



Re-current Epizootics of Highly Pathogenic Avian Influenza in Nigeria: Status of Vaccination as Alternate control

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SUMMARY

Epizootic of Highly Pathogenic Avian Influenza subtype H5N1 in Nigeria was successfully contained during the first wave that lasted from 2006 to 2008 without the use of vaccine. Re-current and more severe outbreak was witnessed in 2015 and there are suspicions that some farmers may have resorted to vaccination to prevent infections in their flocks. We investigate evidence of vaccination in farms and the status of vaccination as alternate control for HPAI in Nigeria. The study was carried out in a cross section of 24 commercial poultry farms in four States in South West and North Central Nigeria. Five hundred and one sera collected randomly were screened by agar gel immunodiffusion (AGID) assay for antibody to group specific influenza A nucleoprotein. One hundred and eight sera obtained from five H5N1 infected poultry farms were also concurrently screened. Reactive sera were further analysed by Hemagglutinin Inhibition (HI) test against H5 antigen using 1% suspension of pooled washed chicken red blood cells. Only 8 out of 501 sera (1.6%) had evidence of influenza A antibody. All of the 8 samples were from one farm with 20 samples collected representing 40% seroconversion at farm level. Three out of those sera were positive for H5 at HI titer of $3\log_2$. All other sera including those obtained from HPAI infected farms were negative for influenza antibody. This study confirms limited antibody response to avian influenza subtype H5 most likely due to vaccination in one commercial flock. Vaccination against avian influenza by farmers desperate to protect their investments may lead to unregulated and suboptimal application of vaccines requiring farmers' and stakeholders' engagement to forestall negative impact.

Keywords: Avian influenza; Control measures; Recurrent outbreaks; Vaccination status.

INTRODUCTION

Highly Pathogenic Avian influenza subtype H5N1 clade 2.2 was first reported in Nigeria in 2006 and the epizootic was successfully contained without the use of vaccine (Fusaro *et al.* 2009; Oladokun *et al.* 2012). Another strain of HPAI belonging to clade 2.3.2.1c was re-introduced in 2015. The infection spread extensively across the country within weeks, also affecting neighbouring West African countries (Niger, Cameroon, Ghana, Ivory Coast and Burkina Faso) from 2015 to 2016 and was still detected in 2017 and 2018 (Monne *et al.*, 2015; Tassoni *et al.* 2016; FAO, 2018; Laleye *et al.*, 2018). The severity and spread of the 2015 outbreaks of HPAI H5N1 in Nigeria was attributed to gaps in the control programme, unlike successes that was recorded when the disease was first introduced in 2006 (Oladokun *et al.*, 2012; Shittu *et al.* 2016). Consequently, the infections spread to all agro-ecological regions accounting for over 500 cases across 20 states within a year (Akanbi *et al.*, 2016).

Nigeria being the most populous country in Africa also have abundant livestock resources including the poultry sector that contribute directly to the socio-economic, livelihoods, food security and health of the population (Robinson *et al.*, 2014). Investments in poultry business are in millions of dollars in products and services with several multi-level and interconnected industries. Poultry therefore contributes hugely to the livelihood and prosperity of the nation making investment in commercial poultry production attractive with high economic value (Akpan *et al.* 2013). Operators in the poultry sector in Nigeria are therefore wary of threats to their businesses and

seek measures to mitigate losses. While rural to urban migration has rapidly increase human population in cities, it is followed by intensification of poultry production in urban and peri-urban areas. Consequently, biosecurity lapses in intensive agriculture are both a threat to animal health and production, and have public health implications. As farming pressure increases, people and their livestock are pushed into ever-closer proximity and disease prevention even becomes more difficult. Other consequences of intermingling include interspecies transmission of zoonotic pathogens like avian influenza at the human-animal interface (Van Kerkhove *et al.*, 2012). Foregoing conditions require holistic control approaches including, biosecurity, modified agricultural practices and vaccination against pathogens of economic and public health importance (Bonfoh *et al.* 2012; Donatelli *et al.* 2016; United Nations, 2018).

