

## Evaluation of the Predisposing Factors of Persistent Avian Influenza (AI) Virus on Farms Post-Outbreak: a Case Study on Farms in Rivers State, Nigeria

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### ABSTRACT

This study was carried out to outline the risk factors that could predispose to persistence of Avian Influenza virus following an outbreak in the state, and ascertain freedom from Avian Influenza through re-evaluation of current status and control measures. Sera (182) were collected from commercial chicken farms, live bird markets (LBM) and Port Harcourt Zoo. Sera when grouped per species 79.1% was from chicken, 14.3% from ducks and 1.6% each from turkeys and guinea fowls; while 0.6% each was from geese, parrots and crown crane. Factors including species of bird in the study area, source of birds, vaccination against AI and other biosecurity measures that could affect persistence of AI virus in poultry and in the environment was investigated in this study. The findings indicated that 43.9% poultry on LBM was from Bauchi state, 16.7% from Gombe State and 39.4% of unknown source. The birds were sourced from commercial hatcheries and LBM. Biosecurity measures and disease prevention by vaccination observed in commercial farms was totally absent in LBM. Screening of sera for antibody against AI using Enzyme Linked Immunosorbent Assay (ELISA), showed a total of 3.8% positive, with 3.3% from duck, 0.5% from geese and 0% from commercial chicken, 3.5% positive samples from LBM and 0.5% from farms. The poultry in farms were sourced from commercial hatcheries and LBM. Biosecurity measures and disease prevention by vaccination observed in commercial farms was totally absent in LBM. Ducks are reservoirs and an epidemiological factor for reoccurrence of outbreak in the state.

**Keywords:** Avian Influenza, ducks, chickens, rivers state

### INTRODUCTION

Many African countries including Nigeria have been affected differently by the outbreak of AI at various degrees in two major waves of infections that affected Africa from 2006 to 2008 and 2014 to 2015 (Kayali *et al.*, 2010; WHO, 2014; OIE, 2016). In Nigeria where AI was considered endemic for HPAI H5N1 in 2015 (FAO, 2015), the first introduction was linked to presence of infected migratory birds and introduction through trade (Ducatez *et al.*, 2006; De Benedictis *et al.*, 2007, Fasina *et al.*, 2008; Fusaro *et al.* (2009); Meseko *et al.*, 2010) The second wave of AI outbreak started in January 2015 and by September 2015, there were over 500 confirmed positive cases from over 1,000 samples screened for Influenza A subtype H5N1 in 20 States including Rivers State (Ifende *et al.*, 2015). This out

break might have originated from reservoirs maintained during the first wave of outbreaks.

Factors responsible for the persistence of AI virus in the environment include its resistance to environmental changes, its survivability, ability to evolve through genetic re-assortment to other subtypes, poor compliance to biosecurity at the poultry farms and live bird markets, husbandry methods and multispecies farming, vaccinated birds that continually shed HPAIV, transboundary spread of HPAIV through trades in poultry, and movement of migratory birds (USDA, 2015, Opukpara, 2016; , Fasanmi *et al.*, 2017). This study was carried out to outline the risk factors that could predispose to outbreak of AI in Rivers state and ascertain freedom from AI through re-evaluation of current control measures, determination of the point seroprevalence of AI in

poultry in the previously affected areas of Rivers State, by screening for presence of Antibody against HPAI in domestic poultry in Obio-Akpor, Ikwere and Port-Harcourt LGAs affected during the 2015 outbreak using Enzyme Linked Immunosorbent Assay (ELISA); Compare the prevalence in domestic poultry in farms to that in market, within the study area.

### METHODOLOGY

Rivers state is located in Latitude 4°45' N and Longitude 6°50' E in the south-south Nigeria with an area of 11,077km<sup>2</sup> (4,277sq mi). The state which is comprised of 23 LGAs is bounded to the north by Anambra, Imo and Abia States, to the west by Bayelsa and Delta States, to the east by Akwa Ibom State and to the south by the Atlantic Ocean. The state has estimated human population of 5,185,400 (2006 Census). The choice for Rivers State, for this study was based on repeated outbreaks of HPAI in 2006 and 2015. Questionnaires were administered to farmers and bird sellers at live bird markets during sample collection for data on aftermath of 2015 outbreak on affected farms, source of birds sold on live bird in Rivers State, record on possible AI vaccination in commercial poultry and biosecurity measures in poultry production and poultry business.

### SEROLOGY

A total of 182 serum samples were collected from chickens, turkeys, ducks and zoo birds. Two (2ml) of blood was collected from the wing vein of the birds following proper restraint, using 5ml syringe and 21gauge needle.

