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AVIAN INFLUENZA A VIRUS SURVEILLANCE IN RESERVOIR DOMESTIC DUCKS (*Anas platyrhynchos domesticus*) IN MAIDUGURI METROPOLITAN COMMUNITY OF NORTH-EASTERN NIGERIA: A NEED FOR ONE-HEALTH APPROACH

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ABSTRACT

The domestic ducks (*Anas platyrhynchos domesticus*) are natural reservoir hosts of avian influenza A virus (AIV) and have since remained significant in the ecology and epidemiology of the virus globally. Continued local surveillance for AIV in this specie is critical to assessing the risks of potential spreading to domestic poultry, other animal species, and zoonotic transmission to humans. In this study, we investigate the status of AIV in domestic waterfowls in Maiduguri metropolis. Twenty-eight cloacal and oropharyngeal swab samples were collected from apparently healthy domestic ducks from November to December 2021 in Bulumkutu (n=2), Giwa Barracks (n=5), Kasuwan Shanu (n=4), Maimalari Barracks (n=5), Mairi (n=5), Premier (n=2) and Shehuri (n=4). Samples were screened for AIV using the real-time RT-PCR molecular assay. A total prevalence of 60.7% (CI: 41.99 –77.32) was recorded. Prevalence based on locations was higher in Bulumkutu 100% (CI:22.36 - 100), Kasuwan Shanu 100% (CI: 22.36 - 100), and Shehuri 100% (CI:47.29 - 100). In Maimalari Barracks and Mairi, prevalence of 60% (18.24 – 92.65) each was recorded respectively. While samples from Giwa Barracks and Gomari had a prevalence of 40% (CI: 7.346 – 81.76) and 33.3% (1.667 – 86.8). AIV was not detected in samples from premier (0%). The result of this study revealed the status of AIV in domestic ducks in Maiduguri metropolis. Thus, this early warning call the need for AIV prevention and control in the region and using the one-health approach to access the zoonotic potential of the virus in the domestic ducks (*Anas platyrhynchos domesticus*).

Keywords: Domestic Ducks, Influenza A, Maiduguri Metropolis, Reservoir Surveillance, One-Health

INTRODUCTION

Avian influenza (AI) is enveloped and single-stranded segmented negative-sense RNA virus, caused by influenza A viruses of the family *Orthomyxoviridae* , genus *Alphainfluenzavirus* (ICTV, 2019, OIE, 2021). The *Alphainfluenzavirus* (Influenza A) viruses are the only Orthomyxoviruses known to naturally infect birds (Swayne and Sims, 2021). Influenza A viruses nucleoproteins and matrix proteins are antigenically related, although they are grouped into subtypes based on their Haemagglutinin (H) and neuraminidase (N) antigens (WHO, 1980). So far, eighteen hemagglutinins (HA; H1–H18) and eleven neuraminidase antigens (NA; N1–N11) have been reported in birds and bats (Tong *et al.*, 2013; ICTV 2019; Swayne *et al.*, 2020 WOAH, 2021). The terrestrial health code of the world organization for animal health (WOAH) define the disease "avian influenza" as an infection of poultry caused by any influenza A virus with high pathogenicity (HPAI) (an intra-venous pathogenicity index of > 1.2 in chickens), and by H5 and H7 subtypes with low pathogenicity (LPAI) (WOAH, 2021). Only viruses in the aforementioned categories are required to be reported to the *WOAH b*y member States, while control of other subtypes in poultry is left to the country's discretion. Some avian influenza virus strains, mostly of the H5, H7, and H9 subtypes, have produced sporadic zoonotic illnesses, and these three subtypes have been identified as potential pandemic hazards should subsequent mutations permit persistent human-to-human transmission (Cox *et al*., 2017). In Nigeria, the first outbreak of highly pathogenic avian influenza virus (HPAIV) H5N1 was first reported in 2006 which caused huge economic losses in the Nigerian poultry industry, and these was attributed to the activities of migratory birds and trade in poultry products as possible sources of the introduction (Meseko *et al*, 2010; Twinning, 2021) and that outbreak persisted until 2008 (Chieloka *et al*., 2020), across twenty-five States in ninety-seven local government areas (LGAs) with over 1.2 million poultry affected, estimated at 1.8 million dollars (Chieloka *et al*., 2020). In 2013, Nigeria was declared free of Highly Pathogenic Avian influenza (FMARD, 2013; Chieloka, 2019). However, isolation of LPAI H5N2 in a pool of ducks at the live bird market, (LBM) in Oyo State Nigeria in 2010 reported by Coker *et al*., (2014), it is possible that this has lent credence to claims that AI including low pathogenic subtypes has wide spread in Nigeria. In 2015, the recurrence of HPAI, H5N1, and the introduction of a reassortant HPAI strain H5N8 isolated from a backyard poultry farm in Kano State and LBM in Lagos State, Nigeria, resulted in the loss of over 3.7 million poultry nationwide, leading to economic losses of over 7.2 million dollars (Monne *et al*., 2015; Chieloka 2020; Chieloka *et al*., 2020). From 2015-2017, the hotspots for avian influenza outbreaks in Nigeria were clustered in six states and five geopolitical zones: Kano State (North-west), Plateau State and Abuja-FCT (North-central), Bauchi State (North-east), Oyo State (South-west), and Rivers State (South-south). In 2021, new outbreak of AIV was reported in more than twenty States across Nigeria with a novel strain of HPAI H5N1/H5N8 clade 2.3.4.4b been introduced into the country

