Research Article

Sero-Survey of Avian Influenza Disease Virus in Live Bird Markets and Farmers Appraisal within Abuja Municipal Area Council, Nigeria

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Abstract | Avian Influenza (AI) virus is a transboundary animal disease of international food and biosecurity importance characterized by recurrent outbreaks in many countries where the virus is still endemic. This purposive sero-surviellance study was conducted to detect the presence of AI antibodies in chickens and AI appraisal of poultry farmers' knowledge, attitude and practice in Abuja Municipal Area Council, Nigeria. One hundred (100) blood samples collected randomly post slaughter from chickens in live bird markets were screened and characterized using Agar Gel Immuno Diffusion and Haemagglutination Inhibition Tests at the Avian Influenza laboratory, of the National Veterinary Research Institute, Vom Plateau State. A structured Questionnaire was also administered to 50 poultry farmers. Results revealed an overall sero-prevalence of 5% with H5 and H9 haemagglutinating inhibiting antibody titre ranging from 1 log2-5 log2. Breed specific sero-prevalence were 4% and 16% for local and exotic (layers) chickens respectively, while broilers showed no detectable antibodies. Questionnaire responses indicated low awareness level on the zoonotic potential of AI but high level of awareness on the economic impact of the disease. In conclusion, this study provides preliminary information on the occurrence of Avian Influenza virus H5 and H5/H9 co-infection and circulation in chickens characterized by poor zoonotic awareness of the disease within Abuja Municipal Area Council. Hence, there is a need to conduct further molecular studies to establish the circulating viral field strains for comparism with empirical vaccine strains and create more public enlightenments and awareness campaign about the disease burden and possible zoonotic impact.

Received | August 30, 2023; **Accepted** | September 23, 2023; **Published** | October 05, 2023
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Citation | Olabode, H.O.K., Mailafia, S., Echioda-Ogbole, M., Ameh, J.A., Amadi, K.I. and Meseko, C.A., 2023. Sero-survey of avian influenza disease virus in live bird markets and farmers appraisal within Abuja municipal area council, Nigeria. *Hosts and Viruses*, 10: 36-42.
DOI | https://dx.doi.org/10.17582/journal.hv/2023/10.36.42
Keywords: Avian influenza virus antibodies, Abuja, Sero-survellaince, Chickens, Live bird markets, Nigeria

Introduction

Avian Influenza (AI), commonly known as bird flu, is a reportable viral transboundary animal disease that primarily affects birds. However, certain strains of the virus can be transmitted to humans (Meseko *et al*., 2018). The virus belongs to the family *Orthomyxoviridae*, genus influenza A (Webster *et*

al., 2006). This virus is also classified as either low pathogenic or highly pathogenic avian influenza viruses (HPAI) (Parvin *et al.*, 2020). The H5 subtypes are highly pathogenic while the H7 subtypes are of low pathogenicity when detected in poultry (OIE, 2014). The disease is caused by the highly contagious influenza A viruses, primarily the H5N1 and H7N9 subtypes (de Bruin *et al.*, 2022). The circulating strains are potential candidate for influenza pandemic which may severely affect human and animal population worldwide especially in resource-poor countries (Joannis *et al.*, 2009). AI is transmitted by direct contact with infected birds or their droppings characterized by sporadic outbreaks with high morbidity and mortality (Fasanmi *et al.*, 2018) leading to great economic losses which can be categorized as financial setbacks for farmers, reduced meat and egg production, and increased prices of poultry products (Elelu, 2017) which ultimately impacted negatively on international poultry industry and livelihood of many individuals (Ramos *et al.*, 2017). Although human cases of avian influenza have been relatively limited (OIE, 2014) the potential for animal to human transmission remains a significant public health concern as H5N1 strain can cause severe respiratory illness with high mortality rate (Monne *et al.*, 2015).

