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ANTIGENIC DETECTION OF INFLUENZA A VIRUS (H7N9 STRAIN) AMONG POULTRY BIRDS IN GBOKO, BENUE STATE.

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ABSTRACT

Influenza A viruses cause contagious respiratory illness resulting in annual epidemics and occasional pandemics. The human death toll of influenza epidemics worldwide is in the range of 250,000 to 500,000 each year. They have also been identified as resident in the animal population where they are native reservoirs. In Nigeria, the poultry and livestock industry has suffered enormous losses to the rampaging and continually mutating Influenza virus that has posed a major zoonotic threat to man. This study involved 92 chickens even with the mildest of respiratory signs that were screened for H7N9 antigen using indirect enzyme linked immunosorbent assay (ELISA). The result from the laboratory assay revealed that only one (1.09%) of the birds (an adult hen) was positive for H7N9. The presence of this virus, though at a low rate could suggest the bird had been naturally infected. The findings in this research also draws our attention to the reality that the H7 strain is present in Gboko, Benue State of Nigeria, especially as sub-optimal levels of the antigen were recorded (7.61%). Establishing this zoonotic virus in birds in this study location, it logically follows that exposed humans should take utmost care to prevent onward transmission to humans. The findings from this research is also a further call to intensify efforts at continuous surveillance to include potential carriers and reservoirs of the virus in order to prevent outbreak in commercial/homestead poultry and human populations in Nigeria and also globally.

Keywords: **Avian influenza, Epidemics, Zoonotic, Poultry, Outbreak, Benue State of Nigeria.**

INTRODUCTION

Avian Influenza (AI) is a highly contagious disease of global phenomenon caused by type A Influenza viruses (Capua and Alexander, 2009). It is not the colossal economic loss incurred in the poultry industry in an event of the disease outbreak that is of concern alone, but also the human health and lives of other susceptible livestock that are put at great risk. Also known as bird flu or avian flu, the disease is primarily of birds of all ages and can lead to high mortality ranging from 50-100% within 2 days in a flock, from its on-set (Swayne and Suarez, 2000).

Type A Influenza viruses are negative-sense, single-stranded, segmented RNA viruses of the genus *Influenzavirus* A and of the family *Orthomyxoviridae*. Being highly contagious pathogens, they can cause respiratory disease in humans, poultry, pigs and horses (Webster *et al.*, 1992). They can be classified into subtypes according to the antigens of the hemagglutinin (H) and neuraminidase (N) projections on their surfaces. There are 16 hemagglutinin (H) and 9 neuraminidase (N) subtypes of the influenza A viruses and AI viruses have representatives in all of these subtypes (Fouchier *et al.*, 2005). Furthermore, they have been grouped for chicken hosts on the basis of their pathogenicity into highly pathogenic avian influenza (HPAI) viruses and low pathogenic avian influenza (LPAI) viruses based on the rate of mortality which could be as high as 75-100% for HPAI and LPAI with a much lower mortality rate (Alexander, 2000). To date, all HPAI viruses belong to either the H5 or H7 subtypes. For example, the classical fowl plague virus is H7N7, but not all H5 and H7 viruses are virulent for poultry (Alexander, 2000).

Nigeria was the first country in Africa to be affected by the Avian Influenza type A H5N1 virus, with HPAI outbreaks initially reported at a commercial farm in Kaduna State in January, 2006 (Fusaro *et al*., 2009; Ekong *et al*., 2012). Between 2006 and 2008, a total of 1,654 suspected outbreaks were reported from 32 of the 36 States and the Federal Capital Territory (FCT), of which 299 were confirmed to be HPAI H5N1 positive from 27 states and FCT (Ekong *et al*., 2012; Henning *et al*., 2012). In Nigeria which has a poultry industry of about 160 million birds estimated at US$ 250 million (Federal Department of Livestock and Pest Control Services, 2007), AI has been reported in several domestic and wild birds such as chickens (Aiki-Raji *et al*., 2015), ducks (Coker *et al*., 2014), waterfowls (Meseko *et al*., 2007), and spur-winged geese or whistling ducks (Snoeck *et al*., 2011).

