AVIAN INFLUENZA (H5N2) ANTIBODIES IN LOCAL CHICKENS AND AWARENESS LEVEL OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN KADUNA STATE

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NIGERIA

JANUARY, 2009

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BY

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(M.Sc./Vet. Med/57369/2005-2006)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE AHMADU BELLO UNIVERSITY ZARIA

JANUARY, 2009

DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Professors J. U. Umoh, P.A. Abdu and I. Ajogi. The work of other investigators referred to in this study was duly acknowledged. No part of this thesis has been previously submitted for a degree or diploma.

_____ _____

Durosinlorun Abdulkareem Date

CERTIFICATION

This thesis titled " AVIAN INFLUENZA (H5N2) ANTIBODIES IN LOCAL CHICKENS AND AWARENESS LEVEL OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN KADUNA STATE'' by **Durosinlorun, Abdulkareem** 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 my late parents, Abubakar Sidiq Durosinlorun Alabi and

Aisha Titilayo Durosinlorun, my wife and children.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my supervisors Professors J. U. Umoh, P.A. Abdu and I. Ajogi for their untiring efforts and guidance throughout the course of this research work. Their advice, suggestions, corrections and contributions have assisted me in bringing this thesis to its present state.

Special thanks goes to Drs Timm Harder and Christian Grund both of the Friedrich-Loeffler Institute of Diagnostic Virology, Germany for providing the antigen at no cost.

My thanks also go to Dr. Lawal Sa'idu, Dr. A. Wakawa, Mr. David Leo and Mal. Lawal Musa who all assisted me during my laboratory work.

My thanks also goes to all the Area Veterinary Officers and Area Livestock Technologists in all the Local Government Areas I visited

for their assistance in sensitizing poultry farmers in their areas. This helped in alleviating some of my problems during sampling.

I am also grateful to my colleagues at work who assisted me during sampling.

A special thanks also goes to my wife for her contributions during the entire work.

Finally my greatest thanks goes to the Almighty God for seeing me through this project and for surrounding me with those whose special qualities have assisted me in completing this work.

ABSTRACT

Six hundred and five sera samples were collected from apparently healthy local chickens in thirty three villages in twelve Local Government Areas of Kaduna State. Six of the Local Government Areas had outbreak of highly pathogenic avian influenza that was reported and confirmed between 2006 and 2007. The remaining six Local Government Areas were randomly selected from those Local Government Areas that did not report outbreak of highly pathogenic avian influenza during the same period. Hemagglutination inhibition test was conducted to detect antibodies to low pathogenic avian influenza virus (H5N2) while structured questionnaires were administered to one hundred and seventy two (172) farmers to determine their knowledge, level of awareness and readiness to disclose outbreak of highly pathogenic avian influenza. The overall prevalence rate of antibodies to low pathogenic avian influenza virus (H5N2) was 18.1%. A higher prevalence rate of (27.3%) was recorded in Local Government Areas that did not report outbreak of HPAI

compared to the prevalence rate of 7.5% in the Local Government Areas that reported outbreak of HPAI. The result of this study shows that low pathogenic influenza virus (H5N2) is circulating among local chicken population in Kaduna state.

There was association between the presence of ducks and detection of antibodies to low pathogenic H5N2 virus (χ^2 =24.257 df =1). Most of the farmers (84.9%) were aware of HPAI. Majority (87.2%) also said they would report outbreak of HPAI but the knowledge of the disease was low (19.8%) among farmers. Educational status (χ^2 = 16.635 df = 5) and occupation (χ^2 = 9.984 df = 4) of farmers had association with the knowledge of HPAI. Majority (78.5%) of farmers heard of HPAI through the radio. This may explain why the knowledge of the disease is low. It is recommended that surveillance to establish the presence or absence of LPAI (H5N2) and other LPAI should be enhanced and sustained. More efforts should also be made to improve the knowledge and recognition of HPAI by local poultry farmers. This will go a long way in aiding the Federal Government's control program of the disease.

LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion
AI	Avian influenza
AIV	Avian influenza virus
CDC	Center for Disease Control and Prevention
DEFRA	Department for Environment, Food and Rural Affairs
CIDRAP	Center for Infectious Disease Research and Policy
ECDPC	European Commission for Disease Prevention and Control
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agricultural Organization
FDLPCS	Federal Department of Livestock and Pest Control Services
FDL	Federal Department of Livestock
H	Hemagglutinin
HNAI	Highly Notifiable Avian Influenza

HPAI Highly Pathogenic Avian Influenza

IVPI Intravenous Pathogenicity Index

LBMS Livebird Market System

LPAI Low Pathogenic Avian Influenza

M Matrix

N Neuraminidase

NAI Notifiable Avian Influenza

NP Nucleoprotein

OIE Office International Des Epizootics

PBS Phosphate Buffered Saline

RBC Red Blood Cell

RT-LAMP Reverse Transcriptase Loop Mediated Isothermal Amplification

RT-PCR Reverse Transcriptase Polymerase Chain Reaction

RNA Ribonucleic Acid

SPSS Statistical Package for Social Sciences

WHO World Health Organizati**on**

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Avian influenza (AI) is caused by specified viruses that are members of the family *Orthomyxoviridae* placed in the genus influenza A (OIE, 2005). The influenza virus genome is an 8-segment single-stranded RNA with high potential for *in situ* recombination. Two segments code for the hemagglutinin (H) and neuraminidase (N) antigens used for viral entry (Causey and Edwards, 2008). The influenza A virus can be divided into 16 subtypes on the basis of the hemagglutinin (H) antigens. In addition to the H antigen, influenza viruses possess one of nine neuraminidase (N) antigens (Capua and Marangon, 2003).

Influenza A virus infects many animal species including birds, seals, whales, humans, horses and swine (Brown *et al*., 1997).

Avian influenza has a wide distribution and has been reported in North and South Africa, Australia, North and South America (USA, Canada, Mexico, Chile, Middle and East Asia (Pakistan, Hong Kong) and Europe (Italy, Germany, UK, Netherlands, Belgium) (Easterday *et al*., 1997; Alexander, 1999; DEFRA, 2003).