(Meseko *et al*., 2023). This was first confirmed in Kano State in January 2021. Clade 2.3.4.4b has been circulating ever since and has become one of the most important threats for both poultry industries and small holders globally (Abolnik *et al*., 2019). At present, H5N1, H5N2, H5N6, H5N8, and H9N2 strains have been reported in Nigeria since the first outbreak in 2006 (Meseko *et al*., 2010; FMARD, 2013; Lalaye *et al*., 2021; Shittu *et al*., 2021; Sulaiman *et al*., 2021; Wungak *et al*., 2021; Ameji *et al*., 2022). Poor inter-State surveillance, control of movement of poultry and their products, illegal trade of live birds where farmers sell their diseased birds upon AIV suspect, lack of incentives or compensation to farmers and inadequate training and re-training of farmers on AIV biosecurity and control measures may all be considered as some of the factors contributing to continous and persistent HPAIV outbreak and spread in Nigeria. The study aimed at investigating avian influenza A virus in domestic ducks (*Anas platyrhynchos domesticus*) within Maiduguri metropolis with the aim to provide information on the appropriate preventive and control measures necessary for public health actions. The domestic ducks (*Anas platyrhynchos domesticus*) are known to be natural reservoir hosts of influenza A viruses and play important role in the ecological transmission of HPAI to domestic poultry.

MATERIALS AND METHODS

Study Area

The study was carried out in Maiduguri the capital of Borno State and is the largest city in North-East geopolitical zone of Nigeria. Maiduguri is located on geo-coordinates 11°30′N 13°00′E. It is bordered by Yobe State to the west, Gombe State to the South-West, and Adamawa State to the South (Inuwa *et al*., 2023). Being the only State in Nigeria to border three foreign countries, it share international border with the Republic of Niger to the North, Chad to the North-East and Cameroon to the East. Borno State northern border represents Nigeria portion of Lake Chad with ecological suitability of wetland for migratory birds. The climate of Maiduguri has March-April as the hottest period of the year with temperature ranging between 30º - 40° C. It is usually cold and dry during the months of November to January (Inuwa *et al*., 2023).

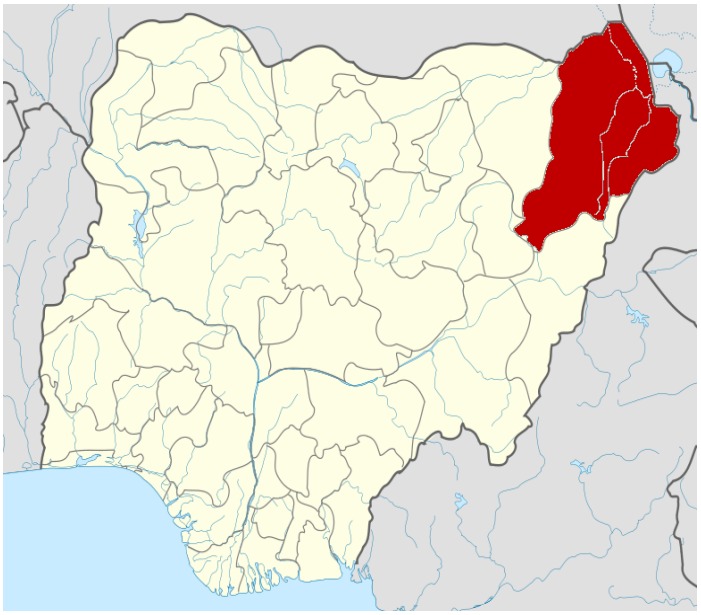

Figure 1: Map of Nigeria showing Maiduguri, Borno State

Sample collection

By convenient method of sampling from end of November to December 2021, representing cold harmattan season, a total number of 28 cloacal and oropharyngeal swab samples each were collected from different household within Maiduguri metropolis (Figure 1) from healthy free range domestic ducks in a virus transport medium (VTM) using a sterile swabs sticks. Samples were collected from: Bulumkutu (n = 2), Giwa Barracks (n = 5), Kasuwan Shanu (n = 4), Maimalari Barracks (n = 5), Mairi (n = 5), Premier (n = 2) and Shehuri (n = 4) (Table 1). Samples were stored and transported in a cool box to the National Veterinary Research Institute, Vom, Plateau State and were kept in -80ºC freezers before further analysis.

