



# Avian Influenza Surveillance in Pigs, Dogs, Chickens and Slaughter Slabs Biosecurity in Kaduna Metropolis, Nigeria

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## Authors' contributions

This work was carried out in collaboration between both authors. Author AA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author DS managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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## ABSTRACT

Avian influenza is a zoonotic disease likely to be exposed to dog, pig and poultry owners & their processors who are in close contact during processing of these animals in Kaduna Metropolis, Nigeria. The study assessed the biosecurity practice of processors in pig, poultry, and dog slaughter slabs; and the prevalence of Avian Influenza (H5 subtype) antibodies in dogs, pigs, and chickens in Kaduna Metropolis. The slaughter slab biosecurity practices were evaluated using questionnaires while the H5 antibodies surveillance was determined by hemagglutination inhibition test. Serum samples from 45 dogs, 104 pigs and 75 chickens were tested for H5 subtype. H5 subtype antibodies prevalence in dogs was 2.2% with a mean titre of  $9.0 \pm 0 \log_2$  while prevalence

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and mean titre in chicken were 6.7% and  $8.5 \pm 0.32 \log_2$  respectively. All pig sera tested were negative for H5 subtype antibodies. The chicken H5 subtype prevalence in central market and railway station live bird markets were 4.3% and 13% respectively. H5 subtype antibodies were present in dogs and chickens in Kaduna metropolis. There is the need for targeted surveillance of avian influenza in dogs and pigs should be incorporated in the national avian influenza surveillance program.

**Keywords:** Avian influenza; biosecurity; chickens; dog; Kaduna metropolis; pig; slaughter slabs; surveillance.

## 1. INTRODUCTION

Avian influenza (AI) is a disease of public health significance though the virus is still restricted to poultry [1]. However, AI currently has a worldwide distribution in many domestic and wild animals including pigs, dogs and humans but chickens, quails and turkeys are the most susceptible [2,3]. The outbreak of HPAI H5N1 in Nigeria in 2006 increases the risk of introduction and spread of the virus in other domestic animals especially with reports of avian influenza virus and antibodies in apparently healthy waterfowls and chickens in Northern Nigeria and Kaduna State respectively [4,5,6]. These extensively raised domestic animals with minimal or no biosecurity, are at risk of exposure to AI viruses from direct contact with infected wild birds or indirect contact with fomites [7]. Since chickens can maintain avian influenza viruses within their production system; and pigs has been proposed as a mixing vessel for human and avian subtypes of AI virus, they constitute a critical control point in the control of AI in Nigeria [8]. The virus could easily mutate and affect humans and other mammals.

Domestic dogs are man's closest associates amongst all animals as they serve as pets, share same accommodation and are physiologically related [9]. Dogs and pigs are naturally predators and their prey usually include birds and other small animals like rodents. These animals can become infected from eating infected chickens or meat provided as their daily rations and can equally scavenge on birds which were infected [10]. With the possibility of interspecies transmission, humans will be at greater risks of acquiring avian influenza infection from these household pets than from chickens [11,12]. Also, Information on AI in dogs and pigs are scarce in Nigeria. Similarly, the recent interspecies transmission from ingestion of infected poultry and birds by domestic dogs and cats in Asia calls for the need to investigate if pigs, and dogs in Kaduna metropolis have been exposed to

influenza A antigen and their possible role in the epidemiology of avian influenza infection in Kaduna Metropolis [10]. The information generated from the study would be important for developing an early warning signal that are essential for designing an effective and comprehensive AI control program in Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study area was Kaduna metropolis in Kaduna State which is in North-central Nigeria with an estimated population of 6 million based on the 2006 census. Kaduna State lies between latitude  $8^{\circ} 45'$  and  $11^{\circ} 30'$  North and longitude  $6^{\circ} 11'$  and  $9^{\circ}$  East. The annual temperature in Kaduna metropolis is  $34^{\circ}\text{C}$  with hottest months being March- April ( $40^{\circ}\text{C}$ ) and the coolest period ( $13.2^{\circ}\text{C}$ ) being December during severe harmattan. Rainfall varies between 1000 mm and 1500 mm and the rainy season last for 100-150 days (Mid-April-ending of October). The dry season occurs between late October-early April [13].