In Nigeria, the introduction of the virus is often linked to multiple sources and animal infections (Meseko *et al.*, 2018a) not limited to the importation of infected birds or exposure to infected and or carrier migratory birds (Meseko *et al.*, 2023). The dense and unregulated poultry markets in the country also contribute to the rapid dissemination of the virus. This has resulted in several outbreaks of avian influenza due to illegal trade of poultry products, lack of awareness among farmers on biosecurity measures, emergence of new strains of avian influenza and poor surveillance and monitoring of the virus (Chieloka, 2021). Sustained national control efforts has led to resounding collaborations with international organizations of UNDP such as the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) since the 2006 outbreaks in order to enhance surveillance, capacity building, and technical support. These collaborations have contributed to improved control and preventive measures (Olabode, 2009), prior to the HPAI (H5N1) resurgence (Monne *et al.*, 2015). However, outbreaks still occur recently amidst the COVID pandemic (Omotayo *et al.*, 2022) which necessitated this surveillance study in AMAC a cosmopolitan settlement in FCT that borders Plateau state a known AI hotspot (Chieloka, 2021). In order to identify circulating avian influenza strains with pandemic potential among poultry flock and assessment of value chain stakeholder awareness about possible zoonosis.

Materials and Methods

Study area

The study was conducted at Abuja Municipal Area Council, FCT Abuja, the area is made up of Nyanya, Garki, Gui, Gwagwa, Kabusa, Gwarimpa, Orozo, Karu, as shown in Figure 1. each with smaller settlements. AMAC is the largest and most developed amongst the six area councils of the FCT. It is located between latitude 7°49 and 8°49 North of the equator and longitude 7°07 and 7°33 East of the Greenwich Meridian. The land mass is about 2,500sq km (Balogun, 2001), with the highest population of 778,567 reported by National Population Census in 2006 and characterized by high human activities and traffic (Mundi, 2000). Hence, the choice of this study area at four (4) different live bird markets with attached slaughter points and volunteered poultry farmers and value chain stakeholders.

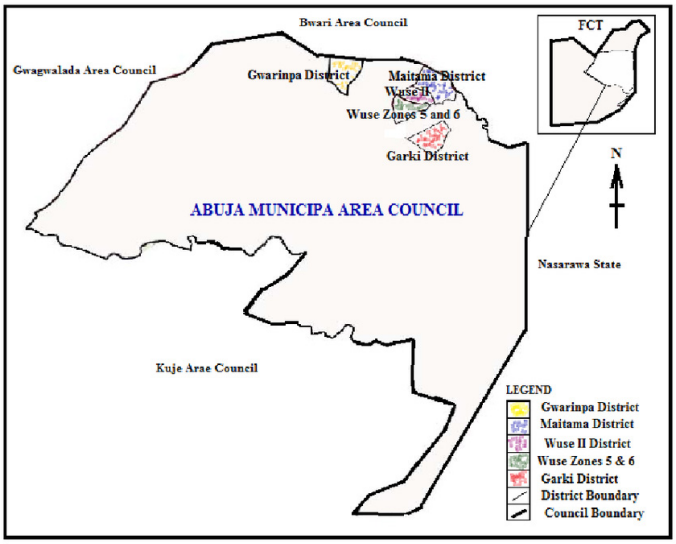

Figure 1: *Map of AMAC showing the study area.*
Source: *Abuja Geographic Information Systems (AGIS) FCT, Nigeria.*

Study design

This pilot survey was carried out from October to November, 2021. Purposive random sampling method was used to collect 100 blood samples from slaughtered chickens comprising of Local chickens (N= 25), layers (N= 25) and broilers (N= 50) from different markets within AMAC (Orozo, Karu,

Kurudu and Nyanya markets). Processed sera were later transported in an ice parked flask for analysis at the Avian Influenza Laboratory, Viral Research Unit, National Veterinary Research Institute (NVRI), Vom, Nigeria. While questionnaire was administered to poultry farmers.

Sample collection and processing

Blood (5ml) was collected aseptically into plain sample bottles that contained no anticoagulant during the slaughter. The samples were kept at 45° slants for sera separation at room temperature for two hours (OIE, 2014). The sera were then transferred into labelled cryovials and stored at 4°C until used for analysis. Prior to the laboratory analysis at NVRI, Vom the sera were inactivated at 56°C for 30 mins in water bath in accordance with laboratory standard operating procedure.

