AVIAN INFLUENZA ANTIBODIES IN CHICKENS, DOGS, PIGS AND BIOSECURITY PRACTICES IN KADUNA, NIGERIA

BY

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BY

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ZARIA, NIGERIA

AUGUST, 2012

DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Medicine, Ahmadu Bello University, Zaria, under the supervision of Prof. P. A. Abdu, Prof. H. M. Kazeem and Prof. S. B. Oladele. The work of other investigators referred to in this study is duly acknowledged. No part of this study has been previously submitted for a degree or diploma.

David SHEHU	_____	_____
Name	Signature	Date

CERTIFICATION

This thesis entitled '**Avian Influenza Antibodies in Chickens, Dogs, Pigs and Biosecurity Practices in Kaduna, Nigeria**' by David SHEHU meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God Almighty, my wife Fidelia and children.

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ABSTRACT

Avian influenza is zoonotic and dog being a close associate to humans, constitutes a source of human exposure. This study assessed avian influenza awareness and knowledge amongst dog owners and biosecurity practices in live bird markets, pig and dog slaughter areas in Kaduna using structured questionnaires and biosecurity checklists. The prevalence of H5 subtype antibodies in chickens, dogs and pigs was also determined by haemagglutination inhibition test. Sera from 75 chickens, 45 dogs and 104 pigs were tested for H5 subtype antibodies. Dog owners (93.1%) kept poultry with 64.6% keeping local poultry. Though 57.7% prevented direct contact of dogs and poultry, 42.3% allowed contact. About 65.4% of dog owners kept pigs with 84.5% managing pigs semi-intensively; 0.8% sought veterinary attention when pigs were sick and 24.1% permitted contact of pigs with poultry. About 27.7% of dog owners did nothing when dogs were sick. Ninety-six per cent were aware of avian influenza and 85.4% first heard about it from the television. Only 2.3% were aware of the susceptibility of dogs to avian influenza, but 0.8% knew that dogs can transmit the virus to humans, and 3.1% knew avian influenza infection in dogs could result in death. Dog owners (95.4%) handled dead poultry with bare hands, while 32.3% do not wash hands with soap and water after handling dead or live poultry. The H5 subtype antibodies prevalence in dogs was 2.2% with a mean titre of $9.0 \pm 0.0 \log_{2,}$ while the prevalence in chicken and mean titre were 6.7% and $8.5 \pm 0.04 \log_{2,}$ respectively. All pig sera tested were negative for H5 subtype antibodies. The chicken H5 subtype antibody prevalence in Central Market and Railway Station live bird markets (LBMs) were 4.3% and 13%, respectively. Awareness of avian influenza of dog owners was high but their knowledge was poor. The H5 subtype

antibodies were present in dogs and chickens in Kaduna. Biosecurity practices of Central Market LBM were adequate, but poor in Barnawa, Kakuri and Railway Station LBMs, dog and pig slaughter areas. Targeted surveillance of avian influenza in dogs and pigs should be incorporated in the national avian influenza surveillance program.

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion
AICP	Avian Influenza Control Project
AI	Avian Influenza
AIV	Avian Influenza Virus
cDNA	clone deoxy nucleic acid
CZC	Country, Zone or Compartment
DIVA	Differentiating Infected from Vaccinated Animals
EDS'76	Egg Drop Syndrome 1976
ELISA	Enzyme Link Immuno-Sorbent Assay
FCT	Federal Capital Territory
FGN	Federal Government of Nigeria
HA	Haemagglutinin
HA0	Haemagglutinin Molecule
HAT	Haemagglutination Test
HAU	Haemagglutinin Unit
HI	Haemagglutination Inhibition
HPAI	Highly Pathogenic Avian Influenza
IVPI	Intravenous Pathogenic Index
LBM	Live Bird Market
LGA	Local Government Area
LPAI	Low Pathogenic Avian Influenza

NA	Neuraminidase
NASBA	Nucleic Acid Sequence Based Amplification
NP	Nucleic Protein
NS1	Non-Structural Protein 1
MP	Matrix Protein
OIE	World Organization for Animal Health
P	Prevalence Value
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PSS	Physiological Saline Solution
RBC	Red Blood Cell
RT-PCR	Reverse Transcriptase-Polymerized Chain Reaction
RNA	Ribo Nucleic Acid
SiRNA	Small Interfering Ribo Nucleic Acid
SPSS	Statistical Package for Social Science
ul	Microlitre
^{o}C	Degree Centrigrade
%	Percentage

CHAPTER 1

INTRODUCTION

1.0 AVIAN INFLUENZA

Avian influenza also known as bird flu, fowl plague or bird pest, is a viral disease caused by a negative-stranded, segmented RNA virus of the Genus influenza virus A and Family *Orthomyxoviridae* (Easterday *et al.*, 1997). It affects the digestive, respiratory or nervous system of most domestic and wild birds with signs ranging from inapparent signs through mild to severe signs with high morbidity and mortality (Whitman and Bickford, 1989; Easterday *et al.*, 1997; DEFRA, 2003). The name influenza was derived from an ancient belief that it was caused by a malign and supernatural influence. It was first reported in Italy in 1887 but was not recognised as influenza virus until 1955 (Easterday *et al.*, 1997).

Avian influenza currently has a worldwide distribution in many domestic and wild animals including whales, horses, seals, pigs, cats, dogs and humans but chickens, quails and turkeys are the most susceptible (Kida *et al.*, 1987; Brown *et al.*, 2007). It is generally believed that aquatic wild birds are reservoirs of the avian influenza virus though there is a hypothesis that the highly pathogenic form is not normally present in wild birds population but arise as a result of mutation after H5 and H7 have been introduced into poultry population from wild birds (Capua and Alexander, 2004).

Avian influenza virus can be classified based on the surface antigen or on its pathotype. Based on its surface antigen, it is classified according to the nature of haemagglutinin and neuraminidase antigen. There are 16 distinct haemagglutinin surface antigens (H1-H16)

and 9 distinct neuraminidase surface antigens (N1-N9) (FAO, 2008). Classification based on pathotype involves the low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) (FAO, 2008). The LPAI cause inapparent to mild signs and is characterized by possession of a single basic amino acid at HA0 cleavage site, while the HPAI is associated with severe signs involving the respiratory, digestive and nervous systems. The HPAI is usually characterized by the presence of multiple basic amino acids at the HA0 cleavage site (Easterday *et al.*, 1997). Highly pathogenic avian influenza is considered to be a virus which fulfils one of the following criteria:

1. Lethal for six or seven out of ten, 4-6 weeks old chickens within 10 days following inoculation with 0.2 ml of 1:10 dilution of the virus in a bacteria free allantoic fluid.
2. Has multiple basic amino acids region at HA0 cleavage site and is H5 or H7 subtype.
3. Avian influenza virus is not H5 or H7 subtype but kills one in five chickens and grows on cell culture in the absence of trypsin (Easterday *et al.*, 1997).

Avian influenza virus infection caused by HPAI H5N1 has been reported in both dogs and cats under natural and experimental conditions (Kuiken *et al.,* 2004; Songserm *et al*., 2006a). Transmission is usually through ingestion of infected chickens' carcass (Songserm *et al*., 2006a).

Highly pathogenic avian influenza virus infection in humans is characterized by fever, sore throat, respiratory distress, pneumonia and death (DAFF, 2008).

In dogs, HPAI H5N1 cause a severe disease characterized by high fever, lethargy, bloody nasal discharge and death within 24 hours. Gross pathological lesions include; severe pulmonary congestion and oedema, congestion of the spleen, liver and kidneys (Songserm *et al*., 2006b).

In cats, the clinical signs of HPAI H5N1 include fever, panting, depression, convulsion, conjunctivitis and ataxia (Songserm *et al.,* 2006a). Gross pathological lesions include; cerebral congestion, pulmonary oedema, severe pneumonia, renal congestion and haemorrhage of intestinal serosa (Songserm *et al*., 2006b).

Experimental studies with Asian H5N1 virus in pigs have demonstrated viral replication in the upper respiratory tract of infected pigs but no transmission to contact pigs (Choi *et al.,* 2005). Clinical signs consisted of mild cough, hyperthermia and anorexia. Microscopically, some of these viruses produced moderate interstitial pneumonia (Choi *et al.,* 2005).

1.1 STATEMENT OF RESEARCH PROBLEM

Interspecies transmission of avian influenza virus has occurred occasionally from aquatic birds to mammals (Shane, 2002). Early warning activities on disease surveillance, reporting and epidemiological analysis are essential for effective avian influenza (AI) control. However, surveillance for this disease has been limited to poultry. Some dog owners may keep dogs for security and feed them with infected chicken carcasses. Dogs may play a role in the adaptation of HPAI strain H5N1 to mammals and its subsequent transmission to humans (Giesem *et al*., 2008). Information for developing an early warning system for influenza is scarce in Nigeria.

Pigs have important role in the ecology of influenza virus because they can be infected by various influenza subtypes (H1-H13) (Kida *et al*., 1994). Since they share the same cellular receptors with those of birds and humans, pigs have the potential to become coinfected with both avian and mammalian strain of influenza viruses. Thus, pigs are considered "mixing vessels", and novel strains of influenza may arise in them by genetic reassortment of existing strains (Ito *et al*., 1998).

1.2 JUSTIFICATION OF THE RESEARCH

Avian influenza is a disease of public health significance though the virus mainly infects poultry (Capua and Alexander, 2004). The virus could easily mutate and affect humans and other mammals (Easterday *et al.,* 1997). Dogs are man's closest associates amongst all animals as they serve as pets, share same accommodation and are physiologically related (Songserm *et al.,* 2006b). These pets are naturally predators and their prey usually include birds and other small animals like rodents. They are also carnivores with large part of their diet being meat (Songserm *et al.,* 2006b). The dogs can become infected with avian influenza virus from eating infected chickens or meat provided as their daily rations (Songserm *et al*., 2006a). They can equally scavenge on birds which were infected. With the possibility of interspecies transmission, humans will be at greater risks of acquiring AI from these household pets than from chickens.

Influenza virus H3N8 of dog was first reported in 2004 (Crawford *et al*., 2005). Influenza H3N8 virus of equine origin caused disease outbreaks in greyhounds in Florida and has since been found in other dogs in United States (Tremayne, 2006). The course of experimental infection of Specific Pathogen Free (SPF) dogs with the subtype H5N1 resembles that of the experimental infection of dogs with the subtype H3H8 (Crawford *et*

al., 2005). All dogs sero-converted and some excreted the virus without obvious disease. Isolation of influenza virus H5N1 from a dog in Thailand has been reported (Songserm *et al*., 2006b).

Cats experimentally inoculated with H5N1 influenza virus have been shown to be susceptible to infection and to shed the virus (Kuiken *et al*., 2004). The interspecies transmission from ingestion of infected poultry and birds by dogs and cats in Asia calls for the investigation of the possible role of these pets in the epidemiology of AI infection in Kaduna.

1.3 AIM AND OBJECTIVES

1.3.1 Aim of the Study

The aim of this study was to establish the possible exposure of chickens, dogs and pigs to avian influenza virus (AIV) and assess AI knowledge and biosecurity practices of dog owners in Kaduna.

1.3.2 Objectives of the Study

The objectives of the research were to:

1. Determine the prevalence of AI antibodies (H5 subtype) in chickens, dogs and pigs in Kaduna.
2. Assess the awareness and knowledge on HPAI and biosecurity practices of dog owners keeping poultry and or pigs in Kaduna.
3. Assess the biosecurity practices in live bird markets, dog and pig slaughter areas in Kaduna.

1.4 RESEACRH HYPOTHESIS

1.4.1 Null hypothesis

Null hypothesis 1: Avian Influenza antibodies (H5 Subtype) are not present in chickens, dogs and pigs in Kaduna.

Null hypothesis 2: Dog owners keeping poultry and or pigs in Kaduna are not aware and knowledgeable on highly pathogenic avian influenza.

Null hypothesis 3: Biosecurity practices in live bird markets, dog and pig slaughter areas in Kaduna are inadequate

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Avian influenza is a viral disease caused by negative-stranded, segmented RNA viruses of the Genus *influenzavirus* A and Family *Orthomyxoviridae*. It affects the digestive, respiratory and nervous system of most domestic and wild birds (Easterday *et al*., 1997). Its clinical signs vary from inapparent signs through mild to severe signs with high morbidity and mortality (Whitman and Bickford, 1989; DEFRA, 2003; Swayne, 2003). Interspecies transmission occasionally occurs from aquatic birds to mammalian species though outbreaks tend to be self-limiting and are not maintained in the new hosts (Shane, 2002).