### 2.2 Sampling Technique

Simple random sample "without replacement" (SRSWOR) was used as sampling technique in the target areas. The sampling units were veterinary clinics, dog and pig slaughter slabs and households. The sampling unit's biosecurity features were assessed using a checklist to ascertain their risk of introduction and spread of AI.

### 2.3 Sample Collection

Samples were collected between August and September 2009. Pigs, dogs, and chickens were selected without replacement noting their age, sex and any abnormal condition prior to sample collection.

About 0.5-1.0 ml of blood was collected from dogs, pigs and chickens through venipuncture from cephalic, ear and wing vein respectively using 22 G needles and 2 ml syringes. Dogs were sampled in the households and veterinary clinics, pig were sampled in slaughter slabs, while chickens were sampled in live bird markets. The blood samples were allowed to clot at room temperature and the sera were separated by centrifugation at about 447g for 5 minutes [14]. The collected sera were then stored in the refrigerator until used.

## 2.4 Preparation of 1% Red Blood Cells

Five milliliter of blood was collected from 5 different adult local chickens in Alsever's solution. The red blood cells were washed three times with physiological saline (PSS) by gentle centrifugation at 2,000 revolutions per minute for 5 minutes. One millilitre of deposited RBCs was mixed with 99ml of PSS to prepare 1% solution [15].

## 2.5 Determination of AI H5 Antigen Titre

The hemagglutination (HA) test was used to determine the H5 antigen titre. The test antigen used was an inactivated H5N9 subtype while the positive serum was also an H5N9 serum both prepared by Istituto Zooprofilattico OIE/FAO Laboratory for AI and NDV delle Venezie.

The HI protocol used was as described by OIE (2009). Twenty-five micro liter of phosphate buffered saline (PBS) will be dispensed into each well of a plastic V- bottom microtiter plate. Twenty-five micro liter of virus suspension was dispensed across the plate and a further 25 ul of PBS was be dispensed into each well. Twenty-five micro liter of 1% washed chicken red blood cells was dispensed into each well. These were mixed by tapping the plate gently and then allowing the RBC to settle for about 40 minutes at room temperature. HA end point was determined by tilting the plate and observing the presence or the highest dilution giving complete HA (no streaming); which represented 1HA Unit and was calculated accurately from initial range of dilutions.

## 2.6 Determination of AI H5 Antibodies Titre

The antibodies were determined by hemagglutination inhibition (HI) test [15]. Twenty-five micro liter of PBS was dispensed into each

well of the plate. Two-fold dilutions of the 25 ul volumes of the serum would be made across the plate. Four hemagglutination units (4HAU) of the antigen suspension in 25 ul was added to each well and left for a minimum of 30 minutes at room temperature. About 25 ul of washed chicken RBC was added to each well and after gentle mixing it was allowed to settle for about 40 minutes at room temperature. The HI titre was the highest dilution of the serum causing complete inhibition of 4HAU of antigen. The wells considered positive to the HI test were those in which the RBC streams at the same rates as the control wells.

## 2.7 Data Analysis

The data obtained from questionnaire was coded and analyzed by SPSS software version 17. The HI titre of avian influenza virus antibodies was analyzed by descriptive statistics.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Live bird markets

The poultry species sold in these markets were local chickens, exotic chickens, ducks, guinea fowls, turkeys, and pigeons. The birds were kept in cages ranging from metal cages through wooden to cane cages. Four LBMs were sampled during the study which included central LBM, railway station LBM, Barnawa and Kakuri LBMs.

The central live bird market in Kaduna North Local Government Area located between N10.51962<sup>0</sup> and E007.42472<sup>0</sup> is one of the markets that received FGN intervention. The marketers housed their poultry in metal cages and are separated by species with one way traffic. The processing area is tiled with bore hole as source of water. Though the processors are expected to de-feather the poultry on the slabs, they do it on the floor of the slaughterhouse.