Avian influenza is also suspected to be introduced through migratory birds from Asia and Europe because Nigeria lies on the path of major flyways (East-Africa-Asia flyway, Atlantic-America and Black Sea/Mediterranean flyway (Ducatez *et al.* 2006; Meseko *et al.*, 2018). The tropical climatic region with abundant wetlands, rivers and lakes, serves as suitable habitat for the stopover for rest and feeding of these migratory birds during intercontinental movement where they contaminate the environment and may infect resident birds with avian influenza (Ducatez *et al.* 2006). There is now more evidence in support of the role of wild migratory birds in the long distance transmission of HPAI from Euro-Asia to Africa mainly in autumn (FAO, 2017; Meseko *et*

al., 2018). The potential risk of re-introduction of HPAI into Nigeria therefore raises concern on the preparedness and ability of the veterinary services and infrastructures to forestall outbreaks and the possibility of the virus becoming endemic in domestic poultry following persistent circulation. Currently the control programme of the Government of Nigeria justifiably excludes vaccination of poultry birds, banking on successes of previous control programme. These include the combinations of modified stamping out by depopulation, paying compensation to farmers, decontamination of premises with improved biosecurity that was able to eliminate HPAI H5N1 clade 2.2 from Nigeria since 2008 (Oladokun *et al.*, 2012, OIE, 2013; Coker *et al.* 2014; Monne *et al.* 2015). It is equally instructive that some countries like Egypt and Indonesia that choose to vaccinate birds against HPAI during the same period were subsequently not free from the disease and had persistent outbreaks with the virus becoming enzootic (Kayali, *et al.* 2016; Tarigan *et al.*, 2018). However, the intensity of 2015 outbreaks and failure of the government to promptly pay compensation to affected farmers, which culminated in delays in culling and lack of incentives for poultry owners to promptly report outbreaks may have contributed in prolonged circulation of HPAI H5N1 clade 2.3.2.1c (Akanbi *et al.* 2016). Subsequently, dual introduction and co-circulation of two distinct genotypes in Nigeria from 2015-2017 resulted in intra clade reassortments (Tassoni *et al.*, 2016; Laleye *et al.*, 2018), and spill over transmission to other livestock (Meseko *et al.*, 2018). These episodes raise concerns over the ability to effectively control HPAI in Nigeria using stamping out and decontamination alone bearing in mind, the poor adherence to biosecurity, especially in backyard

poultry sector and the live bird markets (LBMs). Biosecurity lapses are further compounded by uncontrolled movement of birds and trade in poultry and poultry products across a wide expanse of agro-ecological zones of Nigeria. Added to the burden of circulation of H5N1 clade 2.3.2.1c, there has been sporadic detections of H5N8 clade 2.3.4.4 in farms and LBMs in 2017 and 2018 (OIE, 2017; FAO, 2018). The uncertainty that depopulation and decontamination alone may not stop HPAI incursion into Nigeria and West Africa, the huge economic investment and the associated concerns by farmers may have lured some poultry farmers to resort to clandestine vaccination as alternative or additional measure to control HPAI in order to protect their investment. This study investigated evidence of such vaccination practices in commercial farms and discusses the status of vaccination as alternate control in Nigeria.

MATERIALS AND METHODS

Sample collection

A cross sectional sampling of 108 sera was carried out in four states infected with HPAI, two each in the South West and North Central Nigeria during HPAI epidemic in 2015-2016 (Figure 1). In another sampling frame, we obtained a total of 501 sera from 24 commercial poultry farms comprising of commercial layers, broilers and grower with each sampled farm population ranging from 1000 to 10,000 birds. The first sampling in infected farms served as control for the second set of sampling in farms not known to be infected with HPAI and the samples were randomly collected based on convenience willingness of the farmers involved. Apart from

routine vaccination against Newcastle disease, fowlpox, Gumboro and other endemic diseases, none of these farms admitted

to AI vaccination,. Though the birds had no clinical signs suggestive of AI. Table I provides summary of the sample distribution according to locations.

Preparation of Antigen for AGID

Avian influenza antigen was prepared using the protocol described by the Centers for Veterinary Biologics and National Veterinary Service Laboratories (NVSL), Ames Iowa, with modifications. Briefly described, chicken embryonated eggs were inoculated avian the allantoic route with 0.1 ml of homogenised parenchymatous tissues from AI infected samples obtained from field outbreaks (Monne *et al.*, 2015) and incubated until embryo death was observed. Allantoic fluid obtained from dead embryo were tested for HA activity with 10% pooled chicken RBCs prepared with phosphate buffered saline (PBS). Chorioallantoic membranes from infected eggs were harvested and homogenised according to the methods by Woolcock (2008). The paste obtained was thrice freeze-thawed, followed by centrifugation at 2500rpm for 20 minutes. The pellet was discarded and the supernatant was treated with 0.1% formalin for virus inactivation and standardised with H5N2 antigen and reference control serum kindly provided by Institute Zooprofilattico Sperimentale delle Venezie (IZSVE), Padova Italy. All procedures were carried out in a biosafety cabinet using standard biosafety SOP and protocols.