2.2 HISTORY OF AVIAN INFLUENZA

Avian influenza virus infection was first reported in Italy in 1887 by Perroncito as "epizootic typhoid of fowl" but was later referred to as 'fowl plague' differentiating it from other diseases (Kaleta and Rulke, 2008). However, the agent of 'fowl plague' was known to be a 'virus' in 1901, though its relationship with other mild viruses isolated from influenza infected birds was not known till 1955 (Easterday *et al*., 1997).

History records 17 outbreaks of HPAI from 1959 to 2000 mostly in chickens (Alexander and Brown, 2000). The outbreak of fatal HPAI in poultry and humans in Hong Kong in 1997 by H5N1 subtype of HPAI virus resulted in worldwide interest in HPAI viruses (Alexander and Brown, 2000).

Avian influenza virus belonging to the H5N3 subtype was first isolated in Africa in 1961 from the common tern (*Sterna hirundo*) in South Africa. The outbreak in the common tern was also the first reported case of AI in wild birds (Alexander *et al.,* 2008). However, there have been other outbreaks in South Africa in 2004 and 2006 caused by the H5N2 subtype in Ostriches (Alexander *et al.,* 2008). Similarly, outbreaks caused by H5N2 subtypes was reported in Zimbabwe in 2005 (Alexander *et al*., 2008). In February, 2006, the first HPAI H5N1 outbreak in Nigeria was reported at Sambawa farm in Kaduna State which later spread to 25 States and the Federal Capital Territory (FCT) (Adene *et al*., 2006).

2.3 DISTRIBUTION OF AVIAN INFLUENZA

Avian influenza has a worldwide distribution which includes Asia and Middle East, Europe, Africa, North and South America (Easterday *et al.,* 1997; DEFRA, 2003; NADIS INFO, 2006). In Nigeria, as at October, 2008, HPAI has been reported in 25 States and FCT, involving 97 local government areas (AICP, 2009). Studies in Kaduna State reported sero-prevalence of H5N2 in local poultry (Durosinlorun *et al.,* 2010; Assam *et al.,* 2011a).

2.4 INCIDENCE OF AVIAN INFLUENZA

Avian influenza is a seasonal disease in high risk areas due to the migratory activities of waterfowls (Abdu *et al*., 2005). The incidence varies with geographic region, species, age of bird, time of year and environmental or agricultural system occupied (Swayne and Jackwood, 2008).

It is usually associated with the cold months as the virus is easily destroyed by heat and ultra violet light (Birnbaum and O'Brien, 2008). However, most outbreaks of the AI occur during the harmattan period in Nigeria, though outbreak in waterfowls has been reported in the hot month of April in Zaria (Abdu *et al*., 2005; Wakawa *et al*., 2008).

Some factors have been associated with the increased incidence of AI which comprise increase in global poultry population, increase in intensification of poultry industry with a concurrent increase in free-range production and even climate change leading to alterations in wild bird migratory paths improved diagnostic capability and surveillance (Capua and Alexander, 2004; Lee *et al*., 2006).

2.5 AETIOLOGY OF AVIAN INFLUENZA

2.5.1 Classification

Influenza viruses are classified into types A, B and C on the basis of differences in their antigenic nucleocapsid and matrix (M) proteins, haemagglutinin (HA) and neuraminidase (NA) (Fouchier *et al.*, 2000; Abdu *et al.*, 2006).

Avian influenza viruses are negative-stranded, segmented RNA viruses belonging to the influenza A virus Genus of the *Orthomyxoviridae* Family. The influenza A viruses are divided into subtypes based on their haemagglutinin (H) and neuraminidase (N) antigens. Currently, 16 haemagglutinin (H1-H16) and 9 neuraminidase (N1-N9) subtypes have been described (OIE, 2009).

The haemagglutinin surface glycoprotein projections (spikes) on the viral envelope aid in biding to oligosaccharide cellular receptors of neuraminic acid on host cell surfaces, for subsequent fusion and cell entry (Easterday *et al.*, 1997). The matrix (M) protein is

involved in assembly and budding, while the neuraminidase exerts a sialolytic enzymatic action of cleaving of the host sialic acid receptors, liberating virus progeny captured on surfaces of infected cells (Metselaar *et al.*, 1982; Yeun and Wong, 2005). Neuraminidase also converts the mucus on cell surfaces into a less viscous fluid, preventing self aggregation of progeny virions and thereby promoting the spread of progeny virions released, over a larger area of the mucosa (Metselaar and Simpson, 1982).

The influenza A H5N1 virus codes for 11 structural and non-structural proteins (NSI) (Bjorn *et al.,* 2006; Malin *et al.,* 2007). The non-structural protein is a virulent factor that inhibits the synthesis of virus-induced type 1 interferon, an innate response of infected host cells (George *et al.,* 2007; Malin *et al.,* 2007).

Based on their disease features, AIV are grouped into highly pathogenic avian influenza (HPAI), and low pathogenic avian influenza (LPAI) (Capua and Alexander, 2004). However, HPAI viruses have an intravenous pathogenicity index (IVPI) in 6-week old chickens greater than 1.2 or, cause at least 75% mortality in 4- to 8- week-old chickens infected intravenously; or H5 and H7 viruses with multiple basic amino acids at haemagglutinin cleavage site (OIE, 2009).

Low pathogenic avian influenza infections are caused by viruses from any of the 16 haemagglutinin (H1-H16) and 9 Neuraminidase (N1-N9) subtypes. Currently, all AIV that have multiple basic amino acid sequences at the cleavage site are considered notifiable and the viruses are denoted as Highly Pathogenic Notifiable Avian Influenza (HPNAI) while all H5 and H7 subtypes that are not HPNAI are Low Pathogenic

Notifiable Avian Influenza (LPNAI) (OIE, 2009). This classification is based on the potential of the LPNAI to become HPNAI after circulating in poultry (OIE, 2009).

2.5.2 Antigenic characteristics

Avian influenza has two surface glycoproteins-haemagglutinin and neuraminidase which gives the virus its antigenic subtype specificity (Swayne *et al*., 1998; Abdu *et al*., 2005). There are therefore 144 possible avian influenza subtypes with no cross protection between subtypes (Swayne and Jackwood 2008). These antigenic differences have varied implications for vaccination as specific antibodies are produced against haemagglutinin protein and to a lesser extend neuraminidase protein (Lee and Suarez, 2005). The segmented nature and presence of different surface proteins ensures diversity in viral population. Both random mutations within the genome segments (antigenic drift) as well as reassortment of gene segments (antigenic shift) between different AIV infecting the same animal at a particular point in time are possible (Beard, 1998). The importance and interpretation of antigenic drift in poultry though complicated causes changes at antigenic sites affecting specificity of neutralizing antibodies hence immune response (Suarez, 2008).

2.5.3 Resistance of Avian Influenza virus to Chemical and Physical Agents

Avian influenza viruses are rapidly destroyed by high temperature, extremes of pH, dryness, dry heat, steam and ultra violet lights (Abdu *et al*., 2005; Birnbaum and O'Brien, 2008). Being enveloped viruses, they are relatively sensitive to inactivation by lipid solvents such as detergents (Birnbaum and O'Brien, 2008). The viruses are also susceptible to formalin, ß-propiolactone, oxidizing agents, dilute acids, ether, sodium desoxycholate, hydroxylamine, sodium dodecylsulfate and ammonium ions (Easterday *et*

al., 1997; Birnbaum and O'Brien, 2008). The viruses remain viable for long in tissues, faeces and water (Abdu *et al*., 2005). They can survive low relative humidity and temperature in aerosol; low temperature and high moisture in faeces and remain infective in lake water for up to four days at 25^{o}C and over 30 days at 0^{o}C (Webster *et al*., 1978).

2.6 HOST RANGE

Avian influenza virus infections have been reported to occur naturally in a variety of animal species including humans, pigs, horses, sea mammals and birds (Alexander and Brown, 2000). Birds of all species and ages are susceptible to the virus though chickens and turkeys are the most adversely affected (Hanson, 2005). Ratites, quails, muscovy ducks, geese, guinea fowls, chukars, partridges and pheasants are all susceptible to AIV (Abdu *et al*., 2005). Domestic and wild aquatic birds have been incriminated as the natural reservoirs of AIV since they can harbour and excrete more than one subtype without showing clinical disease and rarely produce precipitin antibodies (Abdu *et al*., 2005).

Contrary to previous reports that HPAI viruses are normally present in the wild birds, evidence confirms that HPAI can arise as a result of mutation after H5 or H7 LPAI viruses are introduced to poultry from wild birds (Gracia *et al*., 1996; Perdue *et al*., 1998).

The findings of Gaidet *et al*. (2008) revealed H5N2 subtype with highly pathogenic genotype in healthy water fowls species sampled in Nigeria. These wild water fowls are likely to transmit the virus to domestic poultry as interspecies infections with AIV has been reported (Shane, 2002).

H5N1 virus infects dogs, horses, cats, swine, human beings and thus is zoonotic with serious consequences (Trevor *et al.,* 2002). Interspecies infection with H5N1 viruses occurs, through the alteration of the HA protein-binding units (Swayne *et al*., 1997; Alexander, 1999; Banks *et al.,* 2001; Shane, 2002; Li *et al.,* 2005).

The respiratory and gastrointestinal tract mucosal cells of swine, quails and human beings have respectively, the alpha 2, 6 and alpha 2, 3-sialic acid receptors of the swine and AIV A H5N1 strains. These species therefore, serve as potential "mixing vehicles" for virus reassortment in concurrent infections (Banks *et al.,* 2001; Perez, 2003). Virus reassortment and possible transmission to humans, favours a potential human influenza pandemic (Larson, 1998; Katz, 1999).

2.7 TRANSMISSION OF AVIAN INFLUENZA

Waterfowls and sea birds may act as reservoirs and introduce the AIV into poultry flocks (Easterday *et al.*, 1997). The virus is present in oral, nasal and ocular secretions and faeces of infected birds (Easterday *et al.*, 1997). The AIV can be transmitted directly by inhalation of these secretions or contaminated dust via aerosol if birds are in close proximity and with appropriate air movement (Abdu *et al*., 2005).

Indirect, transmission is usually through ingestion of contaminated food, water or infected carcass (Easterday *et al.,* 1997). In the incubator, broken contaminated eggs can infect newly hatched chicks (DEFRA, 2003).

The faeco-oral route is the most common route of spread from bird to bird (Abdu *et al*., 2005). Non-composted contaminated litter or manure have been implicated in AI outbreaks (Brugh and Johnson, 1987).

Live bird markets, movement of infected birds, contaminated equipment, clothing, shoes, vehicles and eggs are sources of AIV (Mubiru, 1998). Pigs may also transmit the virus to humans (Shane, 2002). The spread in a flock is usually rapid by direct contact though on some occasions, spread is erratic (Swayne and Jackwood, 2008). The transmission of AI slows as the number of susceptible birds decrease in the population (Swayne and Jackwood, 2008).

2.8 CLINICAL SIGNS OF AVIAN INFLUENZA

The incubation period of AI in individual bird is usually 3–7 days and 14 days between flocks, depending on the subtype, the viral dose, the species and age of the birds and route of infection though it could be up to 14 days within a flock (Easterday *et al*., 1997; Abdu *et al*., 2005).

The clinical signs of AI are variable and influenced by the virulence of the virus involved, the species affected, age, concurrent viral or bacterial infection and the environment (Easterday *et al*., 1997).

Highly pathogenic avian influenza in humans is characterized by fever, sore throat, respiratory distress, pneumonia and death (DAFF, 2008).

The LPAI in chickens and turkeys causes an inapparent infection to drop in egg production with mild respiratory disease of low mortality. Secondary bacterial or viral infection may cause severe respiratory disease with high mortality (Paul and Schrier, 2001; Swayne, 2003). In HPAI onset might be sudden with high morbidity (100%) and mortality (50-100%); ceasation to moderate drop in egg production and the laying of eggs with abnormal shell and pigmentation. Greenish diarrhoea, anorexia, depression, ruffled

feathers, coughing, sneezing, rales, dyspnoea, sinusitis, mucoid ocular and nasal discharges; cyanosis and oedema of the eyes, head, comb, wattles; discoloration of the shanks and feet have been reported (Easterday *et al.*, 1997; Adene *et al.*, 2006). Poultry that survive the acute disease exhibit nervous signs such as excitation, convulsion, circling and ataxia (Swayne, 2003).