The railway station live bird market in Kaduna South Local Government Area located within N10.49427<sup>0</sup> and E007.41858<sup>0</sup> is by the roadside with poultry housed in wooden cages. Though there is a slaughtering area, the floor is not properly cemented, and de-feathering of poultry is done on the floor with the use of wood for heating the water used in de-feathering. The poultry are not separated by species, type, or

age. The water used is tap water though used water drains into a nearby stream. The drainage in the market is poor.

The Barnawa and Kakuri live bird markets both in Kaduna South LGA located within N10.48366<sup>0</sup> and E007.43193<sup>0</sup> and N10.46705<sup>0</sup> and E007.41224<sup>0</sup> respectively have similar features to the railway station live bird market with the only government intervention being weekly fumigation of the markets.

### 3.1.2 Dog slaughter areas

The dog slaughter slabs where samples were collected were Trikania slaughter area located at N10.45353<sup>0</sup> and E007.42472<sup>0</sup> along Abuja Road bye-pass in Chikun Local Government Area and Television slaughter area located at N10.452788<sup>0</sup> and E007.42826<sup>0</sup> in Kaduna South LGA.

Dogs brought for slaughter are from household within and outside the locality and by hunters. These dogs are decapitated, scalded using fire and slaughtered into parts. The slaughtered dogs are prepared within the vicinity of the slaughter slabs and sold as pepper soup. The slaughter areas do not have concrete floor and the butchering table is wooden. There is no drainage and blood, and intestinal contents are spilled on the ground.

### 3.1.3 Pig slaughter area

Pigs were sampled from the television slaughter area located in Kaduna South Local Government Area at N10.44949<sup>0</sup> and E007.39169<sup>0</sup>. Pigs are decapitated, scald with hot water and slaughtered into pieces and sold to the public. The slaughter area is not fenced neither it is the floor concrete. The pigs are housed between two buildings prior to slaughter. The butchering table is wooden and there is no drainage with blood from decapitated pigs and intestinal content spilled on the ground. The butchers do not wear gloves, masks, coveralls, or boots.

### 3.1.4 Surveillance of avian influenza h5 subtype antibodies in dogs, pigs and chickens

All the pigs (104/104) and chickens (75/75) sampled in the study were from the pig slaughtering areas and live bird markets respectively. About 6.8 % (3/44) dogs were

sampled from veterinary clinic and household and 88.6% (39/44) were sampled from the dog slaughter area.

The H5 AI antibodies prevalence in dogs sampled was 2.3% (1/44) with a chicken prevalence of 6.7% (5/70) and none (0/104) of the pigs sampled had antibodies to AI H5 Subtype ( $\chi^2 = 7.47$ ;  $P = 0.024$ ). However, none of the dogs presented in either the veterinary clinic or the dog slaughtering areas had antibodies against AI though, 33.3% (1/3) of dogs in households had AI antibodies ( $\chi^2 = 11.02$ ;  $P = 0.004$ ).

The AI antibodies prevalence in central market LBM was 4.3% (2/47) with Station LBM having 13% (3/20) and 50% (1/2) in Barnawa ( $\chi^2 = 50.47$ ;  $P = 0.00$ ). In central market and railway station LBMs the samples positive for AI H5 subtype antibodies were poultry while the positive sample in Ungwan Rimi was from a household dog.

The AI antibodies titre for the dog was  $9.0 \pm 0.0 \log_2$  while the mean titre for chickens was  $8.5 \pm 0.04 \log_2$  with minimum titre of  $5.0 \pm 0.0 \log_2$  and maximum titre of  $11.0 \pm 0.0 \log_2$  ( $\chi^2 = 74.12$ ;  $P = 0.00$ ).