Laboratory analysis

Agar gel immunodiffusion test (AGID test): The test was carried out using gels of 1% (w/v) agarose, purified type II agar and 8% (w/v) NaCl in 0.01 M phosphate buffer at pH 7.2 and poured to a thickness of 2–3 mm in Petri dishes before incubation in a humidified chamber. Using a template and cutter, wells of approximately 5 mm in diameter were cut into the agar at a distance of about 3 mm from each other. A pattern of wells must have each suspected serum adjacent to a known positive serum and antigen.

Approximately 25–30 µl of each reagent was dispensed per well. The wells were filled with reagent to align the meniscus top with the gel top and avoided over fills and then incubated. Wells were examined for precipitin lines after 24 hours, and weak positive samples or samples for which specific lines had not formed were further incubated longer and examined again after 48 hours. The precipitin lines were observed against a dark background that was illuminated from backside. A specific, positive result was recorded when the precipitin line between the known positive control wells converges with the line between the antigen and the test well. Crossed lines are interpreted to be caused by the test serum lacking identity with the antibodies in the positive control well (OIE, 2014).

Haemagglution inhibition test: This procedure was conducted in accordance with the NVRI Avian Laboratory SOP for subtyping of AGID positive sera with H5, H7 and H9 haemagglutinin antigen. Briefly, 0.025 ml of PBS was dispensed into each well of a plastic V-bottomed microtitre plate. Then 0.025 ml of serum was added into the first well of the plate. Two fold dilutions of 0.025 ml volumes of the serum across the plate were done. 4 HAU of virus/antigen in 0.025 ml was added to each well and left for a minimum of 30 minutes at room temperature (about 20°C). Then, 0.025 ml of 1% (v/v) chicken RBCs was added to each well and mixed gently, and the RBCs was allowed to settle for about 40 minutes at room temperature (about 20°C). The HI titre 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 stream at the same rate as the control wells (containing 0.025 ml RBCs and 0.05 ml PBS only) were considered to show inhibition. The validity of results was assessed against a negative control serum, which was not of a titre >1/4 (>22 or >log2 2 when expressed as the reciprocal) and a positive control serum for which the titre was within one dilution of the known titre (OIE, 2014).

Questionaire survey

Fifty questionnaires were distributed to poultry farmers within AMAC of the FCT. Information collected includes: their knowledge of Avian Influenza, attitude and on- site practice in management of suspected Avian Influenza cases. The indices considered were the mortality patterns, clinical signs, farmers disposition towards ethno-veterinary measures, treatment attempts employed and possible vaccine use by farmers.

Results and Discussion

Out of the 100 sera screened using AGID, 5(5%) were positive for AI antibodies. Distribution of AI antibodies according to bird types showed that layers had 4 (16%) positive sera and local birds had 1 (4%) positive serum, while no detectable antibodies were observed in all broiler samples (Table 1). Further subtying of these positive sera with haemagglutination inhibition test revealed all antibody titre ranged between 1 log2-5 log2 with only one positive serum having H5 subtype with titre of 5log2. However, H5 and H9 subtype confections were detected in other sera. Both subytpes have an antibody titre of 5 log2. All sera were negative for H7 subtype (Table 1).

The questionaire responses showed that the demograghic distribution of the farmers were mostly (72%) married women within the age of 35 years and above (66%). These farmers were mostly Secondary leaving certificate/high school leavers (54%) and Tertiary school graduates (28%) while only a few were either primary school leavers (10%) and 8% were of no educational training (Table 2).