Avian influenza has often occurred as a sporadic disease in different parts of the world. It attracted more public interest in 1997 and 2003 in Hong Kong, China when people died because of contact with affected poultry (Abdu *et al*., 2005).

Many strains of influenza A virus have been isolated from several avian species in various parts of the world (Easterday and Tumova, 1972).

Since the late 1990's multiple subtypes of avian influenza virus have crossed the species barrier to infect humans. In 1997, highly pathogenic H5N1 influenza virus circulated

among chicken and other poultry species on farms and in other poultry markets in Hong Kong (Katz, 2004).

At present, avian influenza is recognized in two forms: highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI). The HPAI viruses have been restricted to subtypes H5 and H7, although not all viruses of these subtypes cause HPAI (Alexander, 2000).

The signs of avian influenza in domestic bird vary depending on the age, health status, species and sex of bird, the dose and strain of the virus and route of virus entry (Hanson, 2005). The signs may appear as respiratory, enteric or reproductive abnormalities (Hansen, 2005).

The LPAI viruses cause drop in egg production and mild respiratory disease with low mortality. However, if there is secondary bacterial or viral infection there may be severe respiratory disease with high mortality (Paul and Schrier, 2001; Swayne, 2003).

The HPAI virus such as H5N1 and H7N7 subtypes cause a disease that spreads rapidly among flocks and sudden onset of high morbidity (100%) and mortality (50-100%), cessation to moderate or severe drop in egg production and the laying of eggs with abnormal shell and pigmentation. Other signs include greenish diarrhea, anorexia, depression and ruffled feathers, coughing, sneezing, rales, dyspnea (due to edema of glottis), sinusitis (particularly in quails, ducks and turkeys) and mucoid ocular and nasal (blood tinged) discharges. Cyanosis and edema of the eyes, head, comb and wattles, discoloration of the shanks and feet, nervous disorder, excitation, convulsion, circling and ataxia in birds that survive the acute phase of the disease have been reported (Whiteman and Brickfold, 1989; Easterday *et al*., 1997; Aiello, 1998; Swayne *et al*.,1998; Alexander, 1999; DEFRA, 2003).

Most avian influenza A viruses are LPAI viruses that are associated with mild disease in poultry. In contrast, HPAI can cause severe illness and high mortality in poultry. More recently, some HPAI viruses (e.g., H5N1) have been found to cause no illness in some poultry, such as ducks (CDC, 2008). LPAI viruses have the potential to evolve into HPAI

viruses and this has been documented in some poultry outbreaks (CDC, 2008). Human illness due to infection with LPAI viruses has been documented, including very mild symptoms (e.g., conjunctivitis) to influenza-like illness. Examples of LPAI viruses that have infected humans include H7N7, H9N2 and H7N2 (CDC, 2008).

In Nigeria, Adeniji *et al.* (1993) demonstrated influenza virus antibodies in chicken sera but the genotype was not described. In the year 2002 Owoade *et al*. reported the presence of influenza serotype H1N1 in Ibadan, Nigeria with a prevalence of 35.3%, 57.1% and 93.4% for broilers, broiler breeders and point of lay pullets respectively.

The first suspected case of HPAI in Nigeria was reported in Sambawa farms, a semi-commercial farm situated in Kaduna state, North Central Nigeria on January 16, 2006. The National Veterinary Research Institute (NVRI) laboratory confirmed the disease to be avian influenza type A virus on February 6, 2006 and the OIE/FAO Reference Laboratory in Padova Italy finally confirmed that it was HPAI caused by H5N1 virus (profile: PQGERRKKRGLFG) on February 7, 2006 (NADIS, 2006).

1.2 **STATEMENT OF THE PROBLEM**

Nigeria has the largest poultry population in Africa (Nawathe and Abegunde, 1980). It has been estimated that the country has about 130 million to 150 million chickens (Abdu *et al.,* 1985; Okoye *et al.,* 1992; Lamorde, 1996). Of these only about 10% are of the exotic breed (Nawathe and Abegunde, 1980). Village chickens account for the remaining population (Abdu *et al.,* 1985; Jagne *et al.,* 1991). The village poultry plays an important role in providing protein and income to the farmer with little or no capital investment (Aini *et al.,* 1987; Ibrahim *et al.,* 1987; Okoye *et al.,* 1992). These chickens have been reported to act as potential reservoirs and carriers of infections to themselves and to the more susceptible exotic breeds in commercial poultry farms (Adu *et al.,* 1986; Nawathe, 1988; Okeke and Lamorde, 1988; Gueye, 2000; Alders and Spradbrow, 2001).

The outbreak of avian influenza in Nigeria was the first reported and confirmed outbreak of H5N1 Asian strain on the African continent. Since the first outbreak in Kaduna in

February 2006, the disease has spread to other parts of the country (NADIS INFO, 2006b). So far a total of 1525 cases have been officially reported to the Federal Government from 97 Local Government Areas in 32 states and the Federal Capital Territory out of which 300 cases were positive in 25 states and the Federal Capital Territory. States that have not had confirmed outbreaks in Nigeria are Akwa Ibom, Bayelsa, Cross River, Delta, Ebonyin, Gombe, Imo, Kebbi, Kogi, Ondo and Osun states. About 1,264,191 birds have been depopulated and compensation paid to farmers is about N631 million (Maina, 2008).

Production losses due to HPAI subsequently result in high cost of poultry and poultry-by products because of the resultant scarcity. Countries and regions affected by avian influenza (AI) stand the risk of losing the right to export poultry and poultry by-products to free countries (Abdu *et al.,* 2005). Thus HPAI has been described as an international problem that requires international efforts and cooperation to solve (Swayne, 2003).

The village poultry could also play an important role in the transmission of disease to the commercial poultry (Elssa, 1985; Spradbrow, 1987).

Before the outbreak of HPAI in 2006, a preliminary survey for antibodies against some viruses in village chickens of various ages carried out in Borno State, Nigeria, showed that 26. 5% of the 320 samples obtained tested positive for influenza type A (El –Yuguda and Baba, 2002).