In broilers, the clinical signs of AI are frequently less obvious, with severe depression, inappetance and a marked increase in mortality being the first abnormality to be observed (Easterday *et al.*, 1997). Oedema of the face and neck, torticolis and ataxia may also be seen. In turkeys, the disease is similar to that seen in layers, but it lasts 2–3 days longer and is occasionally accompanied by swollen sinuses (Swayne, 2003).

Low pathogenic avian influenza virus infections in wild birds are asymptomatic with excretion of viruses in faeces while in domestic ducks and geese, infection varies from asymptomatic to respiratory disease (Swayne and Jackwood, 2008). Highly pathogenic avian influenza virus infection have been reported in wild birds and domestic ducks with clinical signs ranging from respiratory through digestive and nervous signs with high mortality (Kaleta and Rulke, 2008).

In dogs, clinical signs of natural infection include high fever, panting, lethargy and death within 24 hours. In cats, the clinical signs of HPAI include fever, panting, depression, convulsion, conjunctivitis and ataxia (Songserm *et al.*, 2006b). However, signs in experimentally infected pigs were mild cough, hyperthermia and anorexia (Choi *et al.*, 2005).

2.9 MORBIDITY AND MORTALITY

In chickens and turkeys, morbidity (>50%) can be very high with low mortality (<5%) for LPAI virus infections (Swayne and Jackwood, 2008). The morbidity and mortality rates can be higher if secondary bacterial or viral infections are involved (Swayne and Jackwood, 2008). Highly pathogenic avian influenza infections are characterized by high morbidity (100%) and mortality (Swayne and Jackwood, 2008).

2.10 PATHOLOGY OF AVIAN INFLUENZA

2.10.1 Gross Lesions

Lesions caused by AI infections are variable depending on the disease severity and species of bird (Abdu *et al.*, 2005). In gallinaceous poultry, HPAI virus infections result in oedematous, haemorrhagic and necrotic lesions in visceral organs, cardiovascular and nervous systems (Swayne and Jackwood, 2008). In per acute phase, there is usually no gross lesions unlike in the acute phase, chickens have ruffled feathers with swelling of the head, face, upper neck, legs, shanks accompanied by petechial to ecchymotic subcutaneous haemorrhages especially in the un-feathered skin. In chickens, there is usually congested and swollen comb and wattles; oedema of surrounding eyes. The comb tips are usually cyanotic with nasal and oral discharges. Legs are congested and haemorrhagic with severe congestion and oedema of the lungs; enlargement and congestion of the kidneys with urates. Necrotic foci are present on the wattles, skin, liver, spleen, kidneys and lungs (Easterday *et al.*, 1997). Other lesions reported were petechial haemorrhages of serosal and mucosal surfaces of the heart, proventricular junction, small intestines and abdominal fat (Adene *et al.*, 2006).

In dogs, lesions were bloody nasal discharges, severe pulmonary congestion and oedema, congestion of the spleen, kidneys and liver (Songserm *et al.,* 2006b). In cats, lesions were cerebral congestion, pulmonary oedema, severe pneumonia, renal congestion and haemorrhage of intestinal serosa (Songserm *et al.,* 2006b).

2.10.2 Histopathology

In LPAI, there is loss of cilia, swelling, vacuolation and necrosis of epithelia cells of sinuses associated with oedema, heterophils and mononuclear cell infiltration; necrosis and hyperplasia of tracheal epithelium with mononuclear cell and heterophil infiltration (Swayne and Jackwood, 2008). Serofibrinous exudates mixed with macrophages, heterophils and epithelial cells of the bronchial, atria and capillaries of lungs with necrosis of some lobules (Easterday *et al*., 1997). Fibrin thrombi in small arterioles with prominent cuboidalization of atrial epithelium have been reported (Easterday *et al*., 1997). Diffuse severe congestion of pulmonary vasculature. The spleen is congested with fibrin deposition and lymphocytes depletion. There is severe congestion of renal blood vessels (Easterday *et al*., 1997). There is lymphocytic depletion and necrosis or apoptosis of lymphocytes in the bursa of fabricius, thymus and spleen in birds that die of LPAI (Easterday *et al*., 1997).

In HPAI, histopathologic lesions consist of necrosis and inflammatory changes in multiple organs such as the skin, brain, heart, pancreas, lungs, adrenal glands and lymphoid organs (Swayne and Jackwood, 2008). In per acute phase, there is little or no microscopic lesions while in acute cases, there is multiple organs involvement with predominantly necrotic lesions and apoptotic cell death associated with inflammation, haemorrhages and oedema (Easterday *et al.,* 1997). There is oedema of the dermis of the

comb with vesicles in epidermis, micro vesicles in the epithelium; larger vesicles or focal necrosis underneath the comb epithelium, invaded by heterphils and lymphocytes, accompanied by a marked lymphocytic peri vasculitis in the dermis (Easterday *et al.*, 1997). In the wattles, are oedema, hyperemia, haemorrhages and foci of perivascular cuffing. Severe myocarditis and myocardial necrosis, oedema, hyperemia and haemorrhages in the heart (Adene *et al*., 2006).

Microscopic lesions observed in dogs were severe pulmonary oedema and interstitial pneumonia with inflammatory cell infiltration in the lungs with brownish black particle within lung parenchyma. There is focal necrosis in the liver and mild nephritis with tubular degeneration in the kidneys (Songerm *et al.,* 2006b). In cats, lesions were cerebral congestion, pulmonary oedema, severe pneumonia, renal congestion and haemorrhage of intestinal serosa (Songserm *et al.,* 2006b). However, in pigs there is moderate interstitial pneumonia (Choi *et al.,* 2005).

2.11 DIAGNOSIS AVIAN INFLUENZA

Avian influenza diagnosis depends on history, clinical signs, postmortem and histopathological findings; direct demonstration of virus or antigen and indirect measurement of viral exposure through antibody detection (Swayne and Suarez, 2001).

Virus inoculation in 9- to 11-day old embryonated chicken eggs or cell culture is the gold standard test for AIV infection. This would allow for possible viral isolation for further analysis and characterization. However, viral isolation is labour-intensive and slow, requiring up to two- to three-week blind passages before a haemagglutination agent is subtyped by haemagglutination inhibition (HI) and neuraminidase inhibition tests. Viral

isolation of H5 and H7 HPAI viruses are quicker with embryo death within 30 to 72 hours (OIE, 2009).

Molecular diagnosis of AI include conventional or real-time reverse transcription polymerase chain reaction (RT-PCR), or nucleic acid sequence-based amplification (NASBA) assays (Swayne and Akey, 2004; Suarez *et al.,* 2008).

The PCR tests involves making a complementary DNA (c-DNA) copy of the target influenza RNA gene using short nucleotide primers that are specific to the target c-DNA to amplify the target gene which can be detected by gel electrophoresis (conventional RT-PCR) or colorimetrically by release of fluorochromes from probes specifically binding to the target c-DNA (real-time RT-PCR). The PCR can be optimized through the use of primer sequences to examine the gene sequence data from multiple strains of an influenza virus (Spackman *et al.,* 2008). The RT-PCR is more sensitive than viral isolation since it is able to detect nucleic acids of AIV even when the viruses are no longer viable (Spackman *et al.*, 2008).

The serological tests include tests for antibody to group reactive antigens like NP and M such as ELISA and agarose gel immunodiffusion tests; or tests for H5 subtype specific antigen such as the HI test and the virus neutralization test (Alexander *et al*., 2004). Haemagglutination inhibition test is used for surveillance of AI (OIE, 2009). In countries where vaccination is practiced, it is recommended that response to vaccination be monitored serologically using HI test. Where differentiation of flocks between vaccinated and unvaccinated is necessary, H5 HI testing is not able to distinguish between these

flocks so alternative strategies are necessary for differentiation of infected and vaccinated birds (Capua and Marangon, 2003).

2.12 DIFFERENTIAL DIAGNOSIS OF AVIAN INFLUENZA

The AIV infection cannot be diagnosed based only on clinical signs and postmortem lesions. It should be differentiated from diseases exhibiting similar clinical and postmortem lesions such as acute fowl cholera, velogenic Newcastle disease, infectious bronchitis, infectious coryza, chlamydiosis, infectious laryngiotracheitis, adenoviruses causing respiratory disease or drop in egg production (e.g. EDS '76) and mycoplasmosis (Eaterday *et al*., 1997, Swayne and Jackwood, 2008). Avian influenza can be differentiated from other diseases by its high morbidity and mortality rate of up to 100%, ruffled feathers and dark red skin, diffuse haemorahages between the hocks and feet, and blood in the cloaca (Swayne and Jackwood, 2008).

2.13 AVIAN INFLUENZA VIRUS AND PIGS

There are several ways an AIV with pandemic potential can be generated. There is the likelihood of an avian virus directly adapting itself to infect and transmit readily in humans. Secondly, both avian and human influenza viruses occurring in a human concurrently can undergo reassortment with these two viruses. A third alternative is that an animal species that is susceptible to both avian influenza and human influenza viruses might serve as an intermediary host, of which the pig is a likely candidate even though the genetic reassortment in pigs leading to human pandemics has not been substantiated. Pigs are susceptible to both avian and human strains due to the presence of α-2, 3 and α-2, 6 sialic acid receptors in their tracheal epithelium (Ito *et al.*, 1998). Pigs have been

postulated as a “mixing vessel” in the generation of reassortant of AI pandemic strain (Claas *et al.,* 1994). Several types of influenza A viruses, such as classical swine H1N1, avian-like H1N1, human-like H3N2 and reassortant H1N2 have been reported in pigs in several countries (Brown, 2000). The detection of avian influenza in pigs such as report of H3N2 human-like surface genes but with H1N1 internal avian viral genes in Italian pigs, and subsequently children in the Netherlands supported the assertion that pigs are “mixing vessel” for influenza viruses (Castrucci *et al.,* 1993; Claas *et al.,* 1994). There are reports of HPAI H5N1 virus detected in pigs in Fujian province, southern China and in Java, Indonesia (Cyranoski, 2005; Martin *et al.,* 2006). There are also reports of serological evidence of H5N1 infection in pigs in Vietnam (Choi *et al.,* 2005). Experimental infection studies indicated that while avian and human H5N1/2004 viruses were able to replicate in the swine respiratory tract, inoculated pigs developed only mild cough and fever, and no virus was transmitted to contact pigs (Choi *et al.,* 2005). There are no reports that pigs contribute to H5N1 virus adaptation or transmission to humans.

2.14 HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN MAMMALS

H5N1 viruses have been able to cause disease and death in mammals. Two tigers and two leopards in Thailand developed high fever and respiratory distress and died after been fed on raw chicken carcasses from a local slaughterhouse in an area where chickens were dying with respiratory and neurological signs (Keawcharoen *et al.,* 2004). Avian influenza H5N1 virus was isolated from the lung tissue samples from among these felids (Keawcharoen *et al.,* 2004). Kuiken *et al.* (2004) reported that cats can be infected with AIV through direct respiratory route inoculation, feeding of virus-infected birds or horizontal cat-to-cat contact transmission and excreted the virus. The clinical

manifestation includes lethargy, fever, protrusion of third eyelids, conjunctivitis and laboured breathing. The report further showed that, H5N1 virus replicated in the respiratory tract and in multiple extra-respiratory tissues, such as the brain, liver, kidneys and heart (Rimmelzwaan *et al.,* 2006). In Thailand, a domestic cat that consumed an H5N1 infected pigeon carcass developed fever, panting, convulsion and died with the virus and antigen detected in organs (Songserm *et al.,* 2006a). A dog similarly developed fatal infection, with H5N1 virus isolation from organs, following ingestion of a duck carcass from an area with reported H5N1 in ducks (Songserm *et al.,* 2006a; 2006b). Antibodies to H5N1 have been reported in village dogs and cats in Thailand (Butler, 2006). Avian influenza H5N1 infection has also been reported in Iraq in domestic cats associated with bird deaths (Yingst *et al*., 2006). Intranasal inoculation of ferrets showed 2004 H5N1 human isolates from Asia were typically lethal, replicated to high titres in the ferret respiratory tracts and multiple organs, including the brain; the severity of disease (high fever, weight loss, anorexia, diarrhoea and lethargy), broad tissue tropism and high lethality rates in ferrets distinguished these virulent human isolates from most contemporary avian isolates (Govorkova *et al.,* 2005). Marlin *et al.* (2007) also demonstrated in the ferret model that human H5N1 isolates in 2004 exhibited increased virulence compared with 1997 human isolates, which may reflect the higher human case fatality. There is no evidence however to suggest that mammals can play a significant role in H5N1 transmission to humans, although such a chance should not be disregarded in assessing potential risks.