Most of the farmers were broiler keepers (66%) while 26% reared layering birds and only 8% kept local chickens with most of the birds were within 0-2 months of age (66%), and those within 2-4 months were 28% while birds kept above 4 months were 6%. Almost all the farmers were aware of the disease (82%) even though they (96%) never experienced any outbreak in their farms, only 38% of these farmers were aware of the zoonotic effects of the disease to include pnuemonia, cold and flu like nasal discharges while most farmers (62%) did not know that it affects humans. However, most of the farmers (92%) agreed that infuenza is fatal in poultry. In addition, some of the farmers (18%) still thought there was an approved vaccine and vaccination schedule for the disease, while 8% still do not see the negative impact of the virus. Most of the respondents (88%) stated that no government compensations were paid during previous outbreaks while only 12% indicated that monetary compensation were granted to affected farmers (Table 3).

Table 1: *Qualitative and Characteristic distribution of Avian Influenza virus antibodies in AMAC, FCT, Nigeria.*

Agar gel immunodiffusion (AGID) test					Haemagglutination inhibition (HI) test						
Bird type	**Broilers**	**Layers**	**Local**	**Total**	**Titer**	**1log2**	**2log2**	**3log2**	**4log2**	**5log2**	**H Antigen**
Positive	- (0%)	4 (16%)	1 (4%)	5 (5%)		-	-	-	-	1	H5
Negative	50 (100%)	21 (84%)	24 (96%)	95 (95%)		-	-	-	-	-	H9
Total	50	25	25	100	Total	-	-	-	-	1	H5/H9

Table 2: *Demographic data of respondent farmers on the awareness of Avian Influenza disease virus in AMAC, F.C.T, Nigeria.*

Parameter	**Marital status**	**Married**	**Single**	**Divorced**	**Widowed**	**Sex**	**Male**	**Female**
	Frequency	36	14	0	0		14	36
	Percentage (%)	72	28	0	0		28	72
Parameter	**Educational back-ground**	**No formal education**	**Primary school**	**Secondary school**	**Tertiary**	**Age**	**18-35**	**35 and above**
	Frequency	4	5	27	14		17	33
	Percentage (%)	8	10	54	28		34	66

Table 3: *Response of Farmers on the Knowledge, Attitude and Practice of Avian influenza disease on farms in AMAC, FCT, Nigeria.*

Information	**Responses**		**Other names of AI**	**Bird flu**	**Fowl plague**	**Avian flu**
	Yes	**No**		**26 (52%)**	**4 (8%)**	**20 (40%)**
Knowledge of AI	41 (82%)	9 (18%)	Type of bird	Layers	Broilers	Local
Any previous AI outbreak occurrence	2 (4%)	48 (96%)		13 (26%)	33 (66%)	4 (8%)
Any routine AI vaccination	9 (18%)	41 (82%)	Age of Birds	0-2 months	2-4 months	4-6 months
General routine disease vaccinations	39 (78%)	11 (22%)		33 (66%)	14 (28%)	3 (6%)
Zoonotic potential of AI knowledge	19 (38%)	31 (62%)	Clinical signs	High sudden mortality	Coughing	Blood tinged oral and nasal discharges
Knowledge of AI fatality	46 (92%)	4 (8%)		32 (64%)	4 (8%)	14 (28%)
Any available Government compensation	6 (12%)	44 (88%)	Treatment & intervention	Antibiotics	Natural Remedy	Culling
Insurance policy for their farms	0 (0%)	50(100%)		28 (56%)	0 (%)	22 (44%)

The overall sero-prevalence of Avian Influenza (AI) virus antibody during this pilot survey from apparently healthy birds slaughtered at live bird markets is 5%. This finding is lower than previous report of 26.0% in neighboring state of Kogi (Ameji *et al*., 2011) using AGID test, which could be due to differences in the sampling scope. However, AGID test showed no detectable antibodies in sera positive to ELISA test with prevalence of 4.5% in wild birds previously reported (Ameji *et al*., 2017) as ELISA is more sensitive and best use as a screening test although it is often characterized by false positive results (Faraz *et al*., 2010).

The observed high AI antibody detection in layering birds suggests increase vulnerability to natural infection and or due to vaccination considering their purpose and length of time spent on farms. While the occurrence of AI antibody in scavenging local chickens could be due to environmentally acquired infections caused by domestic and wild birds activities (Meseko *et al*., 2018b). The absence of AI antibody in broilers further suggest that duration of flock keeping is shorter than that of both layers and local chickens.