Confirmed outbreaks of HPAI have also been reported in Zimbabwe, South Africa (Pfitzer *et al.,* 2000; OIE, 2004), Niger Republic, Egypt, Burkina Faso, Sudan, Cote d'ivoire, Djibouti, Cameroon (NADIS INFO, 2006a; WHO, 2006b), Sudan, Togo and Benin (OIE, 2008).

From 2003 to June 2008, a total of 243 persons were reported to have died out of 385 that were infected with confirmed HPAI caused by H5N1virus worldwide. One human death due to the disease has been reported in Nigeria in January, 2007 (WHO, 2008).

The concern of the scientific world today is that HPAI virus may mutate into a form that can be transmissible from human to human. With no existing immunity to such a mutant, this might prove devastating (Thanawat *et al.,* 2005).

1.3 **JUSTIFICATION OF RESEACH**

Following the confirmation of HPAI (H5N1) disease on February 7, 2006 by the OIE/FAO Reference Laboratory in Padova, Italy, it is now a known fact that clinical highly pathogenic avian influenza (H5N1) exists in Nigeria. However, there is lack of baseline data on LPAI in the country and Kaduna state in particular. Since the LPAI are known to cause subclinical infection in poultry with low mortality, there is need to carry out this study to ascertain if infection with low pathogenic H5N2 occurs in Kaduna state (Abdu *et al.*, 2005).

The pattern of the spread of HPAI in Nigeria has also being mainly between commercial poultry farms with few backyard poultry affected in only 4 states of the federation namely Kano, Katsina, Gombe, Nasarawa and the FCT (Saidu, 2008).

Also, some LPAI H5 or H7 viruses have the capacity to mutate into more virulent strains that cause extensive economic losses and high mortality (Hall, 2004). Recent AI outbreaks in several countries have increased attention and concern over LPAI H5 and H7 viruses and the possibility of mutation and H5 and H7 should be taken into consideration in eradication strategies (Landman and Schrier, 2004; Hall, 2004). Serology is used for LPAI infections and for post-vaccination monitoring (OIE, 2005).

Global surveillance of influenza A virus activity in birds, humans and pigs is currently a major priority of the World Health Organization (WHO). These efforts are critical for prevention of the first influenza A pandemic of the 21st Century (Webby and Webster, 2001).

1.4 **OBJECTIVES**

This study was conducted to determine:

1. The seroprevalence of low pathogenic avian influenza A virus (H5N2) in village chickens in Kaduna state.
2. Farmer's knowledge, awareness and readiness to disclose the outbreak of HPAI.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

Avian influenza viruses are highly infectious microorganisms that primarily affect birds. Nevertheless, they have also been isolated from a number of mammals including humans (Landman and Schrier, 2004).

Only viruses of the influenza A genus have been isolated from birds and termed avian influenza (AI) viruses, but viruses with all 16 hemagglutinin (H1-H16) and 9 neuraminidase (N1-N9) influenza A subtypes in the majority of possible combinations have been isolated from avian species (Alexander, 2007).

Influenza A viruses have infected many animals, including ducks, chickens, pigs, whales, horses and seals. However, certain subtypes of influenza A virus are specific to certain species, except for birds, which are hosts to all known subtypes of influenza A virus (CDC, 2008).

Influenza A virus, once introduced into poultry, can become endemic within the poultry population. It may be successfully eradicated by human intervention or the virus may fail to successfully spread on its own (Suarez *et al.,* 2003).

Influenza A viruses normally seen in one species can sometimes crossover and cause illness in another species. For example, until 1998, only HIN1 viruses circulated widely in the United States of America's pig population. However, in 1998, H3N8 viruses from horses crossed over and caused outbreaks of influenza in dogs (CDC, 2008).

Influenza A viruses that reside naturally in wild bird species comprise all known subtypes and provide viral genes from which influenza viruses that infect both domestic poultry and mammalian species, including humans may arise (Webby and Webster, 2001).

Avian influenza viruses infecting poultry can be divided into two groups. The very virulent viruses cause highly pathogenic avian influenza (HPAI), with flock mortality as high as 100%. These viruses have been restricted to subtypes H5 and H7, although not all H5 and H7 viruses cause HPAI (with the exception of two H10 subtypes that would have fulfilled the above definition for highly pathogenic notifiable avian influenza (HPNAI), although the reasons for this are not clear) (OIE, 2005). All other viruses cause a milder, primarily respiratory disease - low pathogenic avian influenza (LPAI), except when exacerbated by secondary infection (Alexander, 2007).

Due to the risk of a H5 or H7 virus of low virulence becoming virulent by mutation, all H5 and H7 viruses have been identified as notifiable avian influenza (NAI) viruses (OIE, 2005).

Highly pathogenic notifiable avian influenza (HPNAI) virus is thus defined by the World Organization of Animal Health (OIE) as any influenza virus that is lethal for six, seven or eight 4 to 8-week–old susceptible chickens within 10 days following intravenous inoculation with 0.2 ml of a 1/10 dilution of a bacteria-free, infective allantoic fluid or any virus that has an intravenous pathogenicity index (IVPI) greater than 1.2 (OIE, 2005). The molecular criterion for the identification of HPAI is the presence of multiple dibasic amino acids at the proteolytic cleavage site of the hemagglutinin (H) protein. All HPAI viruses isolated before 2002 fulfilled both the virulence and molecular criteria. Since 2002, however, there have been three outbreaks of HPAI where the viruses responsible for the outbreaks have either fulfilled the virulence criterion or the molecular criterion, but not both (Senne *et al.,* 2006).

2.2 **HISTORY**

Influenza pandemics are rare but recurring events (WHO, 2006a). Pandemics of influenza have been associated with significant morbidity and mortality in humans, including over 20 million deaths during the devastating outbreak of 1918 (Horimoto and Kawaoka, 2001).

In the last century, the sudden emergence of antigenically different strains (antigenic shift) resulted in four pandemics in 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1). More frequent and gradual antigenic changes (antigenic drift) have resulted in more limited outbreaks (Webster, 1998).

