucleoprotein was detected in poultry sera from the surveyed 12 LGAs of the state. This might be due to the high sensitivity of ELISA test to detect antibodies to all avian influenza virus subtypes targeting the nucleoprotein or matrix protein compared to the high specificity of HI test in detecting only a particular haemagglutinin subtype. The ELISA is taken to be better than HI in identifying sera with low antibody titer but the high sensitivity of ELISA sometimes leads to false positive results, often limiting its use only as a screening test (Faraz *et al*., 2010). The seroprevalence of AI obtained by the ELISA fairly agreed with the previous seroprevalence of 22.4% using AGID test in sera of poultry obtained from the study area (Ameji *et al*., 2011). The target subtypes of interest in AIVs surveillance are H5 and H7 due to their potential to cause HPAI in poultry but detection of other subtypes is useful in understanding AIVs epidemiology and impacts of the disease burdens (Komar and Olsen, 2008).

None of the poultry species other than chickens had detectable AI (H5) antibodies by HI test in the present study similar to the previous study in 2010 in the same study area (Ameji, 2010). This consistent trend is a pointer to a different incriminating source of infection other than the duck or any other poultry species in spreading AI infection (Alexander *et al*., 2008). The findings led credence to the speculation that some breeder farmers were vaccinating their chickens against AI at the wake of HPAI H5N1 outbreak in Nigeria in 2006 and were encouraging commercial and backyard farmers that obtained chicks from them to vaccinate their birds as well (Wakawa *et al*., 2012). If the sharp practice was true, it would curtail outbreak but would not stop AI viral replication, circulation in poultry population and antibody response.

On the other hand, using ELISA, an AI seroprevalence of 26.9% was detected in turkey indicating the circulation of AIVs of other subtypes than H5 subtype in turkey population in the study area. Turkeys and chickens are known to be highly susceptible to HPAI of either H5 or H7 subtypes (Swayne, 2008). The circulation of LPAI for long in these species of poultry is a serious risk of an inherent outbreak due to reassortment or mutation of AIVs. Similarly, there was no freedom of AI infection in the surveyed LGAs as antibodies were detected using ELISA in all the LGAs at higher levels than the seroprevalence obtained by HI test due to the ability of ELISA to detect antibodies of low titre and to all subtypes of AIVs (Faraz *et al*., 2010).

Incidentally, there were no AI (H5) antibodies detected by HI test in Ajaokuta and Okene LGAs indicating that poultry from these LGAs were not exposed to AI (H5) viruses. Ajaokuta had the lowest chickens' sera as a result of few backyard farms in the area while poultry sera from Okene LGA were mainly from rural poultry (native breed) which are kept for long period of up to 4 years which might result in loss of antibody titres beyond detectable level if they were earlier exposed to the AIVs. This finding might not mean freedom from disease or AI infection in these two LGAs as the sampled poultry were

apparently healthy. Komar and Olsen (2008) reported that it was more likely to find HPAI (H5 and H7) in sick or dead poultry than apparently healthy birds.

The presence of subtype specific (H5) and type A influenza antibodies in poultry in the study area indicated a previous exposure to AIVs. It is obvious that the detection of AI (H5) antibodies which is more in backyard poultry might be due to vaccination against HPAI (H5N1) in Kogi state. It is recommended that farmers should be discouraged from vaccinating their birds so as not to interfere with the Government AI stamping out policy. It also recommended that further virological investigation and subtype characterization of AIVs should be carried out.

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