2.15 HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 IN HUMANS

Prior to 1997, the transmission of AI viruses to humans was not considered to be a serious human threat, based on the findings that avian viruses do not replicate efficiently and that no fatal cases of human infections had been reported during any outbreaks of AI (Beare and Webster, 1991). Highly pathogenic avian influenza is global as it has caused 307 deaths out of 520 human infections (WHO, 2011). Nigeria was also affected as it recorded the death of a woman in Lagos in 2007 due to HPAI H5N1 infection hence, heightening the fear of the pandemic threat on our shores (WHO, 2007).

2.16 CONTROL OF AVIAN INFLUENZA

The strategy for control of AIV undertaken by a country, zone or compartment (CZC) depends on the status of the infection in that CZC which will determine the control outcome. There are three possible outcome for any control strategy which include prevention, management and eradication (Swayne and Jackwood, 2008). In a CZC free of infection, the goal would be preventing introduction of the disease, unlike when the disease is already present, control program would be aimed at managing the infection to avoid spread and reduce economic losses with long term intention of eradication. Eradication which refers to the complete elimination of the virus from the CZC is the definitive goal of any control strategy (Swayne and Jackwood, 2008).

The aforementioned goals are achievable through development of a broad strategy involving any of the following features: education, biosecurity, diagnostic and surveillance, removal of infected source and reducing host susceptibility to the virus (Swayne and Jackwood, 2008).

The Nigerian control strategy is swift detection and management of AI outbreaks and close monitoring of the situation (AICP, 2008). The features of the control strategy involves establishing a viable epidemio-surveillance and laboratory network for proactive surveillance, ensuring swift disease outbreak reporting within 12 hours; stamping-out, disinfecting affected farms and restriction of movement of animals and personnel (AICP, 2008).

2.16.1 Education

Education which is the provision of knowledge on AI transmission and role of individuals in the prevention, management and eradication is a critical aspect in any AI control program (Swayne and Jackwood, 2008). It involves training of all stakeholders in the poultry industry in the control process regarding how the AIV are introduced, spread and how such events could be prevented. There is also the need for educating these individuals on the control of risky behaviours and actions that would reduce the spread of the viruses. The general public should be involved in the education process by communicating information on risk and dispelling incorrect information and rumours (Swayne and Jackwood, 2008).

2.16.2 Biosecurity

Biosecurity refers to management practices and procedures undertaken to prevent virus introduction or if present from leaving a farm. This involves preventing contamination, controlling movement of birds or products, people and equipments or reducing the amount of virus through cleaning and disinfection (Easterday *et al.*, 1997). Biosecurity consist of two categories – inclusion biosecurity involving quarantine and other measures designed to keep the virus on infected farm or CZC and exclusion biosecurity aimed at

keeping the virus out of AI- free farms or CZC (Swayne and Jackwood, 2008). Cleaning and disinfection of equipments and clothing/ footwear of farm workers are essential to prevent introduction and farm to farm spread (Swayne and Jackwood, 2008).

In Nigeria, the structure of the poultry industry consist mostly of sectors 2 and 3 with little or no biosecurity and constant introduction of new birds from relatively unknown sources which worsen the biosecurity problems (AICP, 2008). The rearing of different species of poultry within a premise increases the ease of interspecies transmission. The government have step up biosecurity measures in live bird markets (LBMs) with routine disinfection and building of eight model LBMs with improved biosecurity features (AICP, 2009). The rural poultry which are mostly raised extensively with minimal biosecurity are constantly in contact with wild birds (FAO, 2008).

2.16.3 Avian Influenza Surveillance

Surveillance in local poultry involves the ability to detect the virus or evidence of infection in a bird population or their environment or a means of verifying freedom from such infections.

Accurate and rapid diagnosis is essential for swift and successful control of AI (Easterday *et al*., 1997). Passive surveillance is critical for identification of LPAI due to the mild nature of the infection. On the contrary, HPAI with high mortality requires active surveillance to identify where AIV infection is located within a CZC or certifying a CZC free from the infection (Swayne and Jackwood, 2008). Surveillance is vital for evaluating the success of a control strategy and for use in decision making as a prelude to improve control strategies (Swayne and Akey, 2004).

The poor veterinary infrastructure in Nigeria poses problems with surveillance though measures have been undertaken to improve surveillance. These include developing a traceability mechanism for monitoring of poultry and poultry product movements; revitalizing veterinary quarantine infrastructure and control posts; promoting training of backyard poultry farmers on HPAI recognition and control including their role in animal disease surveillance (AICP, 2008).

2.16.4 Removal of Susceptible Sources of Avian Influenza Virus

Removal of infection sources or susceptible sources to prevent continued environmental contamination and dissemination of the AIV is important for stopping an outbreak and eradicating the disease from a farm or CZC. Upon identifying an infected flock, high-risk materials such as eggs, infected birds and manure should be eliminated (Easterday *et al.*, 1997). Elimination of risk materials should be carried out through the safest and most economic method which depends on the viral strain, local conditions, level of biosecurity practiced on the farm and available financial and personnel resources (Swayne and Jackwood, 2008).

In HPAI, elimination involves humane depopulation and disposal of carcasses, eggs and manure through composting, incineration, rendering or landfill burial. In LPAI infection, controlled marketing of birds 2-3 weeks following recovery from infection and washing of eggs prior to marketing is practiced in some developed economies (Swayne and Jackwood, 2008). The OIE recommends stamping-out policy in notifiable LPAI infection (WHO, 2008). Stamping-out would only be successful if compensation is paid by governments in a timely manner (Swayne and Jackwood, 2008).

Nigeria practices a modified stamping-out policy where birds on infected farms are depopulated and the farms disinfected. This is followed by compensation which is aimed at encouraging reporting of disease outbreak (AICP, 2008).

2.16.5 Host Resistance to Avian Influenza Virus

Increasing host resistance to AIV reduces the negative effect of the disease or prevents infection if exposed to virus. This is achieved either through using breeds genetically resistant to AIV or producing passive or active immunity to susceptible hosts (Swayne and Jackwood, 2008).

There are reports of Thailand native chicken breeds as being more frequently present in survivors after HPAI H5N1 village outbreak (Boonyanuwat *et al.*, 2006). Small interfering RNA (siRNA) has been used to silence expression of some AI viral genes in avian and mammalian cells thereby conferring resistance to AI viral replication (Li *et al.*, 2005).`

Resistance to AI viruses is usually produced through active and passive immunity, principally against the viral HA and to a lesser extent against viral NA (Swayne and Jackwood, 2008). Immunity is usually through vaccination though chicks can acquire maternal antibodies through egg yolk (Swayne and Jackwood, 2008). Vaccination as an option for AI control has been practiced in Mexico, Italy, Pakistan and Egypt (Capua and Marangon, 2003; OIE, 2009). It is being advocated as a first option for control in areas with high poultry population density in which rigorous biosecurity measures is incompatible with modern rearing systems (Capua and Marangon, 2003). Vaccination when incorporated with other features of AI control such as controlled marketing and

surveillance is thought to be cost-effective and utilizes the industry infrastructure, regulatory power, requires neither ethical destruction of healthy birds nor costly disposal (Halvorson, 2005). This method is said to put more control cost on producers with infected birds (Halvorson, 2005). Vaccinated birds are protected from clinical disease; though they shed the virus at reduced amount into the environment making it impossible to differentiate infected from vaccinated birds when inactivated homologous vaccines are used (Swayne and Jackwood, 2008). The use of inactivated heterologous vaccines allows vaccinated and naturally infected birds to be differentiated using the "Differentiating Infected from Vaccinated Animals" (DIVA) strategy (Swayne and Jackwood, 2008). The advocates for vaccination believes the ability to differentiate vaccinated/infected from vaccinated/non-infected and ethical question of destruction of healthy birds calls for review of stamping-out as the first option in an outbreak of AI. Currently, Nigeria do not vaccinate as an official policy though there is the possibility of adopting the vaccination option (AICP, 2008).

2.17 PUBLIC HEALTH SIGNIFICANCE OF AVIAN INFLUENZA

There is the likelihood that H5N1 infections have become endemic among poultry in certain areas and that sporadic human infections arising from direct contact with infected poultry and/or wild birds will continue to occur. So far, the spread of the H5N1 virus from person to person has been very rare, limited and unsustained, though this epizootic continues to pose an important public health threat (CDC, 2008).

There is little pre-existing natural immunity to the H5N1 virus in human population. Should the H5N1 viruses gain the ability for efficient and a sustained transmission among humans, an influenza pandemic could result with a potential of high rates of ill health and

death worldwide (Katz, 1999). There was also evidence of three cases of human–to–human transmission within families (Capua and Alexander, 2004). In Nigeria, only one human death has been reported in 2007 in Lagos (WHO, 2008).

2.18 ECONOMIC SIGNIFICANCE OF AVIAN INFLUENZA

Poultry are a major source of animal protein for human consumption and AI outbreak would cause a reduction in growth rate, egg production and high mortality which will increase the cost of poultry and poultry by-products. This would be a setback in the food security drive of most developing countries (Adene *at al*., 2006).

The cost of depopulation, disposal of infected/incontact birds together with disinfection of the premises due to AI results in job and income loss and loss of livelihood of millions of poultry workers and owners while being a huge burden on the nation's resources. Avian influenza constitutes a serious threat to poor rural poultry producers, both directly through mortality, but probably even more so indirectly through the rigorous disease control measures applied by animal health authorities. The latter may deprive households of small but valuable amounts of protein in their diets; much needed petty cash income and most importantly, an investment opportunity for escaping poverty (Abdu *at al*., 1992).

Thus AI has been described as an international problem that requires international efforts and cooperation to solve (Swayne, 2003). A total of 915,650 birds have been lost since the beginning of the outbreaks of AI in Nigeria. The sum of N107, 639,866 has so far been paid as compensation to 728 farmers (NADIS INFO, 2006).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The study area included Kaduna North, Kaduna South and Chikun Local Government Areas (LGAs) (Plate I) of Kaduna State located in North western Nigeria, with an estimated population of about 6 million people based on the 2006 census (KDSG, 2007). Kaduna State lies between Latitude 8^0 $45^{'}$ and 11^0 $30^{'}$ North and Longitude 6^0 $11^{'}$ and 9^0 East. It shares boundary with Katsina, Kano, Plateau, Niger, Zamfara, Bauchi, Nasarawa and the FCT. The state has 23 LGAs that are inhabited by different ethnic groups, including Hausa, Fulani, Kaje and Kataf amongst others (RIM, 1993).

The average annual temperature in Kaduna State is 34^oC with hottest months being March- April (40^oC) and the coolest period (13.2^oC) is December during severe harmattan. Rainfall varies between 1,000 mm and 1,500 mm and the rainy season last for 100-150 days (Mid April-ending of October). The dry season occurs late October-early April (RIM, 1993).

The State falls within the Northern Guinea Savannah zone, which comprises two Savannah types namely; shrubs and bush Savannah. The Savannah is characterized by woodland with grasses of different species. Mainly undifferentiated complex igneous and metamorphic rocks underlie the state (KDSFDO, 2007). The State has an estimated DCSpoultry population of 5,200,000, dogs population of 122,089 and pigs population of 249,651 (RIM, 1992; KDSG, 2007). The occupation of the people living in Kaduna State includes crop farming, livestock rearing, trading and fishing (RIM, 1993).



PLATE: Map of Kaduna State showing sampling areas (© KDSG)

Key: Represent sampling areas

3.2 DETERMINATION OF H5 AVIAN INFLUENZA PREVALENCE

3.2.1 Sampling Method

The sampling of the chickens, dogs and pigs that were sampled for H5 antibodies were determined by non-probability sampling (Bernard, 2002).