The three pandemic viruses that emerged in the 20th century- the 1918 ("Spanish influenza") H1N1 virus and 1957 ("Asian influenza") H2N2 virus, and the 1968 ("Hong Kong influenza") H3N2 virus-all spread rapidly around the world but only the 1918 virus was associated with mortality measured in the thousands per 100,000 human population (Belshe, 2005).

In both 1957 and 1968, a new influenza virus emerged because of reassortment events involving two influenza viruses. In 1957, dual infection of an individual animal-probably a human but possibly another species, such as pig- with an avian H2N2 influenza and a human H1N1 influenza resulted in the emergence of a new influenza virus containing the hemagglutinin, the neuraminidase and the gene for one of the polymerase proteins from the avian virus, along with the remaining five genetic segments from the human virus (Belshe, 2005).

The new reassortment virus circulated in humans until 1968 when it was replaced by another virus, the H3N2 Hong Kong virus, created by the replacement of the hemagglutinin (H2) and polymerase genes of the H2N2 virus with two new avian genes, H3 and a new polymerase protein (Belshe, 2005).

Research has shown that avian flu typically occurred every 10–50 years throughout recorded history (Obayelu, 2007). Avian flu has found a permanent ecological niche, becoming entrenched among domestic ducks. Avian flu has been recognized as a highly lethal generalized viral disease of poultry since 1901. In 1955, a specific type of influenza virus was identified as a causal agent of what was then called fowl plague (Obayelu, 2007).

Most of the pandemic influenza virus strains in the last century first appeared in southern China, (Shortridge and Stuart-Harris, 1982). In 1997 in Hong Kong, during the first

known outbreak of human infections with HP H5N1 AIV (Suarez *et al.,* 1998), exposure to live poultry at retail stalls or markets during the proceeding week was identified as a risk factor for human illness (Mounts *et al.,* 1999).

The Asian H5N1 virus was first detected in Guangdong province, China, in 1996, when it killed some geese. However, it received little attention until it spread through live-poultry markets in Hong Kong to humans in May 1997, killing 6 of the infected persons. The culling of all poultry in Hong Kong ended the first wave of H5N1 but the virus continued to circulate among apparently healthy ducks in the coastal provinces (Webster and Govorkova, 2006).

H5N1 (a HPAI virus) strain originated from birds and moved to mammals and began to affect humans after years of mutation. Since 1997, the H5N1 strain has gradually extended its reach and has become established within Asia. In 2003, there was the re-emergence of avian flu in Hong Kong. By 2005, H5N1 was detected in domestic and wild birds in Russia and Kazakhstan and in Mongolia. Isolated outbreaks of H5N1 in birds have also been reported in Romania, Russia and Turkey. The Hong Kong episode has since put the world health officials on alert because the H5N1 strain had fulfilled two of the three prerequisites for a pandemic. First, the strain was a new virus subtype to which the population had little or no immunity. Second, the virus had the ability to replicate in humans and cause serious illness. At present the H5N1 virus has become more robust than the 1997 strain capable of surviving longer under a broad range of environmental conditions (Obayelu, 2007).

In the last 10 years, the incidence of HPAI in domestic poultry has increased substantially. Meanwhile subtypes of LP, such as H9N2, have become endemic in Europe and Asia (Guan *et al.,* 2000).

The H5N1 virus has become increasingly pathogenic in poultry and has increased the range of species it can infect, now including domestic cats (in laboratory experiments) and captive tigers (after being fed infected chicken carcasses in a zoo in Thailand) (Songserm *et al.,* 2006a).

2.3 ETIOLOGY

The influenza viruses that constitute the family *Orthomyxoviridae* are classified into types A, B or C based on differences between their nucleoprotein and matrix protein antigen. AI viruses belong to type A. Influenza viruses are further categorized into subtypes according to the antigens of the hemagglutinin (H) and neuraminidase (N) (FAO, 2008).

Avian influenza viruses are enveloped virions which are 80 to 120 nm in diameter, are 200 to 300 nm long and may be filamentous. Avian influenza viruses consist of spike-shaped surface proteins a partially host – derived lipid-rich envelope, and matrix proteins surrounding a helical segmented nucleocapsid (6–8 segments). The family contains five genera, classified by variations in nucleoprotein (NP and M) antigens; influenza A, influenza B, influenza C, *thogotovirus* and *isavirus* (Anon, 2008b).

Until recently, 15 HA types had been recognized, but a new type (H16) was isolated from black-headed gulls caught in Sweden and Netherlands in 1999 and reported in 2005 (Fouchier *et al.,* 2005). There are therefore 144 possible serotypes. There is no cross protection between serotypes. Subtypes H1, H5, and H7, N1, N2, N3, N7 and N8 are the most pathogenic AI viruses. Others are mildly pathogenic or avirulent although they could later mutate without warning and become virulent without a change in their H and N subtype antigen (Alexander, 1999; Eckroade *et al.,* 2002; Suarez, 2003; Hansen, 2005).

Most AI viruses (H1 – H16 subtypes) are low pathogenic, but some of the H5 and H7 AI viruses are highly pathogenic for chickens, turkeys and related gallinaceous domestic poultry (Swayne, 2005).

The pathogenicity of AI viruses is correlated to the ability of trypsin to cleave the hemagglutinin molecule into two subunits. Highly pathogenic strains of H5 and H7 viruses have several amino acid residues at the cleavage site. Trypsin sensitivity can be used diagnostically to determine whether or not an isolated virus is potentially pathogenic (FAO, 2008).

2.4 **HOST RANGE**

Avian influenza A viruses can infect a variety of domestic and wild avian species (including chickens, turkeys, ducks, domestic geese, quail, pheasants, partridge, psittacines, gulls shorebirds, seabirds, emu, eagles and others) (Horimoto and Kawaoka, 2001). Aquatic birds, particularly ducks, shorebirds and gulls are considered the natural reservoirs for avian influenza viruses (Webster *et al.,* 1992, Fouchier *et al.,* 2004.). These waterfowls generally do not develop disease when infected with avian influenza viruses however H5N1 appears to be virulent for a variety of wild bird species (Horimoto and Kawaoka, 2001).