The evidence of antibody titres (5 log2) against H5 subtype further indicates birds could either acquire these antibodies via vaccination or natural infection. Although vaccination against AI has been banned in Nigeria which suggests a probable strain source from use of imported vaccines since some respondents still believes that vaccination was acceptable for AI. This unofficial vaccination attempts against avian influenza subtype H5 by some commercial flock farmers has been previously suggested (Meseko *et al*., 2020) with limited antibody response. However, this titer does not confer protection against subsequent exposure to AI as HI titre of 4-5log2 and above correlates with protection against field infection (Montomoli *et al*., 2010). The existence of this antibody titers in this study further excludes the possibility of a natural infection because death of birds occurs shortly after infection without sufficient time (2-3 weeks) for humoral antibody development (OIE, 2014).

In addition there also may exist the possibility of a natural infection with a low pathogenic avian influenza virus strain such as H5N2 from other commercial domestic water fowls in the market as previously reported (Coker *et al*., 2014). This would require further surveillance studies. Conversely, the detection of a co-infection with H5 and H9 subtypes in this survey further suggest exposure to natural infection through direct or indirect contact with wild birds reservoirs which harbors both subtypes as no obtainable evidence of H9 vaccination in the study area since samples were collected in the months (October-November) that with concides autumn when migatory birds stopover in Nigeria during their intercontinental movements as earlier reported (Meseko *et al*., 2018b).

This detection of H5 and H9 hemagglutinin (HA) surface protein indicative titers in this pilot survey further confirms occurrence and co-infection of H5N8, H5N6 and H9N2 strains have being circulation in Live Bird Markets (LBM) since 2015-2017 outbreaks (Chieloka, 2021) as LBMs has been previously reported as potential reservoir and source for H9 strains (Sulaiman *et al*., 2021) associated with immense poultry trade activities (Fusaro *et al*., 2019) due to poor biosecurity compliance enhancing the survivability of HPAI virus capacity to evolve into other subtypes through genetic assortment as suggested (Meseko *et al*., 2023), hence the reports of H5/H9 confection in this study. The co-circulation of H5 and H9 subtypes has a potential emergence of a reassortant highly pathogenic AI subtypes/strain of zoonotic epidermics.

The questionnaire responses indicates most poultry farmers are well informed about the disease in the study area (Ijoma *et al*., 2020) but have not experience any outbreak on their farms because they kept broilers with short rearing cycles. However, farmers have poor knowledge as previously reported (Ameji *et al*., 2012) especially about the potential zoonosis. The farmers also agreed that the disease is highly fatal and a topmost cause of serious economic losses to the poultry as previously reported (Ameji *et al*., 2011). However, reports further indicates ineffective and poor government compensation of the farmers which promotes the illegal sales to dead birds to recover some losses and this would further escalate virus spread along trade routes during transportation between farms and live bird markets (Chieloka, 2021) with potential outbreaks both in birds and humans respectively. There also exist few reports of vaccine use and absolute non-compliance with farm insurance policy and guidelines which could possibly be a confounding factor for control and intervention by disease regulatory agencies.

Conclusions and Recommendations

In conclusion, this survey shows evidence of antibody titre to H5 and H9 avian influenza virus in both local and exotic chickens especially layering birds in AMAC, FCT which is fast becoming an endemic disease with potential significant zoonosis and public health consequences due to poor disease awareness.

Acknowledgments

Authors are most grateful to staff of Avian Influence Laboratory, of the Viral Research unit National Veterinary Research Institute (NVRI) Vom Nigeria for their technical supports during the course of the laboratory analysis.

Novelty Statement

The finding of this study reveals the co-infection of H5 and H9 strains of HPAI which may create an environment for possible gene re-assortment and development of wild type strains of serious public health implications.

Author's Contribution

AHI contributed in sample collection. OHOK and MS contributed in the general design and writing of the manuscript. MCA assisted in the laboratory analysis, while EOM and AJA helped in reviewing and editing the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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