A preliminary meeting with the Zonal Veterinary Officer and Desk officer of Avian Influenza Control Project of Kaduna State was done. Sampling locations were identified according to the judgment of local experts, namely; David Isah, Dr. Michael Kapioh and Jamilu Yakubu. The sampling was carried out within the period of five months, between July and November, 2010 in one veterinary clinic, seven slaughter areas and households within the study area.The sampling was done based on availability of animals in the locations. A total of 280 blood samples were collected in the field, out of which 56 sera were not tested due to leakage of serum from the sterile vials. Only 224 samples were analyzed for H5 subtype antibodies.

3.2.2 Administration of Questionnaires

A questionnaire (Appendix 1) was administered to dog owners keeping poultry and or pigs to gather information on the feeding and management aspect of the dogs, knowledge of dog owners on HPAI and other diseases of poultry. The questionnaire was pretested before it was administered. Standardized biosecurity checklists (Abdu, 2010 Unpublished) (Appendix 2 and 3) were completed during the visits to LBMs, dog and pig slaughter areas, and was used to assess the biosecurity practices in the LBMs and slaughter areas.

3.2.3 Blood Sample Collection

About 0.5-1.0 ml of blood was collected from each chicken, dog and pig through venipuncture from wing vein, cephalic and ear vein respectively using 22 G needles and 2 ml syringes. Chickens were sampled in live bird markets, dogs in households, veterinary clinics or slaughter areas while, pigs were sampled in slaughter areas. The blood samples were allowed to clot at room temperature and sera were separated by centrifugation at about 447 g for 5 minutes (Collee *et al*., 1982). The collected sera were then stored in deep freezer at -20^{o}c until tested.

3.2.4 Preparation of 1% Red Blood Cells

Five millilitres of blood was collected from five different adult local chickens in Alsever's solution. The red blood cells were washed three times with physiological saline solution (PSS) by gentle centrifugation at 447g for 5 minutes. One millilitre of fully packed RBCs was mixed with 99 ml of physiological saline (PSS) to prepare 1% solution (Allan *et al*., 1978).

3.2.5 Determination of AI H5 Antigen Titre

The haemagglutination (HA) test was used as described by OIE (2009) 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 in Podova, Italy.

Twenty five microlitre of phosphate buffered saline (PBS) PH 7.2 was dispensed into each well of a plastic V-bottom microtitre plate. Twenty five microlitre of antigen suspension was dispensed into the first well. Two -fold serial dilutions of 25 ul of the

antigen suspension were made across the plate. A further 25 ul of PBS was dispensed into each well. Twenty five microlitre of 1% washed chicken red blood cells was dispensed into each well. These were mixed by gently tapping the plate and then allowing the RBC to settle for about 40 minutes at room temperature. Haemagglutination end point was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration was read to the highest dilution giving complete HA (no streaming). This represented 1 HA unit (HAU) and was calculated accurately from the initial range of dilutions, by moving two spaces behind the highest dilution giving complete HA. The titre of the antigen was determined as 9.0 $\log_2$ and 4 HAU of the antigen was used in the haemagglutination inhibition test.

3.2.6 Determination of AI H5 Antibody Titre

The presence and titres of antibodies were determined by haemagglutination inhibition (HI) test (OIE, 2009). Twenty five microlitre of PBS was dispensed into each well of the plastic V-bottomed microtitre plate followed by addition of 25 ul of test serum into the first well after heat inactivating the sera at 56^{o}C for 30 minutes in a water bath. Two fold dilutions of the 25 ul volumes of the serum were made across the wells in the plate. Four haemagglutination units (4 HAU) of the antigen suspension in 25 ul was added to each well and left for a minimum of 30 minutes at room temperature. A 25 ul of washed 1% chicken RBC was added to each well and after gentle mixing 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 agglutination of RBC by the 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only wells in which the RBCs streams at the same rate as the control well (containing 25 ul RBCs and 50 ul PBS only) were

considered to show inhibition. The result was considered positive if there was inhibition at a serum dilution of 1/16 and above.

3.3 DATA ANALYSIS

The data obtained from questionnaires and serological tests were coded and recorded in Excel data base. Statistical package for social science (SPSS software version 17.0) was used for descriptive statistics and to calculate frequencies and means.

CHAPTER 4

RESULTS

4.1 AVIAN INFLUENZA H5 SUBTYPE ANTIBODIES IN CHICKENS, DOGS AND PIGS

All the chickens (75), about 86.7% (39/45) dogs and pigs (104) sampled in the study were from the live bird markets, dog and pig slaughter areas respectively. About 6.7% (3/45) dogs were sampled, each from Veterinary Clinic and household respectively.

The H5 AI antibodies prevalence in chickens sampled was 6.7% (5/70) with dogs prevalence of 2.2% (1/45) and none (0/104) of the pigs sampled had antibodies to AI H5 subtype. However, none of the dogs presented in either the veterinary clinic or the dog slaughter areas had antibodies against AI. However, 33.3% (1/3) of dogs in households had AI antibodies.

The AI antibodies prevalence in chickens in Central Market LBM was 4.3% (2/47) Station LBM had 13% (3/20) and 50% (1/2) in Barnawa. The AI HA 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 $\log_2$ (Table 4.1).

Table 4.1: Avian influenza H5 sero-prevalence and mean antibody titres in chickens, dogs and pigs in Kaduna

Species	No of Samples Tested	No. Positive (%)	Mean antibody titre (log_2)
Chickens	75	5 (6.7)	8.5 ± 0.04
Dogs	45	1(2.2)	9.0 ± 0.00
Pigs	104	0	0

4.2 MANAGEMENT OF DOG POSSESSING AVIAN INFLUENZA (H5 SUBTYPE) ANTIBODIES

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

4.3 DOG OWNERS AWARENESS AND PRACTICES ON AVIAN INFLUENZA

Among the 130 dog owners who participated in the study, 78.5% (102/130) were males with 59.2% (77/130) having received secondary education (Figure 4.1). Moreover, 36.9% (48/130) were business people with 88.5% (115/`130) of dog owners having a household size of more than 3 persons. However, 82.3% (107/130) of dog owners keep dogs for security, while 17.7% (23/130) keep dogs as pets. The mean number of dogs per household was 1.38 ± 0.05 though 28.5% (37/130) of the respondents had two dogs per household (Figure 4.2). About 50.8% (66/130) of dog owners fed their dogs with food eaten in the household, though some dog owners 20.7% (27/130) fed their dogs with chicken carcass (Figure 4.3). Dogs roam freely 85.4% (111/130) with 10.8% (14/130) confined and 3.8% (5/130) sleeping outside the house.

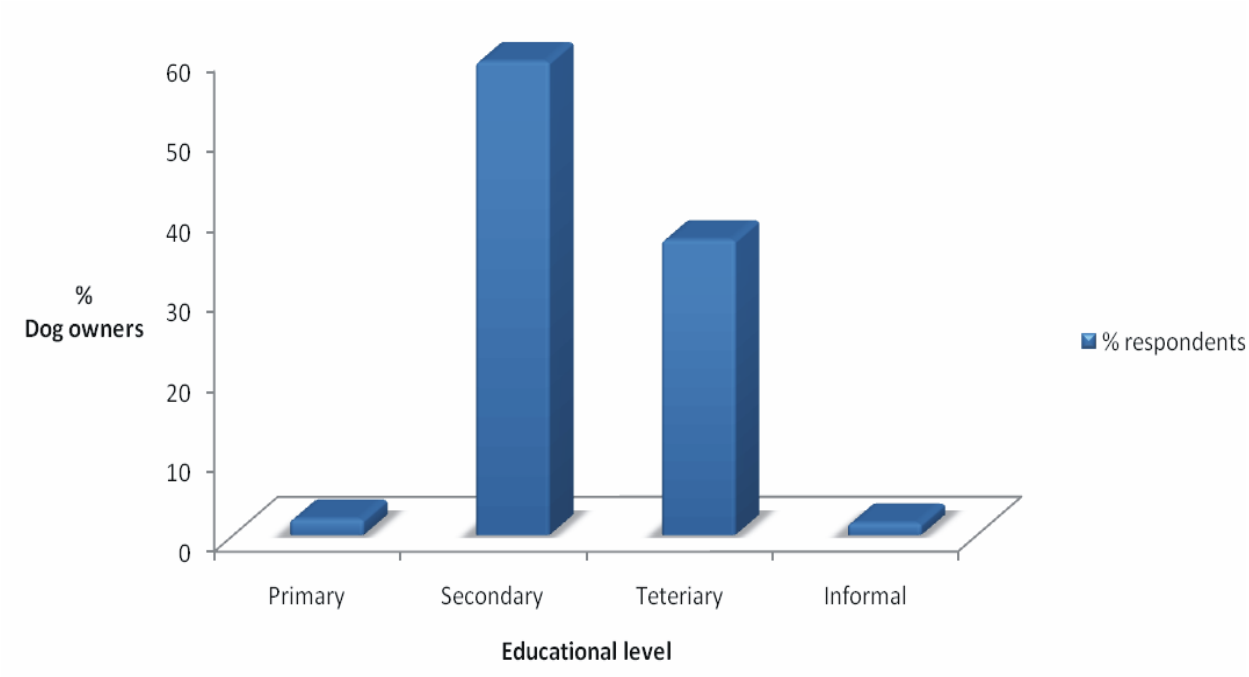


Figure 4.1: Distribution of educational level of 130 dog owners in Kaduna that participated in the study.

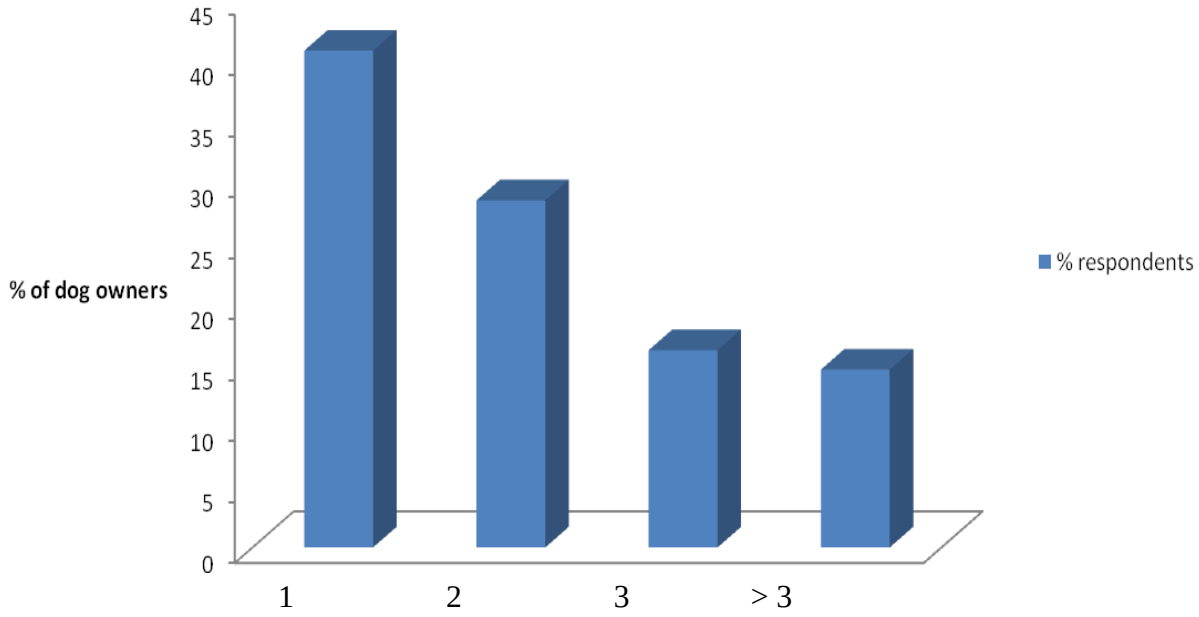


Figure 4.2: Distribution of the number of dogs in 130 dog owners household in Kaduna