The AI antibodies titre for the dog was  $9.0 \pm 0.0 \log_2$  while the mean titre for chickens was  $8.5 \pm 0.04 \log_2$  with minimum titre of  $5.0 \pm 0.0 \log_2$  and maximum titre of  $11.0 \pm 0.0 \log_2$  ( $\chi^2 = 74.12$ ;  $p = 0.00$ ).

### 3.1.5 Management of dog possessing avian influenza (H5 Subtype) antibodies

The dog is owned by a male medical doctor aged between 35-44 years resident at Ungwan Rimi in Kaduna North Local Government Area. The dog owner's family comprised of a male, two females and three children. The dog is a male dog aged five months which is kept as a guard dog and fed with poultry carcass, bones, livestock offal, fish, chicken left over and household food remnants. It is confined within the fenced compound where it is allowed to move freely. The dog is regularly vaccinated against rabies and canine distemper and always consults a veterinarian when the dog is sick. The owner was keeping exotic layer chickens and dead poultry were usually prepared for the dog. The offal of poultry for home consumption are given to the dog.

### 3.1.6 Dog Owners' knowledge and practices on avian influenza

Among the 130 respondents who participated in the study, 78.5 % (102/130) were males with 59.2% (77/130) having received secondary education (Fig. 1). However, 36.9% (48/130) were business people with 88.5% of dog owners having a household size of more than 3 persons. All the respondents kept only dogs as animals of which 74.2% were less than a year old. However,

82.3% (107/130) of respondents kept the dogs for security while 17.7% (23/130) kept dogs as pets. The mean number of dogs per household was  $1.38 \pm 0.05$  though 28.5% (37/130) of respondent had two dogs per household (Fig. 2). Dogs were fed with food eaten in the household (50.8%) though some respondents fed their dogs with chicken carcass (Fig. 3). However, 85.4% of dogs roam freely with 10.8% (14/130) confined and 3.8% (5/130) sleeping outside the house.