Strengthening continued efforts at deepening surveillance in Nigeria and coupled with the large scale of poultry activities in the study area, this study was conducted to detect the presence of Influenza A (H7N9) antigen among poultry birds and to also provide base information on its prevalence or otherwise.

MATERIALS AND METHOD

The study was conducted using cloacal swabs of poultry birds that present with any respiratory sign. These birds were brought mainly from rural areas, the Gboko metropolis and the peri-urban areas of Gboko. 92 samples were obtained by inserting a sterile swab into the cloaca of the birds, allowing it to stay for a few seconds and slowly withdrawing it using a rotating movement, and stored at -20˚C before analysis using the indirect enzyme-linked immunosorbent assay (ELISA).

Reagents provided with the ELISA kit:

1. Antibody coated plate. Anti AI (Ag) antibody on microtiter wells.
2. Conjugate reagent. Anti AI (Ag): Alkaline Phosphatase in Tris buffer with protein stabilizers, inert red dye and sodium azide preservative (0.1% w/v).
3. Substrate tablets. P-Nitrophenyl phosphate (pNPP) tablets to dissolve with substrate buffer.

4. Substrate buffer. Diethanolamine buffer with enzyme co-factors.
5. Stop solution. Sodium hydroxide in Diethanolamine buffer.
6. Phosphate Buffered Saline (PBS) for swab processing and negative control.
7. Positive control. Inactivated Avian Influenza freeze-dried antigen.
8. Wash buffer sachets. Powdered PBS with Tween.

Materials and equipment required (not provided with kit):

1. Precision pipettes and disposable tips.
2. 12 channel pipette/repeater pipette.
3. Microtitre Plate Reader with 405nm filter.
4. Microtitre Plate washer.

Cloacal swab samples were taken into 1ml of PBS and shaken before testing. The Anti AI Ag coated plate was removed from sealed bag and location of samples was recorded on template (i.e. cloaca). 100 µl of PBS was added as negative control into wells A1 and B1. 100 µl of positive control was added into wells C1 and D1. 100 µl of samples were added into the appropriate wells. The plate was covered with lid and incubated at room temperature for 60 minutes. The contents of wells were aspirated and washed 4 times with wash buffer (350µl per well). The plate was inverted and tapped firmly on absorbent paper. 100 µl of Conjugate reagent was added into each well. The plate was covered with lid and incubated at room temperature for 60 minutes. The same procedure to wash the wells as above was followed. 100 µl of prepared Substrate reagent was added into the appropriate wells. The plate was covered with lid and incubated at room temperature for 30 minutes. 100 µl of Stop solution was added to appropriate wells to stop reaction. The microtitre plate reader was blanked on air and the absorbance of controls and test samples recorded by reading at 405nm. Samples with S/N ratio greater than the cut-off value of 1.5 contain AI antigen and are considered POSITIVE. Those with S/N ratio lower than the cut-off value contain sub-optimal Ag levels, while samples considered as NEGATIVE have S/N ratio tending to zero (BioChek®, 2006).

RESULTS

Table 1 shows the result of the samples analyzed in relation to age. Of the 56 adult birds examined, 1 (1.09%) was found to be positive, 48 (52.17%) were negative and 7 (7.61%) contained the antigen at sub-optimal levels. Out of the 36 young birds examined, none was positive, 36 (39.13%) were negative and none was found to contain the antigen (Ag) even at sub-optimal levels.

Table 1: Results of ELISA testing of poultry birds for H7N9 viral antigen in relation to age.

