

## INFLUENZA A VIRUS IN PIGEONS (*COLUMBA LIVIA DOMESTICA*): PREVALENCE AND ASSOCIATED RISK FACTORS IN MAIDUGURI METROPOLIS, BORNO STATE, NIGERIA

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### ABSTRACT

Influenza A virus (IAV) is an important transboundary disease that has continued to pose a threat to both animal and public health worldwide. Pigeons are kept as pets or companion birds; therefore, are in direct contact with humans, poultry, and animal populations. However, there still exist questionable reports about their susceptibility to IAV and their potential role as a link in IAV ecology. This study investigated the influenza A virus in pigeons in Maiduguri metropolis. A total of 71 swab samples were collected from pigeons in two locations: live bird markets (n = 30) and households (n = 41). Total viral nucleic acid was extracted and tested for the IAV matrix gene (M gene) using Real time polymerase chain reaction (rRT-PCR). An overall prevalence of 12.7% (CI: 6.55%, 18.81%) was recorded. The study identified location, age, and breed of pigeons as the three key risk factors associated with influenza A virus infection in pigeons: Pigeons from live bird markets were 5.9 times more likely to be infected with IAV, while younger pigeons were 4.3 times more likely to be infected. Additionally, local breeds were 10.37 times more likely to be infected. These findings significantly contribute to the understanding of the epidemiology of IAV in pigeons and underscore the importance of sustained surveillance and monitoring of the virus in Nigeria.

Keywords: Households, Influenza A Virus; Live Bird Market; Maiduguri, Pigeons

### INTRODUCTION

Globally, influenza represents the greatest public health threat in terms of both seasonal and potential pandemic disease among respiratory viruses (Hayden, 2006; Jallow *et al.*, 2025). There are four genera of influenza viruses, namely: Alpha influenza virus (influenza A virus), Betainfluenzavirus (influenza B virus), Delta influenza virus (influenza D virus) and Gamma influenza virus (influenza C virus) (Mostafa *et al.*, 2024). Influenza A (IAV) viruses belong to the family Orthomyxoviridae and are subtyped based on the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). To date, 19 HA (H1-H19)

and 11 NA (N1-N11) subtypes of IAV have been identified in birds and bats (Mostafa *et al.*, 2018; Fereidouni *et al.*, 2023; Karakus *et al.*, 2024). Based on their pathogenicity in poultry, they are classified into two pathotypes: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) (Swayne *et al.*, 2008).

Most avian species, including domestic, pet, and wild birds, are natural and experimental hosts of avian influenza (AI) viruses. Aquatic wild birds, such as ducks, are a natural reservoir of AI viruses (Swayne & Suarez, 2000).

Before 2002, it was believed that HPAI viruses were incapable of infecting or causing mortality in wild aquatic birds. However, after 2002, numerous reports showed that

wild birds were infected with HPA (Perkins & Swayne, 2002; Ellis *et al.*, 2004; Brown, *et al.*, 2007). There are serious concerns for public health due to the fact that AI viruses can mutate, recombine, adapt and infect other hosts (Boon *et al.*, 2007).

AI viruses proliferate in the gastrointestinal and respiratory tracts and are transmitted via respiratory aerosols or faecal-oral routes (Simancas-Racines *et al.*, 2023). Birds from various orders are susceptible to AI, but susceptibility, severity, and symptoms vary among species. Pigeon covers more than 40 genera and 300 species of the family *Columbidae* within the order Columbiformes globally (Peter *et al.*, 2022). They are kept as pet or companion birds, scavenge, and dwell in areas with water, food, and nesting sites; therefore, they are in direct contact with humans, poultry, and animals. Their ability to fly long distances may serve as great potential host for the spread and transmission of AI viruses between migrating waterfowl populations and poultry, or, in the event of a disease outbreak, to spread viruses between poultry sites (Abolnik, 2014).

Recently, commercial pigeon farming as a source of meat for human consumption has emerged and is on the increase; however, information on their susceptibility to diseases such as AIV infection under natural conditions is limited, as there are still controversies among researchers and reports regarding this issue (Mansour *et al.*, 2014).

Live wild bird markets in Nigeria, are high-risk and hotspot areas for the introduction and transmission of influenza A viruses due to the concentration and interaction of various wild bird species from different sources within and outside the country (Sulaimna *et al.*, 2021). This highlights the need for surveillance and monitoring of influenza A viruses in these markets.

Therefore, this study aimed to investigate the presence of the influenza A virus in pigeons in Maiduguri, Nigeria, so as to enhance early detection to prevent future spread and evaluate risk for poultry producers within the study area.