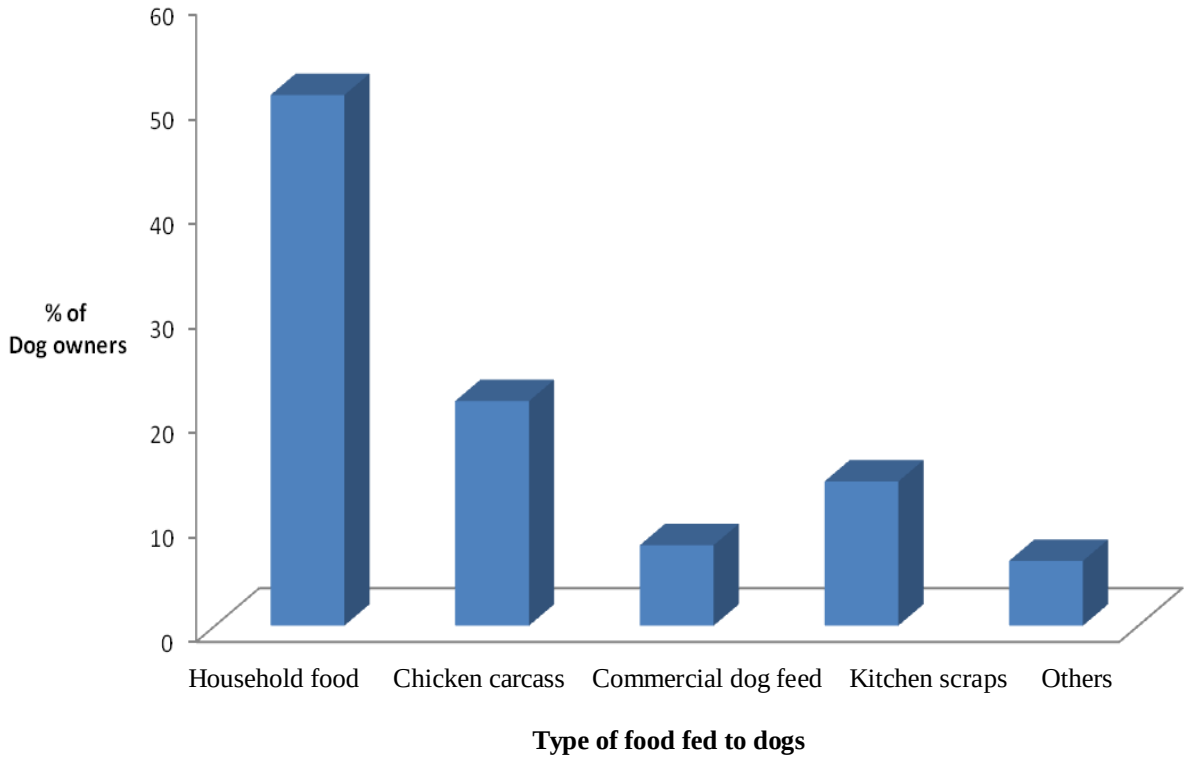


Figure 4.3: Types of food fed to dogs by 130 dog owners that participated in the study in Kaduna

Among the dogs examined, 97.7% (127/130) were apparently healthy. About 46.9% (61/130) dog owners would seek veterinary assistance when a dog is sick, 27.7% (36/130) would do nothing, 18.5% (24/130) would give out and 6.9% (9/130) would treat the dog personally (Table 4.2). Only 32.3% (42/130) dog owners vaccinate dogs against rabies and 2.3% (3/130) knew that dogs can become susceptible to AI (bird flu). Similarly, 0.8% (1/130) and 3.1% (4/130) of dog owners reported knowing that dogs can transmit avian influenza virus to humans and die of avian influenza respectively (Table 4.3).

One hundred and twenty one (93.1%) (121/130) dog owners keep poultry with 29.2 % (38/130) keeping exotic chickens and 64.6% (84/130) local chickens (Table 4.4). About 78.5% (102/130) of dog owners sourced poultry from LBMs with 10.8% (14/130) from agrovet shops and 4.6% (6/130) acquired as gifts (Table 4.5). All dog owners 93.1% (121/130) who keep chickens managed the birds extensively. Among dog owners who keep poultry, 57.7% (75/130) keep their dogs away from poultry while 42.3% (55/130) permitted contact between dogs and poultry feed and offal (Table 4.6).

One hundred and twenty five (96.2%) (125/130) dog owners were aware of avian influenza but none had experienced an outbreak in their poultry flock neither has any of their neighbours flock. About 95.4% (124/130) of the dog owners handled dead poultry with bare hands, while 4.6% (6/130) 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 23.8% (31/130) of dog owners would seek veterinary assistance for sick poultry, while 24.6% (32/130) would sell, 34.7% (43/130) give away, 8.9% (11/130) eat, 4.8% (6/130) will throw away and 0.8% (1/130) would feed to dogs (Table 4.7)

Table 4.2: How dog owners in Kaduna handle sick dogs

Response	No. of respondents	No. Positive (%)
Seek veterinary assistance	130	61 (46.9)
Would do nothing	130	36 (27.7)
Would give out the sick dog	130	24 (18.5)
Treat sick dog personally	130	9 (6.9)

Table 4.3: Dog owners knowledge on avian influenza in Kaduna

Response	No. of respondents	No. Positive (%)
Knew that dog can be sick of bird flu	130	3(2.3)
Knew that dog can transmit avian influenza virus to humans	130	1(0.8)
Knew that dogs can die of avian influenza virus	130	4(3.1)

Table 4.4: Dog owners keeping poultry in Kaduna

Response	No. of respondents	No. Positive (%)
Dog owners keeping poultry	130	121(93.1)
Dog owners keeping exotic chickens	130	38(29.2)
Dog owners keeping local chickens	130	84 (64.6)

Table 4.5: Dog owners source of birds in Kaduna

Source	No. of respondents	No. Positive (%)
Live bird markets	130	102(78.5)
Agrovet shops	130	14 (10.8)
Acquired as gifts	130	6 (4.6)

Table 4.6: Contact between dog and poultry in Kaduna

Response	No. of respondents	No. positive (%)
No contact between dog and poultry	130	75(57.7)
Contact between dog and poultry feed and offal	130	55(42.3)

Table 4.7: How dog owners handled dead and sick poultry in Kaduna

Response	No. of respondents	No. positive (%)
Handled dead poultry with bare hands	130	124(95.4)
Wear hand gloves	130	6(4.6)
Do not wash hands with soap and water after handling dead or sick poultry	130	42(32.3)
Seek veterinary assistance for sick poultry	130	31(23.8)
Sell sick poultry	130	32 (24.6)
Would give away the sick poultry	130	43(33.1)
Would eat the sick poultry	130	11(8.5)
Would throw away the dead poultry	130	6(4.6)
Would feed dog	130	1(0.8)

Sixty five percent (85/130) dog owners kept pigs with a mean herd size of three and 40.0% (52/130) of the pigs aged more than a year and 25.4% (33/130) aged less than a year (Table 4.8). About 51.5% (67/130) of dog owners purchase pigs from friends, while 13.8% (18/130) obtained pigs as gifts (Table 4:9). Only 15.5% (13/130) of dog owners managed pigs intensively, while 84.5% (71/130) managed theirs semi-intensively. About 87.5% (114/130) of dog owners fed pigs with kitchen scraps and bran, while only 13.8% (18/130) use exclusively kitchen scraps.

Eighty seven percent (113/130) of dog owners did not allow direct contact of pigs with poultry while 24.1% (21/130) of the respondents allowed indirect contact. About 96.5% (125/130) of dog owners would sell sick pigs while 1.5% (2/130) ate sick pig. Only 0.8% (1/130) sought veterinary assistance when their pigs were sick. However, 42.3% (55/130) would give out dead pigs while 21.5% (28/130) buried dead pigs, 1.5% (2/130) ate dead pigs while 0.8% (1/130) threw dead pigs (Table 4.10). All the dog owners were not aware that pigs can be infected with avian influenza virus neither were they aware of the mode of transmission of the disease from pigs to humans.

The clinical signs dog owners' observed in sick dogs were loss of appetite 96.2% (125/130), lethargy 70.0% (91/130), emaciation 11.5% (15/130), nasal discharge 3.1% (4/130), cough 6.9% (9/130) and conjunctivitis 0.8% (1/130) (Table 4.11).

Poultry diseases known by dog owners were Newcastle disease 47.7% (62/130), Gumboro 30.8% (40/130), avian influenza 27.7% (36/130), coccidiosis 13.1% (17/130), fowl pox 11.5% (15/130), fowl typhoid and cholera 0.8% (1/130) (Table 4.12).

Table 4.8: Herd size and age of pig kept by dog owners in Kaduna

Response	No. of respondents	No. positive (%)
Mean herd size of three pigs	130	85(65.4)
Aged more than one year	130	52(40.0)
Aged less than one year	130	33(25.4)

Table 4.9: Dog owners source of pigs in Kaduna

Source	No. of respondents	No. positive(%)
Friends	130	67(51.5)
Gift	130	18(13.8)

Table 4.10: How dog owners handle sick and dead pigs in Kaduna

Response	No. of respondents	No. Positive (%)
Sell sick pigs	130	125(96.2)
Eat sick pigs	130	2(1.5)
Seek veterinary assistance	130	1(0.8)
Would give out dead pigs	130	55(42.3)
Would bury the dead pig	130	28(21.5)
Would throw away the dead pig	130	1(0.8)

Table 4.11: Clinical signs of sickness observed by dog owners in sick dogs

Clinical signs	No. of respondents	No. positive (%)
Loss of appetite	130	125 (96.2)
Lethargy	130	91(70.0)
Emaciation	130	15(11.5)
Nasal discharge	130	4(3.1)
Cough	130	9(6.9)
Conjunctivitis	130	1(0.8)

Table 4.12: Poultry diseases known by dog owners in Kaduna

Disease	No. of respondents	No. positive (%)
Newcastle disease	130	2(1.5)
Gumboro disease	130	40(30.8)
Avian influenza	130	36(27.7)
Coccidiosis	130	17(13.1)
Fowl pox	130	15(11.5)
Fowl typhoid and cholera	130	1(0.8)

The dog owners source of news on bird flu were, from friends 61.5% (80/130), from radio 68.5% (89/130), from TV 85.4% (111/130), through posters 33.1% (43/130), from newspapers 2.3% (3/130) and from stickers 3.1% (4/130) (Table 4.13). When asked to state what dog owners remember about 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.

Table 4.13: Dog owners source of news on avian influenza in Kaduna

Response	No. of respondents	No. positive (%)
Friends	130	80(61.5)
Radio	130	89(68.5)
Television	130	111(85.4)
Posters	130	43(33.1)
Newspaper	130	3(2.3)
Stickers	130	4(3.1)

4.4 BIOSECURITY PRACTICES AND INFRASTRUCTURE IN LIVE BIRD MARKETS, DOG AND PIG SLAUGHTER AREAS

4.4.1 Central Market Live Bird Market

The Central Market LBM in Kaduna North LGA located between N10.51962^0 and E007.42472^0 is one of the markets that were upgraded by Federal Government (AICP, 2009). It has maximum holding capacity of 3,000 birds with no wild or migratory birds sighted in the market vicinity. It is not near a residential area and no stagnant body of water nearby. New arrivals are kept separate from the old stock, sick birds are isolated and treated, and disposal of waste and dead birds is by refuse dump. Birds are transported in plastic crates and held in steel holding cages which are washed everyday with detergent. Different poultry species are kept separate with a single slaughtering area (Plate II). Water fowls like ducks are sold in the market. Stray dogs, cats, rodents and flies were found in the market (Plate III), unsold poultry are kept for the next day to sell. There is a market manager and a biosecurity team to oversee daily operations of the market. The drivers, delivery personnel, bird sellers and processors do not wear personal protective equipments (PPE). Once in a month government veterinarians inspect the market and at the end of each market day feed and water containers are washed.

The processors do not wear PPE when slaughtering but claimed to wash their hands with detergent after slaughter. The slaughter area is made up of ceramic tiles and cemented floor. Customers were not allowed into the slaughtering area and there is hot water drum for scalding. Slaughtering is performed on the floor; slaughter and processing waste are deposited inside garbage can and emptied several times daily into the waste container outside the market.



Plate II: Pigeons and guinea fowls in separate metal cage at Central live bird market, Kaduna North Local Government Area. Arrow (a) - Pigeons, b)-Metal cage (c) –Guinea fowls.



Plate III: A stray dog in Central live bird market.
a) Scalding drum, b) Stray dog eating poultry waste.
c) Poultry waste

Vehicles do not pass through a disinfectant dip nor their tyres cleaned with a pressure sprayer. They are restricted to delivery and storage areas. The market is divided into three sections; delivery and storage, selling and slaughter areas. There is adequate drainage, roof over entire market floor and a wall around the market. The entry and exit gates have locks for security. There is electrical lighting, reliable water supply, a generator and there is no wooden material in the market.