FIGURE 1.: Map of Nigeria showing AIV infected but seronegative States and States

Table I: Distribution of samples and results of HPAI AGID and HI serology tests

State	No. sera collected	Serology results	
		AGID	HI
Lagos	161 (7 farms)	*8 (5%)	3(2%) : GMT (1.41)
Ogun	120 (6 farms)	0	0
Plateau	140 (7 farms) 70 (3 farms) ⁺	0	0
Bauchi	80 (4 farms) 38 (2 farms) ⁺	0	0
Total	501(24 farms) 108 (5 farms) ⁺	8 0	2 0

suspected to be involved in AIV vaccination in poultry that were sampled and tested.

Agar gel immunodiffusion

Sera were screened by AGID test to detect group specific ribonucleoprotein (RNP) antigen to influenza A and matrix (M) protein according to the protocol described in the OIE manual (2015). Tests were organised by placing test serum adjacent to a known positive antigen (prepared and standardised in-house as described earlier). Thereafter the reactants were incubated at 35°C in a humidified incubator and examined after 24 hours for precipitin lines formed to the homologous antigen of the test antibody.

Hemagglutination Inhibition

Hemagglutination-Inhibition (HI) test was carried out on influenza A positive sera from the AGID assay using H5 subtype as antigen. The test was performed in a 96-well V-bottom microtitre plate with 1% suspension of pooled washed chicken red blood cells prepared in PBS as indicator. Positive HI titers of 4log₂ indicated inhibition at a serum dilution of 1/16 (2⁴) when expressed as the reciprocal (OIE, 2015). In this assay, 0.025 ml of PBS was dispensed with a micropipette into each well of a V-bottomed microtiter plate followed by 0.025 ml of serum into the first well of the plate. Two fold serial dilutions of the serum were made across the plate. Thereafter, 0.025 ml of 4 HAU of antigen was added to each well and incubated at room temperature for 30 minutes. Subsequently 0.025 ml of 1% pooled chicken RBCs prepared in

PBS was added to each well and thoroughly mixed. Second incubation of 30 minutes at room temperature was observed for the time it took control RBCs to settle to a distinct button in the microtiter plate. The HI titer (the highest dilution of serum that caused complete inhibition of 4 HAU of antigen) was assessed by tilting the plates and only wells in which the RBCs streamed at the same rate as the control wells were considered HI positive.

RESULTS AND DISCUSSION

This investigation showed evidence of antibody response to avian influenza in commercial flock in South West Nigeria as eight (1.6%) sera out of 501 collected from apparently healthy birds had evidence of influenza A antibody. Distinct line of precipitation was recorded when antigen and antiserum in the immune diffusion test combined. AGID test on 108 sera obtained from poultry farms known to be infected with HPAI H5N1 by RT-PCR reported in another study (Shittu *et al.* 2016) and tested in the current study were negative. Further analysis of influenza A positive sera by HI showed 3 sera (2% by state and 15% by farm) were positive for H5 antibody; 2 samples at marginal titre of 3 log₂ and only one sample had HI titre of 4 log₂ and the overall GMT of 1.41 was calculated using the method by Perozo *et al.* (2008). The H5 antibody detected in this investigation is most likely due to seroconversion induced by vaccination which is speculated to have been clandestinely applied by some farmers in the region. One Avian Influenza vaccine bottle was recovered from one farm that declined further inquiry (Figure 2).

There are unconfirmed reports of vaccination in southwestern region, the hub of poultry production in Nigeria by farmers that are desperate to protect their business. This may lead

to such clandestine, unregulated and inappropriate application of non-standardized vaccine and vaccination practice with resultant misapplication and poor antibody responses. In our study, antibodies were detected but the titer was marginal at less than $4\log_2$, a conventionally consideration for HI positivity but less than protective titre (OIE/FAO, 2019). The potency of



FIGURE 2. : A bottle of Avian influenza vaccine recovered from a poultry farm is South West Nigeria.

influenza A vaccine is generally evaluated by testing the ability of the vaccine to induce a significantly high HI titer of $4-5\log_2$ which also correlate with protection against field infection (Montomoli *et al.*, 2010). According to Hannoun (2004), HI antibody titers are read as the reciprocal of the highest serum dilution causing complete inhibition of agglutination and the results can be presented as the percentage conversion which have been defined using vaccine or wild strains. The vaccine (s) that was probably used in the poultry farm evidently seroconverted but did not induce significant HI titer though sera were positive by both AGID and HI and only one farm out of 24 had evidence of

seroconversion to influenza antigen. The 40% seroconversion recorded in that particular farm showed that the vaccine used (proprietary identity could not be linked to that shown in figure 1) was broadly administered in the farm. In this investigation (i) the first category of samples collected from known HPAI infected farms were all negative and justifiably so as HPAI susceptible chickens usually die before developing antibodies (OIE, 2014) . (11) In the second category of samples collected from apparently healthy birds, antibody was detected and not caused by infection as no previous or current outbreak was attributed to farms in the location (Akanbi *et al* 2016). Though sampling was not representative of all farms in southwest region or Nigeria as a country and sampling frame was also not systematic but targeted at suspect area, vaccine use may not be as widely practised as envisaged which is understandably so because vaccination against AI is not permitted officially by the government authorities. The finding also poses questions on the quality and potency of unofficial vaccines due to breaks in cold chain, since they are usually smuggled into the country in order to evade regulatory agencies at ports of entry.