Chickens and turkeys are the most adversely affected birds by AI virus (Hanson, 2005). Chickens (in live bird markets), turkeys and ducks are potential sources of AI. Domestic and wild ducks (particularly Mallard ducks) have asymptomatic infection and may excrete AI virus for long periods They are carriers and harbor more than one AI virus subtype and rarely produce precipitin antibodies (Hanson, 2005). They are, thus, the natural reservoir of AI viruses. Ratites (ostriches and rheas), quails, muscovy ducks, geese, guinea fowls, chukars, partridges and pheasants, pets, sea and shorebirds are susceptible to AI to a varying degree (Hanson, 2005).

Certain mammals also are susceptible to influenza. Influenza A viruses have traditionally been known to cause disease in horses, pigs, whales and seals. However, the range of several influenza A subtypes is expanding to different mammalian species. H5N1 virus has now been shown to infect cats, leopards, tigers, civet cats and dogs (Keawcharoen *et al.,* 2004; ECDPC, 2005; Thanawongnuwech *et al.,* 2005; Songserm *et al.,* 2006a; Songserm *et al.,* 2006b; Webster *et al.,* 2006; Yingst *et al.,* 2006).

A particular isolate may produce severe disease in turkeys but not in chickens or any other avian species. Therefore, it would be impossible to generalize on the host range for avian influenza, for it will likely vary with the isolate. This assumption is supported by reports of farm outbreaks where only a single avian species of several species present on the farm became infected (FAO, 2008).

Avian influenza is seasonal in high risk areas due to migratory activity of waterfowl, the only species of birds in which the AI virus was found to be present year round (Hansen, 2005). Avian influenza prevalence is highest in birds on free range due to continuous field exposure to the virus (Abdu *et al.,* 2005).

2.5. **GEOGRAPHICAL DISTRIBUTION**

AI viruses are probably ubiquitous in wild water birds. Pathogenic strains could emerge and cause disease in domestic poultry in any country at any time without warning. In fact, outbreaks have occurred at irregular intervals on all continents. The most serious outbreaks in recent times have been reported in Hong Kong 1997-1998 and 2003, Chile 2002, The Netherlands 2003 and South East Asia 2004-2006 (FAO, 2008).

2.6 **EPIDEMIOLOGY**

The immediate source of infection for domestic poultry can seldom be ascertained. However, most outbreaks probably start with direct or indirect contact of domestic poultry with water birds (FAO, 2008). Many of the strains that circulate in wild birds are either non pathogenic or mildly pathogenic for poultry (FAO, 2008). However, a virulent strain may emerge either by genetic mutation or by reassortment of less virulent strains. Scientific evidence indicates that the former mechanism occurred in 1983-1987 in the Eastern part of the United States of America (FAO, 2008).

Only domestic poultry are known to have played a role in the transmission cycle of the AI virus from animals to humans (Wikipedia, 2006). Wild birds are the primary natural reservoir for influenza A and are often the vector that introduces new outbreaks into domestic flocks. The virus can be highly contagious in domestic poultry which are not as resistant as wild birds. Once present in domestic flocks, human activity becomes a risk for the transmission (Obayelu, 2007).

Airborne transmission may occur if birds are in close proximity and with appropriate air movement (FAO, 2008). Birds are readily infected via instillation of the virus into the conjunctival sac, nares or the trachea (FAO, 2008). Preliminary field and laboratory evidence indicates that AI virus can be recovered from yolk and albumen of eggs laid by hens at the height of the disease (FAO, 2008). The possibility of vertical transmission is unresolved; however, it is unlikely infected embryos could survive and hatch. Attempts to hatch eggs in disease isolation cabinets from a broiler breeder flock at the height of disease failed to result in any AI-infected chickens (FAO, 2008). This does not mean that broken contaminated eggs could not be the source of virus to infect chicks after they hatch in the same incubator (FAO, 2008).

Humans become infected with avian influenza virus through direct contact with bird feces and respiratory secretions, droplets and by mechanical transfer through contact with contaminated formites but not through eating of cooked chicken (WHO, 2004a).

When a bird is infected with avian flu, it sheds the flu virus in its feces, saliva and mucus and other birds become infected by eating or inhaling the virus. Wild migratory water fowl can acquire HPAI infection with signs of clinical disease and spread this to domestic flocks. The virus can survive at cool temperatures, in contaminated manure for at least three months. In water, the virus can survive for up to four days at 22^{o}C and more than 30 days at 0^{o}C (Ausvetplan, 2002). Contaminated farm equipment, feed, cages or shoes can carry the virus from farm to farm (WHO, 2004b). The virus can also be carried on the bodies and feet of animals, such as rodents (WHO, 2004b). In a food handling/preparation setting, there is some concern that AI could be transmitted from uncooked birds or bird products. AI can contaminate eggs and poultry meat (frozen and/or commercially packaged). For example, the HPAI virus can survive nitrogen carcasses and blood for as long as 3 weeks (WHO, 2004b). Broken contaminated eggs in incubators infecting healthy chicks (OIE, 2002) and garbage flies (suspected of transmitting the virus during the 1983–84 epidemic in Pennsylvania) have also been implicated in the spread of AI virus within and between flocks (Beard, 1998).

In Africa, it appears that the AI virus has spread predominantly through trade of poultry for human consumption (FAO, 2006). Nigeria was the first African country to report outbreaks of H5N1 in poultry, in February 2006. One study showed that three different sub lineages were independently introduced into Nigeria through routes that coincide with flight paths of migratory birds, although the another state that independent trade imports could not be ruled out as the source of spread (Ducatez *et al.,* 2006).

Studies show that H5N1 can move from poultry to migratory birds and back again (i.e. "relay transmission"), which may account for some of the continuing geographic spread (WHO, 2006a).