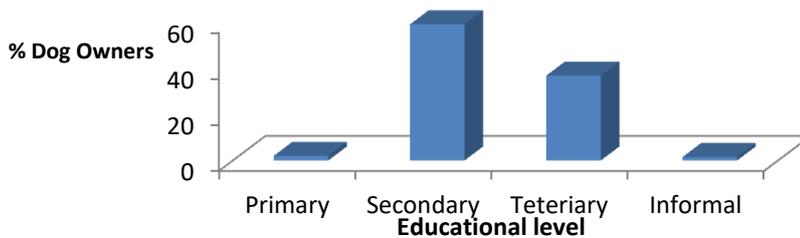


Fig. 1. Distribution of educational level of respondents

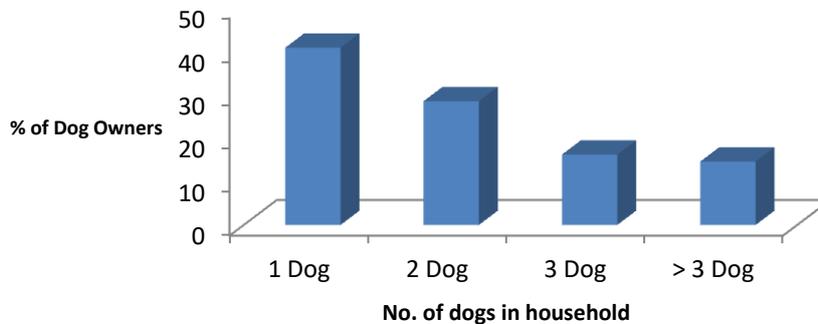


Fig. 2. Distribution of number of dogs in respondents' household

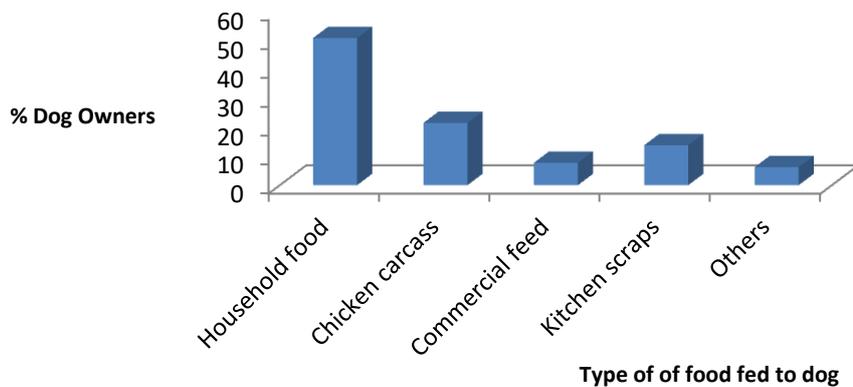


Fig. 3. Variation of food fed to dog by respondents

Among the dogs examined, 97.7% (127/130) were apparently healthy. About 46.9% (61/130) respondents would seek veterinary assistance when a dog is sick, 27.7% (36/130) would do nothing, 18.5% (24/130) would give out the dog and 6.9% treat the dog personally. Only 32.3% (42/130) respondents vaccinate their dogs against rabies and 2.3% (3/130) knew that dogs can be sick of bird flu. Similarly, 99.2% (129/130) and 96.9% (126/130) of respondents reported not knowing that dogs can transmit avian influenza virus to humans and die of avian influenza respectively.

One hundred and twenty-one (93.1%) of respondents kept poultry with 31.4% (38/121) keeping exotic chicken and 68.6% (83/121) local chickens. About 84.3% (102/121) of respondents sourced their poultry from live bird markets with 11.6% (14/121) from agrovet shops and 4.9% (6/121) were acquired as gifts. All respondents (121/130) who kept chickens managed the birds extensively. Among respondents who kept poultry, 62% (75/121) kept their dogs away from poultry while 45.5% (55/121) permitted contact between their dog and poultry feed and offal.

One hundred and twenty-five (96.2%) dog owners are aware of avian influenza, but none had experienced an outbreak in their flock neither has any of their neighbors. About 52.4% (124/130) of the respondents handled death poultry with bare hands while 47.6% (59/130) would wear hand gloves. About 32.3% (42/130) dog owners would not wash their hands with water and soap after handling a sick or dead poultry. Only 25% (31/130) of dog owners will seek veterinary assistance for sick poultry while 25.8% (32/130) would sell, 34.7% (43/130) give away, 8.9% (11/130) eat, 4.8% (6/130) will throwaway and 0.8% (1/130) would feed dog.

Eighty-five (65.4%) dog owners kept pigs with a mean herd size of three and 57.1% (52/85) had pigs aged more than a year and 36.3% (33/130) aged less than a year. About 78.8% (67/85) of respondents purchase pigs from friends though 21.2% (18/85) obtained as gifts. Only 15.5% (13/130) respondents managed their pigs intensively with 84.5% (71/85) managing semi-intensively. About 87.5% of respondents fed their pigs with kitchen scraps and bran though only 22.5% (18/85) use exclusively kitchen scraps.

Eighty-seven per cent of respondents do not allow direct contact of pigs with their poultry with 24.1% (21/85) allowing indirect contact. About

96.5% of respondents would sell their sick pigs with 2.4% (2/85) eating and only 1.2% (1/85) seeking veterinary assistance. However, 64% (55/85) would give out dead pigs with 32.6% (28/85) burying, 2.3% (2/85) eating and 1.2% (1/85) throwing away. None of the respondents were aware that pigs can be infected with avian influenza virus neither were they aware of the mode of transmission from pigs to humans.

Clinical signs of respondents' sick dogs are 96.2% (125/130) noted loss of appetite, 70.8% (91/130) lethargy, 11.5% (15/130) emaciation, 3.1% (4/130) nasal discharge, 6.9% (9/130) cough and 0.8% (1/130) conjunctivitis.

Poultry diseases known by dog owners were 63.1% (62/130) Newcastle disease, 30.8% (40/130) Gumboro 27.7% (36/130) avian influenza, 13.1% (17/130) coccidiosis, 11.5% (15/130) fowl pox and 0.8% (1/130) fowl typhoid and cholera.