RNA Extraction

Viral RNAs from cloacal and oropharyngeal swabs were extracted from 100 µl of each sample after votexing using RNeasy® Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions, then eluted in 50 µl sterilized RNase free water and was stored at −80°C before use.

Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) Assay

Real-time RT-PCR reactions was performed using Qiagen QuantiTect® Multiplex RT-PCR Kit as previously reported by Spackman *et al*., (2002). This protocol amplifies different regions within the matrix (M) gene which is well-conserved among avian influenza A viruses of all subtypes. The master mix contained: 12.5 µl of 2× RT-PCR QuantiTect Multiplex master mix, 300 nM of each primer, 100 nM Taqman® hydrolysis probe, 0.2 µl of Enzyme mix and 5 µl of viral RNA extracts in a final volume of 25 µl. Nuclease free water and previously established known positive sample for avian influenza were used as negative and positive controls respectively. The real-time RT-PCR was performed on Rotor Gene Q system (Qiagen, Germany) with the following thermal cycling condition: 50°C for 20 minutes, 95°C for 15 minutes, then 40 cycles of 45 seconds at 94°C and 45 seconds at 60°C.

Data Analysis
Descriptive statistics such as frequency and percentages were used to present estimated of prevalence in the study.

RESULTS AND DISCUSSION

In this study, a total prevalence of 60.7% (17/28) was recorded. Based on locations of sampling showed a higher prevalence in Bulumkutu 100% (CI:22.36 - 100), Kasuwan Shanu 100% (CI: 22.36 - 100), and Shehuri 100% (CI:47.29 - 100) was recorded. In Maimalari Barracks and Mairi, prevalence of 60% (18.24 – 92.65) each was recorded respectively. While samples from Giwa Barracks and Gomari had a prevalence of 40% (CI: 7.346 – 81.76) and 33.3% (1.667 – 86.8). AIV was not detected in samples from premier (0%) (Table 1). Total prevalence of 60.7% recorded in this study is higher than previous reports across some states in Nigeria 4.65% in Gombe State by Meseko *et al*., 2010, 0% in reported Sokoto by Nwankwo *et al*. (2012), 0% reported across 8 states in Nigeria by Vakuru *et al*. (2012), 0% also reported in Benue State by Semeka *et al*., (2013), 13% was reported by Meseko *et al*. (2010) in samples collected from domestic ducks in Ibadan, Oyo State.

Table 1: Real-time RT-PCR Results for AIV from Different Locations in Maiduguri Metropolis

Sample Location	No. Samples	No Positive	Prevalence (%)	Confidence Interval (95%)
Bulumkutu	2	2	100	22.36 - 100
Giwa Barracks	5	2	40	7.346 – 81.76
Gomari	3	1	33.3	1.667 – 86.8
Kasuwan Shanu	2	2	100	22.36 - 100
Maimalari Barracks	5	3	60	18.24 – 92.65
Mairi	5	3	60	18.24 – 92.65
Premier	2	0	0	0.0 – 77.64
Shehuri	4	4	100	47.29 - 100
	28	**17 (60.7)**		**41.99 – 77.32**

In another study carried out by Adole *et al*, (2019) in free-range domestic ducks in Benue State, using the conventional RT-PCR technique, 0% prevalence was recorded. The high prevalence recorded in this study may be attributed to the period of sampling. As this was carried out in ending month of November to December which coincide with the cold hamattan season which favour ecological and maintenance the ecology of the AIVs. Variations in molecular assays might have also contributed to the higher prevalence recorded in this study than in previous reports from other parts of the country. The real-time RT-PCR is a very rapid molecular diagnostic technique with high sensitivity and specificity (Starick *et al*., 2005; Bitrus *et al* 2020; WOAH, 2021). With the exception of one location where AIV was not detected out of the eight areas sampled, AIV was detected in all the other seven locations. The study was limited with small sample size as well as our inability to subtype the virus detected. However, this information has revealed the status of AIV in domestic duck population in Maiduguri metropolis. An implication that other poultry species, livestock and even humans are at high risk of infection and future outbreak for influenza A virus. This may have negative impact on both public and animal health as well as food security. This may exacerbate the negative socio-economic impact of the populace already experiencing security challenges in the area.

CONCLUSION

Domestic ducks remain natural reservoirs for AIVs causing repeated outbreaks and continue spread of the virus in backyards, commercials and live bird markets. This study therefore recommends wide surveillance using the one-health approach, virus isolation and characterization to identify the current subtypes of AIVs circulating in waterfowls in the study area for early warnings and control of AIVs in the region. One-Health approach application in the management and control of diseases such as the avian influenza will be very crucial in addressing zoonotic potential of the virus in the domestic ducks (*Anas platyrhynchos domesticus*) which are known to be natural reservoir hosts of influenza A viruses continued playing key role in HPAI maintenance and transmission. It is therefore paramount to take local surveillance of zoonotic disease important to ensure both local and global health security.

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