## MATERIALS AND METHODS

### STUDY AREA

The study was conducted in Maiduguri Metropolis, which comprises Maiduguri Metropolitan Council and parts of Jere, Konduga, and Mafa Local Government Areas. Maiduguri, the administrative hub of Borno State, is located in North-eastern Nigeria. Specifically, it lies between latitudes 11° 46' 18"N and 11° 53' 21"N, and longitudes 13° 02' 23" E and 13° 14' 19"E. As the economic and cultural centre of North-eastern Nigeria, Maiduguri boasts a vast land area of 137,356 square kilometres (Abatcha *et al.*, 2024). The city is rapidly urbanizing, with a population of 822,000 as of 2022 (Abatcha *et al.*, 2024).

Maiduguri's climate is characterized by a lengthy dry season and brief wet season. The climate of Maiduguri is characterized by a prolonged dry season and a brief wet season. The dry season lasts from October to April, while rainfall occurs from late May to early July, peaking in August and continuing until September (NiMet, 2021). According to data from the Nigerian Meteorological Agency, the standard rainy season duration in the area ranges between 110 to 120 days, with fewer than 90 days considered abnormal (NiMet, 2021).

Pigeons are widespread in Maiduguri, with an estimated millions of pigeons residing in the city and surrounding areas (Biu and Umoru, 2010).

### STUDY DESIGN

A cross-sectional study was conducted to determine the prevalence of avian influenza virus in pigeons within live bird markets (LBMs) and households (HH) in the study area. We conducted the sampling in two stages. At the first stage, three LBMs, which included Baga Road, Monday Market, and Custom LBMs, were selected, and five cages were randomly chosen from each. Two pigeons were then sampled from each of the cages, yielding a total of 30 pigeons sampled in the 3 LBMs.

In the second stage of sampling, 21 HH were randomly selected from four areas within the Maiduguri metropolis, where 2 pigeons were sampled from each of the HH: Moduganari Area (n = 6), Damboa Road (n = 5), Bulunkutu (n = 5), and Dala (n = 5).

All pigeons sampled were all apparently healthy.

### SAMPLE SIZE ESTIMATION

The sample size was calculated using the formula for a 95% confidence interval outlined by Thrusfield (2005) with an earlier prevalence of 2.0% determined by Kayali *et al.* (2011) in Pigeon in Egypt. The calculated sample size was 30. However, for precision 72 Samples was Collection.

### SAMPLE COLLECTION AND PRESERVATION

Samples were collected from the Oropharyngeal mucosa by gently inserting and rotating a sterile swab stick to be in contact with the mucosa.

A total of 71 samples (Figure I) were aseptically collected randomly. The swab stick was gently removed and immediately submerged into viral transport medium (VTM) supplemented with antibiotics mixture containing penicillin, streptomycin, gentamicin and amphotericin B. The samples were properly labeled, sex, age and management systems were recorded.

After collection, the samples were immediately transported in an ice packed box to the Veterinary Microbiology laboratory, University of Maiduguri And stored at – 20°C.

## LABORATORY ANALYSIS

### RNA EXTRACTION

Viral RNA was extracted from 140µl each oropharyngeal swabs using the QIAamp® Viral RNA Mini kit (Qiagen, Hilden Germany) as described by the manufacturer, The RNAs were eluted in 60 µl elution buffer and stored in -80°C freezer until further analysis.

### REAL-TIME RT-PCR ANALYSIS

Avian influenza A virus was detected in a real-time RT-PCR using primers: M+25F 5'-AGATGAGTCTTCTAACCGAGGTCG-3' forward and M-124R: 5'-TGCAAAAACATCTTCAAGTCTCTG-3' reverse with probe FAM M+64-TCAGGCCCCCTCAAAGCCGA-TAMRA -3'.

Targeting matrix gene as previously described by Spackman et al. (2002), Akanbi et al. (2024), Olawuyi et al. (2024) and Atuman et al. (2025), using the QuantiTect Multiplex RT-PCR Kit (Qiagen, Germany). The total reaction master mix volume was 25 µl containing 12.5 µl of 2× RT-PCR buffer, 1.5 µl of each primer (5 µM), 2.5 µl of probe (1 µM) and 1.8 µl nuclease-free water to make up to the final volume. The prepared master mix was vortex and spinned for few seconds. Twenty (20 µl) each of the master mix were aliquoted into a sterile 0.2 mL PCR tubes and 5 µl of RNA were added. The RT-PCR was carried out in Rotor Gene Q real-time systems using the following cycling conditions were used: 50°C at 20 minutes, 95°C for 15 minutes; 40 cycles of 94°C for 45 seconds, 60°C for 45 seconds.