4.4.2 Railway Station, Barnawa and Kakuri Live Bird Markets

The Railway Station LBM in Kaduna South LGA is located within N10.49427^0 and E007.41858^0 is by the roadside with poultry housed in wooden cages and having maximum holding capacity of 500 birds. The market is near residential area with no wild or migratory birds sighted in the vicinity and no stagnant body of water nearby. The market manager said new arrivals of birds are kept separate from the old stock, sick birds are sold at a cheaper price, wastes and dead birds are disposed in refuse dump. Birds are held in wooden holding cages and cleaning of the market is not adequate (Plate IV). Different species of poultry are kept together with no separate slaughtering area (Plate V). Water fowls like ducks are sold, no stray dogs and cats but rodents/ flies are present in the market, and unsold poultry are kept for the next day to sell. The market manager oversees daily operations of the market. There are poultry sellers and slaughtering team but no biosecurity team. No veterinary inspection by government or private veterinarian. The drivers, delivery personnel, bird sellers and processors do not wear PPE. They separate new arrivals from old stock.

The slaughter area is cemented, has hot water drum for scalding, no ceramic tiles, no adequate drainage and customers are allowed into the slaughtering area (Plate VI).

Slaughtering is performed on the floor, the water in defeathering tank is replaced regularly, slaughter and processing waste are deposited inside garbage can which are emptied several times daily into the waste container outside the market.



Plate IV: Wooden cage for holding poultry at Station live bird market Kaduna South Local Government Area. a) Birds in wooden cage. (b) - Poultry faeces.



Plate V: Mixed bird species at Station live bird market Kaduna South Local Government Area a)- Turkey, b)- Broilers



Plate VI: Station live bird market slaughter area, Kaduna South Local Government Area.
(a)-Wooden table for dressing of poultry,
(b)-Poultry processor,
(c)-Broilers to be slaughtered
(d)- Lack of drainage, (e)-Foot of a customer
(f)- Scalding drum

Vehicles do not pass through disinfectant dip or tyres cleaned with a pressure sprayer, they are not allowed into areas other than delivery and storage. The market is divided into delivery and storage, selling floor and slaughter area. The market is a section of the main market with no perimeter wall around it and no entry and exit gates. No deep storage freezer and generator. There is a roof over the market floor; there is electricity lighting and reliable water supply.

Barnawa and Kakuri LBMs are in Kaduna South LGA located within $N10.48366^0$ and $E007.43193^0$ and $N10.46705^0$ and $E007.41224^0$ respectively have maximum holding capacities of 200 birds and 400 birds respectively. They have similar features with Station LBM except that cows are also sold in Barnawa LBM.

4.4.3 Trikania and Television Dog Slaughter Areas

The dog slaughter areas where samples were collected were Trikania slaughter area located at $N10.45353^0$ and $E007.42472^0$ along Abuja road in Chikun LGA and Television slaughter area located at $N10.452788^0$ and $E007.42826^0$ in Kaduna South LGA. The maximum number of dogs slaughtered in a day is eight for Trikania and three for Television. The slaughter areas are not in the general market, they are near residential areas with no wild or migratory birds sighted in the vicinity, and no stagnant body of water nearby. No stray dogs and other livestock species, and no rodents control program. Butchers do not wear PPE during slaughter but claim to wash their hands and tools after slaughter. Dogs brought for slaughter are from households within and outside the locality and by hunters. These dogs were decapitated, scalded using fire and cut into parts. The slaughtered dogs were prepared within the vicinity of the slaughter areas and sold. The slaughter areas do not have concrete floor and the butchering table is wooden. There was

no drainage, blood and intestinal contents were spilled on the floor (Plate VII**).** No veterinary inspection by government or private veterinarian is undertaken.

4.4.4 Pig Slaughter Area

Television pig slaughter area is in Kaduna south LGA located at N10.44949^0 and E007.39169^0, having maximum holding capacity of 50 pigs. It is near a residential area with no wild or migratory birds sighted in the slaughter vicinity and no stagnant body of water nearby. Pigs were decapitated, scalded with hot water, cut into pieces and sold to the public. The slaughter area was fenced with its floor made of concrete. The pigs were housed between two buildings prior to slaughter (Plate VIII). No stray dogs, cats and other livestock species were sighted, and no rodent control program. The butchers do not wear PPE but claim to wash their hands and tools with detergent after slaughter. The market is divided into; holding, slaughter and sales. Slaughter area is cemented, has drainage and hot water drum for scalding but intestinal contents were spilled on the floor (Plate IX). It is cleaned regularly with waste disposed by refuse dump; customers are not allowed into the slaughter area. Sales area consists of a shed and wooden table. No inspection by either government or private veterinarian is carried out.



Plate VII: Trikania dog slaughter area, Chikun Local Government Area, Kaduna.

a)- Two men under a shed waiting for prepared dog meat

b)- Butchering table,

c)- No concrete floor and drainage.



Plate VIII: Pig holding area at Television market, Kaduna South Local Government Area.
(a)-A man in pig holding area
(b)-Arrow showing pigs in holding area.



Plate IX: Pig slaughter area at Television market
a) - Pig to be slaughtered, b) - Concrete floor,
c) - Intestinal content.

CHAPTER 5

DISCUSSION

The study revealed that a dog in Kaduna has been exposed to AI H5 subtype. The virus might be either of low pathogenicity, causing a non-fatal infection unlike reports from Thailand where the infection was fatal in dogs (Songserm *et al*., 2006b). The study confirms that the practice of feeding dogs with dead poultry predisposes dogs to AI infection which increases the likelihood of human exposure from their dogs, as dogs have been reported to excrete AI virus through secretions (Songserm *et al*., 2006b). However, none of the dogs slaughtered in dog slaughter areas and the pigs had been exposed to AI H5 subtype as revealed by the study.

The presence of AI (H5 subtype) antibodies among chickens in LBMs might either be as a result of the chickens being exposed to the field AI virus which is of low pathogenicity or the antibodies might be as a result of vaccination of birds against AI which is being practiced contrary to government policy (AICP, 2008).

The study also revealed that few dog owners keeping poultry and pigs instead of seeking veterinary assistance for their sick dog would rather give them out as gift. This practice is risky as it might expose the human population to AI. This is because avian influenza is zoonotic and has been reported to infect dogs with active excretion of virus in body secretions (Songserm *et al*., 2006b). Most of the dog owners were not aware that their dogs were at risk of being infected by HPAI virus. This lack of knowledge on the susceptibility of dogs to HPAI virus 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 it has been reported that dogs were infected on consumption of HPAI infected poultry Dogs have been reported to excrete HPAI H5N1 virus which could be a source of human infection (Songserm *et al.,* 2006b). This is because human beings are also susceptible to the virus and dogs being the closest associate of pet owners may expose the owners to the virus, hence the need to educate dog owners. There is need to educate dog owners on the possibility of human death as a result of exposure to HPAI virus from dogs. This will improve on their risk perception and ensure that appropriate protective measures are taken to protect both the owners and their dogs.

Since most of the dog owners keep local poultry which are usually managed extensively, there is increased possibility of interspecies transmission of avian influenza virus from local poultry to dogs and pigs, more so, studies in Kaduna State reported influenza A antigen in local poultry (Assam *et al.,* 2011a). Sourcing of poultry from LBMs might spread avian influenza virus from LBM to households since antibodies to AI viruses have been reported in poultry from LBMs (Aye, 2010). This will increase the risk 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 (Durosinlorun *et al.*, 2010). This might also be due to the fact that dog most dog owners are educated and exposed to many sources of information on avian influenza.

The dog owners engage in risky practices, such as touching dead poultry with bare hands and not wearing PPE which increases risk of human exposure and spread of disease to their dogs. The unwillingness to report sick poultry to veterinarians by dog owners would

reduce the likelihood of reporting HPAI outbreak, thereby increasing spread among poultry and increase human and environmental exposure to HPAI virus. The selling of sick poultry would spread the AI virus through excretion and exposure to poultry at home, while eating of sick poultry exposes processors to the virus while contaminating the environment.

Pigs described as the likely mixing vessel for avian influenza viruses and they were kept together with poultry by dog owners 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 (Swayne and Jackwood, 2008). Since young pigs with undeveloped immune system are kept, there is likely increase chance of the virus establishing and multiplying in these pigs thereby propagating the virus (Swayne and Jackwood, 2008).

The study revealed that there is likelihood of pigs infected with avian influenza virus to spread the virus to humans through the sale of sick pigs, processing sick pigs and the practice of giving out dead pigs. There is a 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 revealed that the signs that the dog owners observed on sick dogs include nasal discharges, coughing and conjunctivitis which are signs also attributed to avian influenza (Songserm *et al.*, 2006b). The differential diagnosis of AI in dog includes; canine

distemper, canine influenza and rabies. Since these sick dogs are rarely referred to a veterinarian it reduces the probability of detecting the cause of these signs.

The study revealed 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 then the veterinarian would differentiate between ND and AI.

The study revealed that Television was a major means of HPAI information dissemination for dog owners. This result is contrary to previous studies that radio was a major medium of HPAI information for local poultry farmers (Durosinlorun *et al*., 2010; Assam *et al*., 2011b). This is because unlike local poultry farmers, dog owners are likely to be more affluent and could afford TV and generators if there is power failure. Although dog owners are expected to be educated, newspapers were a poor source of HPAI information probably due to reduce attention given to HPAI outbreaks by the print media (Assam *et al*., 2011b).

Contrary to a previous study, posters were a poor source of HPAI information among dog owners. Poor access to the HPAI posters may be responsible. Stickers as medium of HPAI information dissemination were equally poor as reported by Assam *et al*. (2011b). High mortality was what most dog owners could remember because most news report indicated high number of poultry dying from HPAI. Although, the zoonotic potential of HPAI was known by dog owners its potential for causing human fatality was not, and this aspect needs to be better articulated so as to improve the human risk perception of HPAI.

The biosecurity practices and infrastructure in Central Market LBM are adequate as the use of metal cages ensures adequate cleaning and the separation of poultry based on species reduces interspecies cross infection with AI virus and other poultry disease agents. The failure of processors in all LBMs to use the slabs for defeathering poultry is inappropriate. There is need for improvement in the infrastructure of Station, Kakuri and Barnawa LBMs.

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

The study determined the prevalence of H5 subtype antibodies in chickens, dogs and pigs by haemagglutionation inhibition test. Most dog owners were not aware that dogs can die of avian influenza and transmit avian influenza virus to humans. None of the dog owners were aware that pigs can be infected with avian influenza virus neither were they aware of the mode of transmission from pigs to humans. The biosecurity practices in Central Market LBM was adequate because it was upgraded by the Federal Government, but Barnawa, Kakuri and Station LBMs as well as dog and pig slaughter areas had poor biosecurity measures in place.

6.2 CONCLUSIONS

From this work it was concluded that:

Avian influenza (H5 subtype) antibodies were present in a dog from household and chickens from Central, Station and Barnawa LBMs in Kaduna. Dog owners in Kaduna are aware of HPAI but their knowledge of the disease is poor, resulting in risky practices such as selling and eating of sick and dead poultry, improper disposal of dead poultry and unwillingness to report sick poultry to the veterinary authorities. The inadequate knowledge of dog owners on HPAI is reflected in the low risk perception of HPAI amongst them. Television was the major source of HPAI information for dog owners. Biosecurity practices in dog and pig slaughter areas and Barnawa, Kakuri and Railway Station LBMs were poor.

6.3 RECOMMENDATIONS

From this work it was recommended that:

1. Veterinarians should pay attention to AI in dogs brought from households for treatment.
2. Dog owners rearing poultry should keep the two away from one another.
3. Dog owners should ensure proper cooking of chicken meat before giving to dogs.
4. HPAI information targeting dog owners should be aired through the television.
5. There is need for government to intervene and improve the infrastructure and biosecurity practices in the Barnawa, Kakuri and Railway Station LBMs.