Sera obtained from known HPAI infected farms were negative for AI antibody and not surprisingly so because of the patho-biological characteristics of HPAI H5N1 virus. In previous studies, naturally infected birds with HPAI H5N1 die shortly without developing antibody and were seronegative (Joannis *et al.*, 2008) because death occurred shortly after infection without sufficient time (2-3weeks) for humoral antibody development (OIE, 2014). Though in an AGID and HI experimental set up by Brown *et al* (2006) they were able to detect post-inoculation antibodies in surviving waterfowls. The period of

seroconversion is usually up to two weeks and HPAI H5N1 infection in domestic poultry especially chickens and turkey is usually 1 to 3 days period from infection to clinico-pathological findings. The rapid onset of mortality does not allow antibody development and detection. Hence all the sera from infected farms collected before depopulation and analysed in our study were negative. This also showed that the antibody detected in the second category of samples (apparently healthy birds) in the study is not likely due to infection but vaccination due to its specificity to H5. Though we are not able to determine what types of vaccines were used (inactivated or live attenuated) in this study but there is laboratory evidence that the vaccine antigen is H5 specific at least in three samples. In previous studies, analysis of antibody levels following vaccination with inactivated virus or natural exposure to pathogen showed higher post-vaccination HI antibody titers associated with lower rates of infection on subsequent exposure to influenza virus (Hannoun, 2004). Lack of protective HI titer as observed in this study portends a more dangerous scenario where farmers may be under false security while avian influenza circulates in poultry flocks with all the attendant risks.

HPAI control strategies may include vaccination of poultry in countries where stamping out alone is not sufficient because vaccines also offer effective tool to reduce virus shedding and risk of transmission as well as lower potential zoonotic transmission (Lee and Suarez, 2005; Ellis *et al.*, 2006; Capua and Marangon 2006). Nigeria was under pressure to adopt vaccination as a control strategy in 2006 with reference to some countries like Vietnam and China that had used vaccine with certain degree of success (To *et al.*, 2007). This

was the same time when vaccination was adopted in Egypt in 2006; unfortunately the impact of vaccination on the control of AI vaccination in Egypt has been poor despite continuous vaccination of poultry birds. Frequent outbreaks in poultry have also been source of human infections and deaths (Peyre *et al.*, 2009). The limitations identified in countries where vaccination has failed to control HPAI such as Egypt include mass vaccination without outbreak investigation and management, failure to maintain strict bio-security measures, lack of post vaccination monitoring, concurrent disease conditions, insufficient trainings in the application of vaccination and above all, weak institutions and infrastructures (Domenech *et al.* 2009; Peyre *et al.*, 2009). Similarly, failure of vaccination programme against avian influenza in Indonesia was attributed to a number of reasons including the use of an unlicensed virus seed strain and induction of low levels of protective antibody because of an insufficient quantity of vaccine antigen, appearance of drift variant field viruses that partially or completely overcame commercial vaccine-induced immunity (Swayne et al. 2015).

In the absence of sustained disease surveillance, laboratory and field trials on the efficacy of vaccines as well as such post vaccination monitoring for the differentiation of infected from vaccinated animals (DIVA), unregulated vaccination in Nigeria and anywhere should be discouraged. This would minimise the likelihood of avian influenza epizootics in poultry, human exposure and potentials for influenza pandemic, because unregulated application of vaccine is a recipe for disaster (Capua *et al.* 2004; James-Berry 2013). While vaccination has been shown to boost immune response in the vaccinated, increase resistance to field outbreaks, reduce virus

shedding and transmission, it can be a powerful tool to support eradication programmes if used in conjunction with other control methods and designed as is fit for peculiar agro-ecology (Capua and Marangon 2006).

In the best interest of avian influenza control in Nigeria, while still implementing measures that works, poultry farmers' engagement should be initiated without further delay. Government should therefore regularly hold stakeholder's consultation, discuss the merits and demerits of vaccination programmes and get a consensus on vaccination no vaccination through consultation. This is imperative in building trust in order to have full cooperation to agree on the best approaches. Such may include canvassing reasons why vaccination option may still be delayed or to monitor regulated use of vaccines, field application and other considerations including exit strategy. It is important to forestall unending cycles of vaccine usage as it is currently experience with Newcastle disease. Negligence on the part of government and inappropriate application of vaccines by poultry farmers may create more problems for the poultry sector, food security and public health.

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