The dog owners' source of news about Avian Influenza were, 61.5% (80/130) first heard from friends, 68.5% (89/130) radio, 85.4% (111/130) TV, 33.1% (43/130) posters, 2.3% (3/130) newspapers and 3.1% (4/130) stickers. When asked to state what they remember of bird flu, 93.8% (122/130) stated high mortality, 72.3% (94/130) that it affects humans, 3.1% (4/130) high morbidity and 6.9% (9/130) it kills human beings.

### 3.2 Discussion

The study revealed that dogs in Kaduna metropolis had been exposed to AI H5 subtype similar to reports from the Europe and America of evidence of carnivores' exposure to H5 antigen [16,17]. The virus might be either of low pathogenicity causing a non-fatal infection unlike reports from Thailand where the infection was fatal to dogs [10]. The study confirms that the practice of feeding dogs with dead poultry predisposes dogs to AI infection like reports from UK and Russia [18] which increase likelihood of human exposure from their dogs as dogs have been reported to pass out virus through secretions [10,19,20]. However, none of the dogs slaughtered in dogs slaughter slabs had antibodies to AI H5 subtype which might be because the dogs sold for slaughter not having access to AI infected poultry since most of the dogs are local dogs sourced from rural areas. However, either none of the pig sampled had been exposed to AI H5 subtype as revealed by

the study or though the pigs were exposed to the virus the antibodies produce had weaned out at the time they were sampled for this study.

However, the prevalence of AI (H5 subtype) among chickens in LBMs might either be because of the chickens being exposed to the field AI virus which is of low pathogenicity, or the antibodies might be because of vaccination which is been practiced contrary to government policy [21].

The biosecurity practices and infrastructure in central market LBM are adequate as the use of metal cages ensure adequate cleaning of cages and the separation of poultry based on species prevent interspecies cross infection of AI and other poultry diseases. In station, Barnawa and Kakuri LBMs wooden cages are used, and the markets do not have concrete floor making cleaning difficult and inadequate. However, the failure of processors in all the LBMs to use the slabs for feathering poultry is inappropriate leading to spread of AI virus [22]. There is need for infrastructural intervention by government in the other LBMs.

The study revealed that few dog owners keeping poultry and pigs would seek veterinary assistance for their sick dog and would rather give it out, similar to practice engaged by local poultry farmers [22]. This practice is risky as it might expose the human population if the disease affecting the dog is zoonotic such as avian influenza which has been reported to infect dogs with active excretion of virus in body secretions [9]. Most of the owners are not aware their dogs are at risk to be infected by HPAI though carnivores have been reported to transmit the virus [23]. This lack of knowledge on the susceptibility of dogs to HPAI would prevent owners from protecting their dogs and engaging in practices such as feeding dogs with dead uncooked poultry which might expose the dogs to HPAI as dogs are reported to be infected on consumption of HPAI infected poultry [9]. Dogs have been reported to excrete HPAI H5N1 virus which could be a source of human infection as humans are also susceptible to the virus and dogs being the closest associate of pet owners will expose the owners to the virus hence the need to know that dogs can transmit HPAI to humans [9]. There is need to educate the dog owners on the possibility of humans dying of HPAI as this will improve on their risk perception and ensure appropriate protective measures are taken to protect both the owners and their dogs.

Since most of the dog owners kept local poultry which are usually managed extensively, there is increased possibility of interspecies transmission of avian influenza from local poultry to dogs and pigs since studies in Kaduna State reported influenza A antigen in local poultry [5]. Sourcing of poultry from LBMs might spread avian influenza virus from LBM to households since AI viruses have been reported to be circulating in LBMs as revealed in this study and previous studies, hence, will increasing the likelihood of human exposure.