Age	Collection Site	Number Examined	Results		
			Positive (%)	Negative (%)	Sub-optimal (%)
Adult	Cloaca	56	1 (1.09)	48 (52.17)	7 (7.61)
Young	Cloaca	36	0 (0.00)	36 (39.13)	0 (0.00)
Total		92	1 (1.09)	84 (91.30)	7 (7.61)

Table 2 shows the number of samples that were observed in relation to sex. No cock (0) was found to be positive. All 41 (44.56%) were found to be negative and none (0) was found to contain sub-optimal antigen (Ag) levels. Out of the 51 hens examined, 1 was found to be positive (1.09%), 43 (46.74%) were negative, and 7 (7.61%) were found to contain sub-optimal antigen (Ag) levels.

Table 2: Results of ELISA testing of poultry birds for H7N9 viral antigen in relation to sex.

Sex	Collection Site	Number Examined	Results		
			Positive (%)	Negative (%)	Sub-optimal (%)
Cock	Cloaca	41	0 (0.00)	41 (44.56)	0 (0.00)
Hen	Cloaca	51	1 (1.09)	43 (46.74)	7 (7.61)
Total		92	1 (1.09)	84 (91.30)	7 (7.61)

DISCUSSION

Influenza A (H7N9) was detected in just one (1.09%) of the chickens sampled (Adult hen). Although the need for additional studies is recognized, this result shows that viral antigen can be found among poultry even though relatively low titres of this virus were found in the birds sampled. This finding could also suggest natural infection of the bird.

Several different subtypes of influenza viruses have been isolated from domestic chickens. The two highly pathogenic subtypes H5 and H7 have been isolated from chickens for many years (Stubbs, 1965) and this work has also shown presence of the H7 strain here in Gboko, Benue State. Other influenza viruses isolated in chickens include the following subtypes: H1 (Siebinga & De Boer, 1988), H2 (Schafer *et al.,* 1983), H4 (Donis *et al.,* 1989), H6 (Lin *et al.,* 1994) and H10 (Feldman *et al*., 1988). Unlike the highly pathogenic strains, these less pathogenic viruses

have been detected sporadically and appear to be confined to the poultry species from which they had been isolated.

The transmission of influenza viruses to domestic chickens, the establishment of stable lineages of influenza virus strains in chickens and the possible transmission of these viruses to humans suggest that the role of domestic chickens in the ecology of influenza viruses may be expanding (Coker, 2014). This evidence is bared in this study thus the importance of poultry birds in the epidemiology of the human infection cannot be overemphasized. As domestic chickens become an important source of protein for humans, their increasing population raises the possibility of an increased role for them in the ecology of influenza. As was observed from the results, the viral antigen was readily available in high titre in one of the birds. This suggests that the viral antigen could as well have been present in more than half of the chickens sampled at sub-optimal levels and this could translate to a possible potent transmission of the virus to humans.

Since March 2013, sporadic transmission of avian influenza H7N9, not previously known in humans, was reported in several fatal cases of avian influenza infection in China (WHO, 2013). Though the Asian H7N9 virus is a low pathogenic strain in poultry, the clinical outcome of infection in humans is severe. This is as a result of genetic changes in amino acid sequences which have been associated with adaptations leading to enhanced virus binding to, and replication in, mammalian respiratory cells with increased severity of infections (Gao *et al.,* 2013).

This study suggests that the circulation of non-pathogenic influenza viruses in commercial poultry may be significantly underestimated because these infections are asymptomatic and are often not detected. This theory raises some concern because of the episodes of the transmission of low pathogenicity H7N9 influenza viruses to humans (Schmirring, 2013). This study therefore serves as a reminder that avian influenza circulates in Nigeria.

CONCLUSION

This report shows the importance of continuous surveillance of the avian influenza virus especially in geographical areas with a history of outbreaks. This study also emphasizes the need to include potential carriers and reservoirs of the virus in surveillance programs in order to prevent outbreak in commercial poultry and human populations in Nigeria and also globally.

CONFLICT OF INTEREST

The authors hereby state that there is no conflict of interest in the course of this study.

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