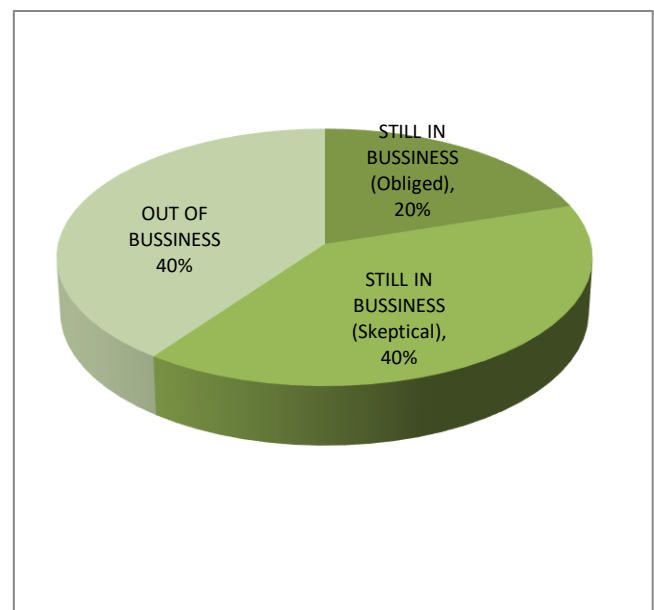
The blood collected was centrifuged or allowed to stand for 30 minutes for clotting to occur and the serum carefully decanted into eppendorf tubes on ice, then transported to the laboratory within 48 hours and kept at -20 °C until when needed. Antibody detection for avian influenza H<sub>5</sub> using enzyme linked immunosorbent assay (ELISA) was done using the Avian Influenza ELISA antibody test kit by Affinitech Ltd, Bentonville, AR 72712, USA. All procedures were carefully carried out following the manufacturer's instructions. A dual wavelength microtitre plate-ELISA reader was used to read the ELISA plates, with 405 nm primary filter and 630 nm reference filter blanked on air. Positives were taken from serum with 15 ELISA Units (EU) and above.

### RESULTS

It was observed that the species of birds on the live bird market included geese, guinea fowl, pigeon and duck while the commercial poultry farms reared chicken and turkey. The zoo had in addition crown crane, pea fowl and parrots (Table I). From the questionnaire, most of the birds sold in the live bird markets were sourced from the northern states majorly Bauchi and Yobe, with the higher number (43.9%) from

Bauchi (Table II). There were no observable measures of biosecurity with regards to disease prevention and control on the live bird markets. However, on commercial farms, there was some level of biosecurity including movement restriction, functional foot dip, screens, practice of routine fumigation; vaccination against NewCastle disease, infectious bursal disease (IBD), fowl pox and fowl typhoid was carried out by commercial farms. No vaccination against AI was reported (Table I). Farms affected by the 2015 AI outbreak in Rivers State were contacted during this study and 40% was out of business after the devastating effects of the outbreak. Out of the 60% still in business, 20% accepted the survey, the other 40% declined (Figure I).

On serological testing the highest percentage of positive samples came from LBM (58.3%) then (0.5%) in commercial farm and none was detected in sera of zoo birds (Table IV). Ducks had the highest positives among species (3.3%), and guineafowl had the highest positives within species (33.3%), (Table V). The positive samples of the LBM (100%) were from the Trans-Amadi LBM (Table III) majorly sourced from Bauchi State (Table IV).



**Figure I: Chart showing socioeconomic impact of the Rivers State 2015 AI outbreak on farmers.**

**TABLE I: TYPE, SOURCES AND MANAGEMENT OF BIRDS IN SELECTED FARMS IN RIVERS STATE**

Location	Types of Bird	Source of Bird	Biosecurity	Vaccinations		
				AI	Others	
LBM	Trans-amadi	Duck, geese, pigeon, Guinea Fowl	Bauchi, Yobe,	None	None	None
	Creek Road	Duck	Bauchi, Yobe,	None	None	None
	Rumuokoro	Commercial poultry	Commercial Farm	None	None	None
	Rumukwurushi	Commercial poultry	Commercial Farm	None	None	None
Farms	Obio-Akpor LGA	Commercial poultry, Turkey, Duck	Hatchery, Live Bird Market	Movement restriction, Functional Foot Dip, Bird house Screens, Routine Fumigation	None	Fowl Pox, Newcastle Disease, IBD, Fowl Thyphoid
	Ikwere LGA	Commercial poultry, Turkey, Duck	Hatchery, Live Bird Market	Movement restriction, Functional Foot Dip, Bird house Screens, Routine Fumigation	None	Fowl Pox, Newcastle Disease, IBD, Fowl Thyphoid
Zoo	Trans-amadi	Pea fowl Crown crane Parrot pigeon	Diverse	Movement restriction, Functional	None	Fowl Pox, Newcastle Disease, IBD, Fowl Thyphoid