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APPENDIX 1

Study of the knowledge of avian influenza by dog owners that may have poultry or pigs

SECTION A: Owner Personal Data

S/N_____ State _____Town _____ Location_____

1. Age: 15-24 [ ] 25-34 [ ] 35-44 [ ] >45[ ]

2. Gender: Male [ ] Female [ ]

3. Educational level: Primary [ ] Secondary [ ] Tertiary [ ]
Unconventional [ ]

4. Occupation: Civil Servant [ ] Housewife [ ] Farmer [ ] Student [ ]
Business [ ] Others (specify) _____

5. Household population: No. of adult [ ] No. of children [ ]

6. Gender: How many males [ ] How many females [ ]

SECTION B: Pets Data

1. Which type of pet do you own?
Dog [ ] Cat [ ] Others (Specify) _____
Vadilation-

2. Pet Name
Dog _____ Cat _____
Vadilation-

3. Pet Age
Dog _____ Cat _____
Validation-

4. Purpose of keeping pet dog/cat:
Pet [ ] Guard [ ] Hunting [ ] Consumption [ ] others (specify) _____
Validation-

5. Number of pets in household:
Dogs [ ] Cats [ ] others (specify): _____
Validation-

6. What do you feed your pets with?
Commercial pet food [ ] Carcass of poultry [ ] Poultry offal [ ] Bones [ ]
Carcass of livestock [ ] Livestock offal [ ] Scavenge for food [ ] Food remnant [ ]
Fish [ ] Chicken left over [ ] others (specify) _____
Validation-

7. Type of housing provided.
Confine [ ] Free movement [ ] Outside [ ] Live indoors with owners [ ]
Others specify_____
Validation-

8. Health status of pet: Dog [ ] Cat [ ]
Apparently healthy [ ] Sick [ ] others (specify) _____
Validation-

9. If pet is sick, which signs were observed?
Loss of appetite [ ] Conjunctivitis [ ] High fever [ ] Lethargy [ ] Coughing [ ]
Nasal discharge [ ] Ocular discharge [ ] others (specify) _____
Validation-

10. What do you do with a sick pet?
Seek vet assistance [ ] Treat it personally [ ] Give it out [ ]
Consume it [ ] Do nothing [ ] others (specify) _____
Validation-

12. Do you routinely vaccinate your pets against diseases?
Yes [ ] No [ ]
Validation-

13. List the disease that you routinely vaccinate your pets against:
_____
Validation-

14. Do you know that dogs and cats can be sick of bird flu?
Yes [ ] No [ ]
Validation-

15. Do you know that dogs and cats can transmit to humans bird flu?
Yes [ ] No [ ]
Validation-

16. Do you know that dogs and cats can die of bird flu?
Validation-

17. If the answers to items 14, 15 and 16 are yes how do dogs and cats become infected?

_____

_____

Validation-

SECTION C: Birds Data

1. Do you own birds? Yes [ ] No [ ]
Validation-

2. Species of birds:

Wild pet birds [ ] Local chickens [ ] Local Turkeys [ ] Local Ducks [ ]

Exotic chickens [ ] Exotic Turkeys [ ] Exotic Ducks [ ] others (specify) _____

3. If wild pet bird, which type? _____

4. Number/age of wild pet birds [ ] Local chickens [ ] Local Turkeys [ ] Local

Ducks [ ] Exotic chickens [ ] Exotic Turkeys [ ] Exotic Ducks [ ]

Validation-

5. Sources of birds _____
Validation-

6. Management system for birds:
Intensive [ ] Semi-intensive [ ] Extensive [ ] others (specify) _____
Validation-

7. Do they have any direct contact with dogs and cats? Yes [ ] No [ ]
Validation-

8. Do they have indirect contact with dogs and cats? Yes [ ] No [ ]
Validation-

9. Do you know of any poultry disease? Yes [ ] No [ ]
Validation-

10. If yes indicate which one: _____
Validation-

11. Do you know of bird flu? Yes [ ] No [ ]
Validation-

12. How do you come to know about bird flu?
TV [ ] Radio [ ] Newspapers [ ] Friends [ ] Posters [ ] Church [ ]
Mosque [ ] others (specify):_____
Validation-

13. What do you know about bird flu?
i. Clinical signs (specify): _____
ii. Transmission (specify): _____
iii. Public Health significances (specify): _____
Validation-

14. Have your birds suffered from bird flu? Yes [ ] No [ ]
Validation-

15. Have your neighbours birds suffered from bird flu? Yes [ ] No [ ]
Validation-

16. If you have to handle a dead bird, what do you wear?
Use bear hands [ ] Laboratory coat [ ] Hand gloves [ ] Mask [ ] Goggles [ ]
Validation-

17. After handling sick or dead bird, do you wash your hand with soap and water?
Yes [ ] No [ ]
Validation-

18. What do you do with sick birds?
Eat [ ] Bury [ ] Seek vet assistance [ ] Put in a dust bin [ ] Feed pets [ ] Sell [ ]
Give away [ ] Feed Fish [ ] Burn [ ] Do nothing [ ]
Validation-

SECTION D: Pigs Data

1. Do you own pigs? Yes [ ] No [ ]
Validation-

2. Number of pigs _____
Validation-

3. Age: 0 Month-6 Months [ ] 6 Months-1year [ ] > 1 Year
Validation-

4. Source of pig:
Established Farm [ ] Market [ ] Gift [ ] Buy from friends [ ]
Others (specify) _____
Validation-

5. Management system of pigs:
   Extensive [ ] Intensive [ ] Semi- intensive [ ]
   Validation-

6. If Extensive or Intensive what is the source of feed?
   Commercial Feed [ ] Kitchen Scraps [ ] Dead poultry [ ] poultry Manure [ ] others (specify) _____
   Validation-

7. Do they have direct contact with poultry? Yes [ ] No [ ]
   Validation-

8. Do they have indirect contact with poultry? Yes [ ] No [ ]
   Validation-

9. What do you do with a sick pig?
   Seek vet assistance [ ] Treat it personally [ ] Give it out [ ]
   Eat [ ] others (specify) _____
   Validation-

10. What do you do with dead pig?
    Bury [ ] Burn [ ] Salvage [ ] Throw away [ ] Sell [ ]
    Validation-

11. Do pigs suffer of bird flu? Yes [ ] No [ ]
    Validation-

12. How do pigs become infected with bird flu? _____
    Validation-

13. Can infected pigs infect humans? _____
    Validation-

14. What are the signs of bird flu in pigs? _____
    Validation-

APPENDIX 2

Biosecurity checklist for live bird market

Identification number:

Date:

State:

A. Site

1. Name of Market:

2. Local government area:

3. Town/ village:

4. Upgraded:

    - Upgraded by NAICP:
    - Upgraded by state government:
    - Upgraded by fowl sellers:

5. Traditional:

6. Maximum holding capacity of Market:

7. Is it in the General Market?

8. Is it near a residential area?

9. Are there wild or migratory Birds in the market vicinity?

    - If so which species?

10. Is there a stagnant body of water nearby?

B. Biosecurity Practices

1. Are the new arrivals kept separate from the old stock?

2. Are sick birds killed and disposed of?

- If no where they:
    a. Sold at a cheaper price
    b. Salvage slaughtered
    c. Isolated and treated
- If yes
    - Were the sellers compensated?
    - How much?

3. How is the disposal of wastes and dead birds carried out in the Market?

a. Refuse dump
b. Dug pit
c. Burial
d. Burning
e. Others (specify)

4. Are birds transported in plastic transport crates?

5. Are birds held in steel holding cages?

6. Are holding cages disinfected?

- If yes
    - How often?
    - Which disinfectant was used?
    - Was cleaning adequate?

7. Are different poultry species kept separate (other side of the market or separate shed)?

    - Please describe
    - Is there a separate slaughtering for the different poultry species?

8. Are water fowls sold in the market?

- If yes which species

9. Are ornamental and cage birds sold in the market?

- If yes which species?
- Can you sight free flying wild birds in the market?

10. Are stray dogs and cats in the market?

- If yes which area?

11. Are rodents/flies in the market?

- If yes which area?

12. Are there any non poultry animals in the market?

    - If yes what species?
    - What happens with unsold poultry?

a. Returned to owner

b. Kept for the next day to sell

c. Slaughtered

13. Is periodic tracheal and cloacal sampling performed?

- If yes
    - How often?
    - How many samples

14. Is there a market manager overseeing the daily operations of the LBM?
    - What are their responsibilities?
15. Is there a biosecurity team?
    - What are their responsibilities?
16. Is there a poultry seller team?
    - What are their responsibilities?
17. Is there a poultry slaughtering team?
    - What are their responsibilities?
18. Does Veterinary inspection take place?
        - If yes how often?
        - Is the vet government or private?
        - What sample do they take?
19. Do the following wear PPE and which PPE?
        - Drivers
            - Type of PPE?
        - Repairmen
            - Type of PPE?
        - Delivery personnel
            - Type of PPE?
        - Bird seller
            - Type of PPE?
        - Processors
            - Type of PPE?

20. Is the market closed every fortnight for a disinfection and cleanup?

21. At the end of each marketing day is the entire market (including feed and water containers washed down and disinfected)

- If yes which disinfectant is used?
- Was cleaning adequate?

22. Is an all in all out system used?

23. Do operators keep a record book of supplies transporters and wholesalers they use?

C. Slaughter

1. Does the butcher wear protective clothing?

- If yes which protective clothing?
- 2. What is the mode of slaughter?

3. Does the butcher disinfect his hands after slaughter?

- If yes which disinfectant was used?

4. What are the slaughtering slabs made of?

5. Are customers allowed into the slaughtering area?

6. Are there hot water drums for scalding?

If yes what temperature are they?

7. Does the slaughtering area have ceramic tiles?

8. Is any slaughtering performed on the floor?

9. Is the defeathering tank maintained and replaced regularly?

10. Are slaughter and processing waste deposited inside garbage cans which are emptied several times daily into the waste containers outside the market?

D. Transportation

1. Do vehicles pass through a bath or have their tyres cleaned with a pressure sprayer?

2. Were vehicles thoroughly disinfected after the delivery?

3. Did the gateman check vehicles traffic logbook and veterinary movement permit?

4. Are transporters allowed into areas other than the delivery and storage space?

E. Equipment and building design

1. Is there a one-way customer flow from entry to exit?

2. If yes is it strictly followed?

3. Is the market divided into three sections?

    - Delivery and storage
    - Selling floor
    - Slaughter area?

4. Are the market rules posted at the entry?

5. Is there cement floor for the entire market floor?

6. Is there adequate drainage (including sink pits and gutters)

7. Is there a roof over entire market floor to provide shade?

8. Is there a perimeter wall all around the market?

9. Is there an entry and exit gate?

    - If yes do they have locks for security?

10. Is there electrical lighting?

11. Is there a reliable water supply?

12. Is there a pump and hoses to produce high water pressure?

13. Is there deep storage freezers?

14. Is there a generator?

15. Is there any structures in contact with poultry that are made of wood?

APPENDIX 3

Biosecurity checklist for dog and pig slaughter areas

The purpose of this form is to determine conditions presently in the slaughter areas that may increase the risk of introducing and spreading disease.

Identification number**:**

Date:

State:

Local government area:

A. Site

1. Name of slaughter area:

2. District:

3. Maximum holding capacity of slaughter area:

4. Is it in the General Market?

5. Is it near a residential area?

6. Are there wild or migratory birds in the slaughter vicinity?

7. Is there a stagnant body of water nearby?

B. Isolation and Traffic Control

1. Slaughter area fenced to separate it from other areas in the large market.

2. Entry and exit doors separate

3. Unloading area for trucks

4. Sellers have distinct booths

5. Good distance between booths

6. Strict control of entrance/exit

C. Management

1. Sales and Slaughter record kept

2. Slaughter license displayed**.**

3. Healthy pigs bought from reliable source.

4. All pigs slaughtered and processed at market.

5. All pigs slaughtered in one day**.**

6. Stray cats, dogs and pigs in the market.

7. Other livestock species in the market.

8. Rodent control program**.**

D. Sanitation

1. Does the butcher wear protective clothing?

2. Are the protective clothing clean?

3. Does the butcher disinfect their hands after slaughter?

   If yes which disinfectant was used?

4. What is the mode of slaughter?

5. Are the tools always disinfected after slaughter?

   If yes which disinfectant was used?

6. What is the slaughter areas made of?

7. Are customers allowed into the slaughtering area?

8. Are there hot water drums for scalding?

   If yes what temperature are they?

9. Do they regularly clean and disinfect the slaughtering areas?

10. How is the disposal of waste carried out in the slaughter area?

    - Refuse dump
    - Burning
    - Burial
    - Dug pit
    - Others (specify)

11. Does veterinary inspection take place?

    If yes how often?

12. Is the vet government or private?