Wild waterfowl and shorebirds are known to be natural reservoir for influenza A viruses. Surveillance studies in waterfowl and shorebirds in North America show that influenza A viruses are repeatedly recovered from these birds. However, the virus recovery is influenced by geography, season, age and species of birds. In addition to the natural reservoir, the live-bird marketing system (LBMS) in certain regions of the United States has been recognized as a man–made reservoir of influenza viruses and has been linked to several outbreaks LPAI in poultry (Senne *et al.,* 2006).

Swine appear to be important in the epidemiology of infection of turkeys with swine influenza virus when they are in close proximity (FAO, 2008).

2.7 **INCUBATION PERIOD AND CLINICAL SIGNS**

The incubation period of AI is 3-5 days in general but may be longer. The longest incubation period is 21 days as defined by the OIE Terrestrial Animal Health Code (FAO, 2008).

Clinical signs are variable and are influenced by factors such as the virulence of the infecting virus, species affected, age, sex, concurrent diseases and environment (FAO,

2008).The signs may appear as respiratory, enteric or reproductive abnormalities (Easterday *et al.,* 1997; Hansen, 2005).

The AI virus in most cases is carried by some birds without displaying any symptoms of the disease and can spread over great distances while remaining healthy. However, birds affected with all forms of AI disease may show one or more of the following: sudden death of the affected bird without clinical sign on the first day especially those that are infected with the "HPAI" characterized by very high and rapid mortality, with rates approaching 100 per cent, lack of energy and appetite, decreased egg production, soft-shelled or misshapen eggs, ruffled feathers, swollen heads, cyanosis of the combs or wattles and possibly neurologic signs and diarrhea. Purple discoloration of the wattles, combs and legs, nasal discharge and coughing, sneezing, lack of coordination are other clinical signs. Any poultry establishment experiencing an unusually high mortality rate (e.g. > 1% daily for 2 days in commercial settings and > 5% for village poultry farms) and where the mortality is associated with one or more of the above signs is suspected to have been infected with avian flu (Obayelu, 2007).

In broilers, the signs of disease are frequently less obvious with severe depression, inappetance, and a marked increase in mortality being the first abnormalities observed. Edema of the face and neck, torticollis and ataxia may also be seen. The disease in turkeys is similar to that seen in layers, but it lasts 2-3 days longer and is occasionally accompanied by swollen sinuses. In domestic ducks and geese, the signs of depression, inappetance and diarrhea are similar to those in layers, though frequently with swollen sinuses (FAO, 2008).

The symptoms of avian influenza in humans on the other hand ranged from fever, cough, sore throat and muscle aches to conjunctivitis, pneumonia, acute respiratory distress, viral pneumonia and other severe and life-threatening complications (CDC, 2006).

2.8 **GROSS AND MICROSCOPIC LESIONS**

Birds that die of peracute disease may show minimal lesions, consisting of dehydration and congestion of viscera and muscles (Swayne, 2005).

In LPAI virus infection, lesions in the respiratory tract include congestion and inflammation of the trachea and lungs. In layers and breeders, there may be decreased egg production or fertility, ova rupture (evident as yolk in the abdominal cavity) or involution or mucosal edema and inflammatory exudates in the lumen of the oviduct. some layer and breeder chickens may have acute renal failure and visceral urate deposition [visceral gout] (Swayne, 2005).

For HPAI viruses, lesions include: in peracute cases, gross lesions may be lacking before death. However, in acute cases, lesions may include cyanosis and edema of the head, comb and wattles; edema and discoloration of the shanks and feet due to subcutaneous ecchymotic hemorrhages, petechial hemorrhages on visceral organs and in muscles and blood-tinged oral and nasal discharges. In severely affected birds, greenish diarrhea is common (Swayne, 2005).

There is extensive subcutaneous edema, particularly around the head and hocks. Yellow or grey necrotic foci may be present in the spleen, liver, kidneys and lungs. The air sacs may contain exudates. The spleen may be enlarged and hemorrhagic (FAO, 2008).

The location and severity of microscopic lesions are highly variable and may consist of edema, hemorrhage and necrosis in parenchyma cells of multiple visceral organs, skin and the central nervous system (Swayne, 2005).

2.9 **DIAGNOSIS**

2.9.1 **RAPID TESTS**

Rapid tests have been developed in order to detect in the field, without delay, any type A influenza virus in order to quickly detect the presence of antigen and antibodies (Edan *et al.,* 2003).

Rapid tests include:

1. Directigen ^{TM} Flu A+B test: to detect antigens

This is a rapid chromatography immunoassay for the direct and qualitative detection of influenza A and B viral antigen from nasopharyngeal washes/aspirates. The test is highly sensitive and can be used for distinguishing influenza A viral antigen from influenza B viral antigen in one test. However, the kit is very expensive (Edan *et al.,* 2003).

2. Flu Detect ^{TM} avian influenza Antigen test

This test is used to detect influenza A viral antigens (all 16 subtypes of influenza A virus) in 15 minutes. It can be used in the field and also in the laboratory. The samples are tracheal, oropharyngeal and /or cloacal swabs. The accuracy of this test is quite high (Edan *et al.,* 2003)

3. ELISA Kit introduced by IDEXX

The ELISA test kit can be used for detecting antibodies against the type A avian influenza viruses in the suspected serum (Edan *et al.,* 2003).

Viral antigens are attached to the bottom of holes so that after the incubation period, they could conjugate with the antibodies against the avian influenza virus found in the suspected serum. However the sensitivity of this rapid test is quite low resulting in false-negative results. Thus the results of this quick tests should always be combined with epidemiological and clinical data and laboratory confirmation (Edan *et al.*, 2003).

2.9.2 **GENE SEQUENCE DETECTION AND ANALYSES**

Specific primers for H and N types can be used for RT-PCR and RRT-PCR, but this does not provide fine detail. Further genetic analyses require access to a DNA sequencer. This procedure enables characterization of viruses as highly pathogenic or potentially highly pathogenic from the genetic sequence of the cleavage site of the HA gene. It also provides powerful information that enables epidemiological relationship of the virus to be established (FAO, 2004).

AI viruses are identified by demonstrating the presence of influenza A matrix or nucleoprotein antigens using agar gel immunodiffusion (AGID) tests, enzyme-linked immunosorbent assays (ELISA) or viral RNA using influenza A specific reverse transcription polymerase chain reaction (RT-PCR) tests (Swayne, 2005).