**TABLE II: LBM BIRD SOURCES**

Source State	No. of Samples	Percentage (%)
Bauchi	58	43.9
Yobe	22	16.7
Uncertain	52	39.4
Total	132	100

**TABLE III: PERCENTAGE OF SEROPOSITIVE FROM THE DIFFERENT SOURCES OF NON-CHICKEN BIRDS SAMPLED FOR AVIAN INFLUENZA USING ELISA**

Bird Source	No. Tested	% Tested	No of Sero-positives	% Sero-positive	Total % Sero-positive
Bauchi	9	50	6	20.6	33.3
Yobe	3	16.7	0	0	0
Uncertain	6	33.3	1	3.8	5.5
Total	18	100	7	-	38.8

**DISCUSSION**

The refusal of 40% of farms previously affected and contacted for this study to be involved in this surveillance indicates that affected farmers have not completely recovered from the psychological trauma caused by previous outbreak. They have nevertheless return into business unlike the 40% percent of farms that have quit production. This agrees that Avian Influenza has socio-economic impact, some farmers are unable to return to business either because of loss of capital or psychological trauma as reported by Abdu (2010). A great percentage of birds particularly domestic ducks were seen to be sourced from the northern part of the country (Bauchi and Yobe) (Table II). Cases of HPAI in Nigeria since the 2006 incidents were predominantly from the north, mainly: Bauchi, Kaduna, Katsina Kano and Plateau. These states have high poultry production activities and are geographically close in proximity to the Hadejia Nguru wetland, where migratory birds from Europe go during winter (Cecchi *et al.*, 2008). In Nigeria, 65-80% of the estimated poultry populations are kept in backyard and

rural production system (Adene & Oguntade, 2006). In these villages, backyard and free-range poultry, multiple species including waterfowls are kept in poor biosecurity settings. Some of these birds intermingle with resident and migratory wild waterfowls that are frequently sold in live bird markets (LBMs) and large number are transported to southern parts of Nigeria along the poultry value chain (Coker *et al.*, 2014).

**TABLE IV: PERCENTAGE OF SERO-POSITIVE OF AVIAN INFLUENZA ANTIBODIES USING ENZYME LINKED IMMUNOSORBENT ASSAY AMONG AND WITHIN SPECIES**

Species	No of Serum Samples (%)	No of sero-positive	Sero-positive within species (%)	Total Sero-Positive (%)
Chicken	144 (79.1)	0	0	0
Turkey	3 (1.6)	0	0	0
Duck	26 (14.3)	6	23.0	3.3
Guinea Fowl	3 (1.6)	1	33.3	0.5
Geese	1 (0.6)	0	0	0
Parrot	1 (0.6)	0	0	0
Crown Crane	1 (0.6)	0	0	0
Total	182 (100)	7	-	3.8

Bauchi is known to be among the states in the north with the highest risk of Avian Influenza especially in waterfowls, due to the interactions with migratory birds in the Hadaji. Guinea fowl had the highest intra-species percentage seropositive of 33.3% (Table III), while ducks had the highest inter species percentage seropositive of 3.3% which agrees with the fact that waterfowls especially ducks have a role to play in the epidemiology and spread of Avian influenza as they can be carriers (Fasanmi *et al.*, 2016). Chicken were completely free (0%) from the antibodies. The seropositive samples in this study all originated from the Trans-amadi LBM, this may be because this LBM has the highest interaction of birds of different species in the state. Here only non-chicken birds were sampled to increase the chances of finding any positives where there are, and since other LBMs besides creek road only had chicken. The few bird sellers in Creek Road market which had non chicken species refused blood collection, and so only swabs were collected. Rumuokoro had low number of birds sampled because the poultry traders still harbour the fear of stigmatization, after the ordeal of the

2015 outbreak in the state. They feared that buyers will suspect a disease most likely Avian Influenza outbreak if they notice that their birds are being sampled.

## CONCLUSION

It is understood that the prevention of AI is important for animal, human health, and for economic development. It will be necessary in this regard to develop and implement policies that will encourage restriction on movement of ducks to avoid contact with migrating birds, and also from mingling with other poultry birds. Movement of ducks from the North should also be controlled to prevent spread of the virus to other parts of the country. Multispecies farming in commercial poultry farm should be discouraged to prevent introduction of the infection, especially from ducks to more susceptible poultry.

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