### STATISTICAL ANALYSIS

Data generated from this study were analysed using the Epi info version 7.2.5. The prevalence was estimated as the proportion of positive samples for influenza A Virus in Pigeons. Variables were assessed for association with influenza A infection using Chi square test.

Odds ratio was used to determine the degree of the association at 95% Confidence Interval. Values of  $p < 0.05$  were considered significant

### RESULTS

A total of 71 pigeons from various locations in Maiduguri, Nigeria, were sampled, and 9 (12.7%) tested positive for influenza A virus (Table I) with cycle threshold (Ct) range of 30 - 35.

Analysis revealed that pigeons from LBM had a significantly higher prevalence (23.3%, 7/30) compared to those from household settings (4.9%, 2/41), with a significant association ( $p = 0.0305$ ; OR: 5.9; CI: 1.14-31.02).

Age was also a significant factor, with young pigeons showing a higher prevalence (33.3%, 5/15) compared to

adult pigeons (7.1%, 4/56), and a significant association ( $p = 0.0168$ ; OR: 4.3; CI: 1.3022-13.3597).

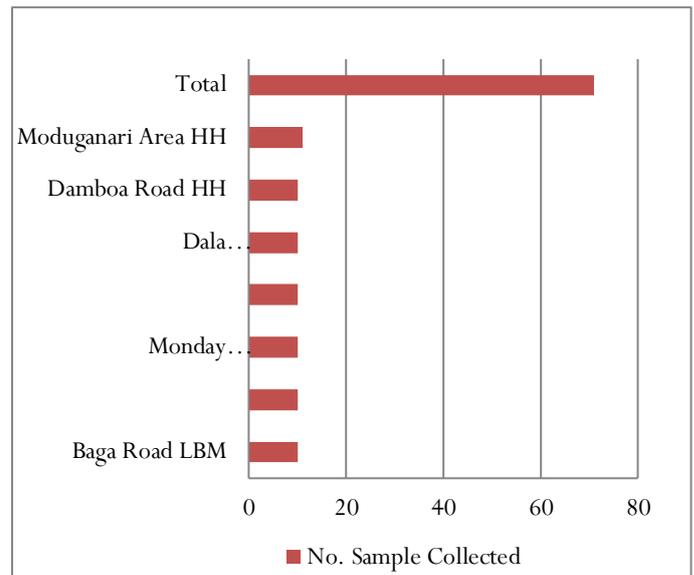


Figure I: Bar chart with locations and number of samples collected

Furthermore, breed was found to be a significant factor, with local pigeons having a higher prevalence (22.9%, 8/35) compared to exotic pigeons (2.8%, 1/36), and a significant association ( $p = 0.0136$ ; OR: 10.37; CI: 1.22-88.02).

Although male pigeons had a higher prevalence (16.2%, 6/37) compared to female pigeons (8.8%, 3/34), no significant association was found between sex and influenza A virus positivity ( $p = 0.4818$ ; OR: 2.0; CI: 0.46-8.72).

Table I: Distribution of Influenza A Virus in Pigeons by Location

Sampling Site	Location	No. Examined	No. (%) positive	Ct value range
Live Bird Market (LBM)	Baga Road	10	4 (40)	32-35
	Customs	10	2 (20)	31-33
	Monday Market	10	1 (10)	30
Households	Bulumkutu	10	1 (9.1)	32
	Dala	10	0 (0)	
	Damboa Road	10	0 (0)	
	Moduganari Area	11	1 (10)	31
Total		71	9 (12.7)	

**Table II: Chi square for risk factors and occurrence of Influenza A Virus in Pigeons in Maiduguri**

Factor		No. Tested	No. Positive (%)	p-value	OR	95% Confident Interval
Location	HH	41	2 (4.9)	0.0305	5.9	1.14-31.02
	LBM	30	7 (23.3)			
Age	Young	15	5 (33.3)	0.0168	6.5	1.48-28.52
	Adult	56	4 (7.1)			
Sex	Male	37	6 (16.2)	0.4818	2.0	0.46-8.72
	Female	34	3 (8.8)			
Breed	Local	35	8 (22.9)	0.0136	10.37	1.22-88.02
	Exotic	36	1 (2.8)			

HH: Households

## DISCUSSION

Influenza A is a zoonotic disease affecting various hosts, including domestic and wild birds, as well as mammals. This study revealed a 12.7% prevalence rate for Influenza A virus (IAV), with notably high Ct values indicating low viral load, which made subtyping and isolation of the positive samples to be very difficult. This is consistent with previous findings of Liu *et al.* (2009).