Reverse transcriptase loop mediated isothermal amplification (RT-LAMP) is a rapid and sensitive laboratory diagnostic system for the H5N1 HPAI (Masaki *et al.,* 2006).

Molecular tests are rapid, highly sensitive and specific and differentiates type A influenza viruses. They however require expensive equipments and special facilities. False negatives are also possible due to genetic variation (Senne, 2008).

2.9.3 **SEROLOGY**

1. Hemagglutination Inhibition Test

Hemagglutination test is based on the principle that certain viruses clump red blood cells from some species (active hemagglutination). This clumping action can be inhibited by antibody specifically directed against the virus (i.e. hemagglutination inhibition) and can be used to measure the presence and concentration of such antibody (Jawetz *et.al.,* 1989).

The Hemagglutination Inhibition Test is the subtype specific test recommended. It is sensitive and specific when an epidemiologically appropriate antigen is used. It can be

used for monitoring the response to vaccination and where birds survive infection (e.g. with LPAI or HPAI in ducks), to monitor circulation of virus (FAO, 2004).

2. Agar Gel Immunodiffusion Test

Agar gel immunodiffusion test is one simple method of demonstrating precipitation of antigen by antibody. Round cells, about 5mm in diameter and about 1 cm apart, are cut in a layer of agar. One well is then filled with soluble antigen and the other with antiserum. The reactants will diffuse out radially. Where the reactants meet in optimal proportions an opaque white line of precipitate will appear (Tizard, 2000).

The Agar Gel Immunodiffusion Test is a group-specific test for antibodies. It is relatively useful on a flock basis for serology for LPAI but of limited use for HPAI strains when mortality is high (FAO, 2004).

AGID is easy, inexpensive and requires few reagents/equipments. It is however semi qualitative, of moderate sensitivity and the interpretation can be subjective. It requires about 24 hours and further testing of positive samples (Senne, 2008).

3. Competitive ELISA using Group Antigen

ELISA depends on the conjugation of an enzyme to either an antigen or an antibody. The enzyme is detected by assaying for enzyme activity with its substrate (Jawetz *et.al.,* 1989).

The C ELISA is a test system that can be used for all species. It is very sensitive and specific for chicken but considered to be of limited use for sero surveillance for H5N1. It can be used to detect antibodies in ducks, but its use in this species has only limited validation (FAO, 2004).

Its advantages include the availability of commercial kits and it can be semi automated. It however requires expensive equipment and there is possibility of false positive reactions thus positives require confirmation (Senne, 2008).

4. DIVA (Differentiating infected from vaccinated animals) System

a. Antibody detection using immunofluorescence

This test uses cells infected with a baculovirus vector expressing neuraminidase antigen of interest. Sera are tested by reaction with antigen fixed cells. The result is read using a fluorescent microscope and thus requires subjective evaluation (FAO, 2004).

b. Antibody detection using Inhibition of neuraminidase

This is essentially a biochemical assay inhibited by antibody. The test uses bête-propiolactone-inactivated antigrn.The result is a visible colour change that can be observed by the eye. This method has been miniaturized to conduct tests in a 96-well micro plate format (FAO, 2004).

2.9.4 **VIRUS ISOLATION AND CHARACTERIZATION**

AI viruses can be readily isolated from tracheal and cloacal swabs. They grow well in the allantoic sac of embryonating chicken eggs and agglutinate red blood cells. The hemagglutination is not inhibited by Newcastle disease or other paramyxoviral antiserum (Swayne, 2005).

Isolation is the basic minimum requirement for virus detection. Tracheal and cloacal swabs as well as lung and spleen specimens are samples of choice for H5N1. Specimen on transport medium are inoculated into specific pathogen free (SPF) embryonated eggs, but commercial eggs from known unvaccinated source free of AI can be used as well. At least two passages four days apart should be attempted before a test is declared negative (FAO, 2004).

Virus isolation is a gold standard diagnostic test for avian influenza and is sensitive to all subtypes. It is however expensive and labour intensive. Special facilities are needed and false negatives may be seen due to sample mishandling (Senne, 2008).

Characterization of isolates is done by:

1. Hemagglutinin typing

Hemagglutinin (HA) typing is carried out on allantoic fluid that shows hemagglutinating activity. It requires a panel of reference sera to likely virus subtype (H5, H9 and NDV). It is a relatively simple procedure that does not require any sophisticated equipment (FAO, 2004).

2. Neuraminidase typing

Neuraminidase (N) typing is carried out on allantoic fluid when hemagglutinating activity is inhibited by reference H type serum. It requires a panel of reference antisera for likely N types. It incorporates a biochemical assay that requires specific skill and hence training (FAO, 2004).

2.10 **DIFFERENTIAL DIAGNOSIS**

LPAI must be differentiated from other respiratory diseases or causes of decrease in egg production including: Infectious bronchitis, infectious laryngotrachitis, lentogenic Newcastle disease, mycoplasmosis, infectious coryza, ornithobacteriosis, turkey coryza and the respiratory form of fowl cholera and aspergillosis (Swayne, 2005).

HPAI must be differentiated from other causes of high mortality such as velogenic Newcastle disease, peracute septicemic fowl cholera, heat exhaustion and severe water deprivation (Swayne, 2005).

Avian influenza should be suspected in any disease outbreak in poultry that persists despite the application of preventive and therapeutic measures for other diseases (OIE, 2005).

Because of the broad spectrum of signs and lesions reported with infections of avian influenza viruses in several species, a definitive diagnosis must be made by virologic and serologic methods (Easterday *et.al.,* 1997).

2.11 **PREVENTION AND CONTROL**

Since avian influenza virus is highly contagious and easily spread, the commonest method of control is the culling (depopulation or killing) of the infected flocks. Another method is the quarantine of affected areas until the disease is no longer present. While vaccination is possible and has been tested on a small scale, it is not widely considered a viable control method (Obayelu, 2007).