Awareness of HPAI among dog owners was high which might be due to the effectiveness of the information dissemination by the AICP. This might also be due to the fact dog owners are highly educated which exposes them to many information sources likely to discuss avian influenza. Similar to studies of poultry farmers' practices, dog owners engage in risky practices such as touching dead poultry with bare hands and not wearing protective clothing which increases risk of human exposure and spread of disease to their dogs [20,24]. Similar to local poultry farmers, the study revealed that dog owners are unlikely to report sick poultry to veterinarians thereby reducing the likelihood of reporting HPAI outbreak, which would increase spread among livestock and pets with a resultant increased human and environmental exposure to HPAI virus.

The study reported that among the signs owners reported experienced in their sick dogs are signs such as nasal discharges, coughing and conjunctivitis which are signs attributed to avian influenza [9]. Since these sick dogs are rarely referred to a veterinarian it reduces probability of detecting the cause of these signs.

Furthermore, findings of the study reported that more dog owners were aware of Newcastle disease than highly pathogenic avian influenza implying less likelihood of reporting HPAI. Since ND is a differential diagnosis of HPAI, if dog owners are educated to report all ND outbreaks, so the veterinarian will differentiate which case is HPAI.

There is increased risk in keeping pigs and dogs since pigs are being described as the likely mixing vessel for avian influenza [21]. Hence dog owners keeping pigs or poultry are likely to provide a delicate mix where reassortment of avian influenza virus and canine influenza viruses can take place to produce a progeny

virus with improved infectivity to dogs and likely humans. Since young pigs with poorer immune system are kept, there is increase chances of the virus establishing and multiplying in these pigs thereby propagating the viruses which might be introduced to pigs through kitchen scraps fed.

The study further revealed that there is likelihood of pigs infected with avian influenza to spread the virus through sale of sick pigs and human exposure from processing sick pigs especially the practice of giving out dead pigs. However, there is need for respondents to consult veterinarians on sick pigs. There is need to articulate the relationship of avian influenza and pigs in avian influenza communication materials. This will improve the risk perception in pig farmers and avoid introduction of avian influenza from poultry to pigs and from pigs to humans.

The study confirms that radio was a major means of HPAI information dissemination like previous studies though unlike local poultry farmers, television was also an appropriate medium of HPAI information for dog owners [6,25]. This is because unlike local poultry farmers, dog owners are more affluent and can afford TV and generators if there is power failure. However, though dog owners are expected to be highly educated, similar to previous studies, newspapers were a poor source of HPAI information probably due to reduce attention given to HPAI outbreaks by the print media [25].

Contrary, to a previous study, posters were a poor source of HPAI information among dog owners probably due to poor access to the HPAI posters though stickers as medium of HPAI information dissemination was equally poor as reported by Assam et al. [25]. However, high mortality was what most dog owners could remember probably due to most news report indicating the number of poultry dying from HPAI. Nevertheless, dog owners were knowledgeable on the zoonotic potential of HPAI though not its potential for causing human fatality which needs to be better articulated to improve the human risk perception of HPAI.

#### 4. CONCLUSION

The biosecurity practices in dog and pig slaughter areas and Barnawa, railway station and Kakuri LBMs was poor. Dog owners in Kaduna metropolis are aware of highly pathogenic avian influenza though their knowledge was poor resulting in HPAI risky

practices such as selling and eating of sick and dead poultry; throwing away of death poultry and not willing to report sick poultry to the veterinary services. The inadequate knowledge of dog owners on HPAI is reflected in the low-risk perception of HPAI amongst the dog owners. Radio, television, and friends were HPAI information source to dog owners while posters, stickers and newspapers were not appropriate media to target dog owners. Avian influenza (H5 subtype) antibodies were present in dogs and chickens in Kaduna metropolis.

There is need for government to intervene and improve the infrastructure and biosecurity of the dog and pig slaughter areas and the railway station, Kakuri and Barnawa live bird markets. The risk perception of dog owners in Kaduna metropolis should be improved to ensure reduction in risky practices. Any HPAI information targeting dog owners should be aired through the radio or television. Targeted surveillance of AI in dogs and pigs should be incorporated in the national AI surveillance program.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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