Pigeons' reduced susceptibility to AIV infection might have contributed to the low viral load (low ct) observed in this study. This may be attributed to the inefficient AIV attachment and replication in their upper respiratory tract. Pigeons have a unique respiratory tract that consists of  $\alpha$ -2,6-linked sialic acids (SA), instead of  $\alpha$ -2,6-linked SA. This differs from that of chickens where  $\alpha$ -2,3-linked sialic acids is the major receptor in the respiratory tract (Rogers *et al.*, 1983; Liu *et al.*, 2009).

Furthermore, pigeons possess the retinoic acid-inducible gene I, which triggers a strong antiviral interferon response, potentially controlling AIV replication (Barber *et al.*, 2010). These factors collectively may contribute to the low viral load observed in this study.

The prevalence of influenza A virus infection in this study is similar to the 12.5% reported by Nkwankwo *et al.* (2012) in Sokoto State, Nigeria, but higher than the 2% reported by Kayali *et al.* (2011) in Egypt.

Comparing results in this study to other parts of the world, the prevalence in this result appeared higher than the 1.9% and 1.4% reported by Zhou *et al.* (2012) and Teske *et al.* (2013) in China and Germany, respectively. The difference in prevalence rates could be due to climatic conditions, sampling season, and management systems. In this study, the pigeons were raised under a semi-intensive management system, whereas those in Germany and China were raised under an intensive management system.

The prevalence of the influenza A virus varied across the live bird markets, with the Monday Market LBM having the highest rate at 40%, followed by Baga Road LBM at 20% and Custom LBM at 10%. The higher prevalence recorded in Monday Market LBM is not surprising; given its role as the central hub that receives all birds in the State prior to distribution to other markets. LBMs have been reported as a potential reservoir for influenza viruses (Sulaiman *et al.*, 2021).

A significant association was found between the prevalence of influenza A virus in pigeons and their source, with pigeons from LBMs being nearly 6 times more likely to carry the virus (23.3%) compared to those from household settings (4.9%). The higher prevalence of influenza A virus in pigeons from LBMs may be attributed to the markets' role in receiving birds regularly from various sources, increasing the risk of viral transmission.

The results of this study revealed a significant difference in the prevalence of influenza A virus infection between young and old pigeons ( $p = 0.00233$ , OR: 4.3; CI: 1.3022-13.3597). The higher prevalence of the virus in younger pigeons may be attributed to their immature immune system, which offers less effective protection against the virus. Our findings in this study corroborate with the report of Sitati *et al.* (2022), who reported a higher rate of positivity in chicks below 4 months of age compared to older chickens in Kenya, although the difference was not statistically significant. However, this study's results contradict those of Cheema *et al.* (2011), which suggested that older chickens are more vulnerable to AIV than younger chickens in Pakistan.

The results of this study have important implications for the control and prevention of influenza A virus infection in pigeon populations. The significantly higher prevalence of the virus in younger pigeons suggests that targeted interventions, such as vaccination programs, may be most effective when focused on this age group. Additionally, pigeon owners and breeders can take steps to reduce the risk of infection in young pigeons, such as implementing strict biosecurity measures and ensuring proper ventilation and sanitation in lofts.

Although male pigeons had a higher infection rate (16.2%) than female pigeons (8.8%), the difference was not statistically significant, indicating that sex is not a determining factor in influenza A virus infection dynamics in pigeons. This finding aligns with Sitati *et al.* (2022), who reported no significant sex-based difference in influenza A virus infection rates in chickens in Kenya. The higher prevalence in the male may also be attributed to the samples collected. More males were sampled than the females.

The prevalence of influenza A virus infection varied significantly between local and exotic breeds of pigeons. Local pigeons had a higher infection rate (22.9%) compared

to exotic pigeons (2.8%), with a significant difference observed between the two breeds ( $p = 0.0136$ ; OR: 10.37; CI: 1.22-88.02). The higher prevalence of influenza A virus infection in local pigeons may be attributed to their flying behaviour, which increases their exposure to other birds and potential sources of infection. This finding agrees with the report by Sitati *et al.* (2022), who also reported a statistically significant difference between breed and influenza A virus infection in chickens. Various chicken breeds have varying levels of resistance to the virus, as documented by Sarwar *et al.* (2013).

The results of this study highlight the need for continued surveillance of Influenza A in healthy pigeon populations and provide baseline information about the circulation of the virus in the study area.

## CONCLUSION

The study found a 12.7% prevalence of the Influenza A virus in apparently healthy pigeons in Maiduguri, Nigeria, with age (young) and source of bird (LBMs) being significant risk factors. Further research on the specific subtypes and molecular characterization of the Influenza A virus in pigeons is needed. Public health education and strengthening of surveillance systems are also recommended.

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