After the contaminated flock is depopulated, buildings and equipment are vigorously disinfected before new birds are allowed, a process that takes at least several weeks. The virus can also be killed by common disinfectants or heat. For instance, heat of 76^{o}C, has been recommended for chicken, turkey dark meat, 82^{o}C, ground chicken, turkey:74^{o}C and eggs: 71^{o}C (WHO, 2004a). While the best method to prevent or limit the impact of HPAI outbreaks on public health is to promptly contain and control outbreaks in poultry, conduct efficient surveillance and report potentially infected poultry flocks to the right authority. There is the need to implement biosecurity measures that reduce human exposure to potentially infective birds, bird debris such as litter, feathers, dust and husbandry equipment (Obayelu, 2007).

Experimental work has shown, for both NAI and LPAI that vaccines protects against clinical signs and mortality, reduces virus shedding and increases resistance to infection, protects from diverse field viruses within the same Hemagglutinin subtype, protects from low and high challenge exposure and reduces contact transmission of challenge virus (Capua *et.al.,*2004; Swayne, 2003; Swayne and Suarez 2000). However, the virus is still able to infect and replicate in clinically healthy vaccinated birds (OIE, 2005). In some countries, vaccines designed to contain or prevent NAI are specifically banned or

discouraged by government agencies because it has been considered that they may interfere with stamping-out policies (OIE, 2005).

Specific protection is achieved through autogenous virus vaccines or from vaccines prepared from AI virus of the same hemagglutinin sub type. Antibodies to the viral neuraminidase may provide some protection (Swayne, 2005).

Three types of vaccines have been used to control AI. They include:

Inactivated homologous vaccines

Homologous vaccines were originally prepared as "autogenous" vaccines (i.e. vaccines that contain the same AI virus strain as the one causing the problems in the field). They have been used extensively in Mexico and Pakistan during AI epidemics (Swayne and Suarez, 2000).

The efficacy of these vaccines in preventing clinical disease and in reducing the amount of virus shed in the environment has been proven through field evidence and experimental trials (Swayne and Suarez, 2000). The disadvantage of this system is the inability of differentiating vaccinated from field–exposed birds unless unvaccinated sentinels are kept in the shed. However, the management (identification, bleeding and swabbing) of sentinel birds during vaccination campaign is time consuming and rather complicated since they are difficult to identify and they may be substituted with seronagative birds in the attempt to escape restrictions imposed by public health officials (Capua and Marangon, 2003).

Inactivated heterologous vaccines

Heterologous vaccines are manufactured in a similar way as the inactivated homologous vaccines. They differ in the fact that the virus strain used in the vaccine is the same type as the field virus but has heterologous neuraminidase (Capua *et al.,* 2000).

Following field exposure, clinical protection and reduction of viral shedding are ensured by the immune reaction induced by the homologous H group, while antibodies against the neuraminidase induced by the field virus can be used as a marker of field infection (Capua *et al.,* 2000).

The degree of protection of heterologous vaccines is not strictly correlated to the degree of homology between the hemagglutinin genes of the vaccine and challenge strains (Swayne and Suarez, 2000). This is definitely a great advantage because it enables the establishment of vaccine banks since the master seed does not contain the virus that is present in the field and may contain an isolate available before the epidemic (Capua *et al.,* 2000).

Recombinant Vaccines

Recombinant vaccines for AI viruses have been produced by inserting the gene coding for the influenza virus hemagglutinin into a live vector and using the recombinant virus to immunize poultry against AI (OIE, 2005).

Several recombinant fowlpox viruses expressing the H5 antigen have been developed (Beard *et al.,*1991,1992; Webster *et al.,* 1996; Swayne *et al.,* 1997, 2000b), and one has been licensed and is being used in Mexico (Swayne and Suarez, 2000).

Experimental data have also been obtained for fowlpox recombinants expressing the H7 antigen (Boyle *et al.,* 2000). Other vectors have been used to successfully deliver the H5 and H7 antigens, using the infectious laryngotrachitis virus (LÜschow *et. al.,*2001; WHO 2006a). The only field experience with a recombinant virus to control AI has been obtained in Mexico where it has been used in the vaccination campaign against a LPAI H5N2 virus (Villareal-Chavez and Rivera Cruz, 2002).

Advantages of recombinant live vector vaccines include the fact that they are live vaccines able to induce both humoral and cellular immunity, they can be administered to

young birds and induce early protection and they enable differentiation between infected and vaccinated birds (OIE, 2005).

However, these vaccines have limitations in that they will replicate poorly and induce only partial protective immunity in birds that have had field exposure to or vaccination with the vector virus, i.e. fowlpox virus or infectious laryngotrachitis viruses for currently available recombinant vaccines ((LÜschow *et al.,*2001; Swayne *et al.,* 2000).In addition, because the vectors are live viruses that may have a restricted host range (for example infectious laryngotrachitis virus does not replicate in turkeys) the use of these vaccines must be restricted to species in which efficacy has been demonstrated (OIE,2005).

It is important that vaccination alone is not considered the solution to the control of NAI or LPAI subtypes if eradication is the desired result. Without the application of monitoring systems, strict biosecurity and depopulation in the face of infection, there is the possibility that these viruses could become endemic in vaccinated poultry populations (OIE, 2005). Long-term circulation of the virus in a vaccinated population may result in both antigenic and genetic changes in the virus and this has been reported to have occurred in Mexico (Lee *et al.,* 2004).

2.12 **ZOONOTIC RISK**

Avian influenza viruses exhibit host adaptation and rarely infect humans, usually as isolated individual cases without human to human transmission. In the 1997 Hong Kong outbreak, the risk factor for human infection was direct contact with infected poultry, but not the handling, cooking or consumption of poultry meat (CDC, 2008).

In 2004, HPAI of strain H5N1 infected poultry and wild birds in nine Asian countries. In Thailand and Vietnam, 37 human cases were confirmed, with a case fatality rate of 68% (Swayne, 2005).

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

There is little pre-existing natural immunity to H5N1 virus in human population. If H5N1 viruses gain the ability for efficient and sustained transmission among humans, an influenza pandemic could result, with potentially high rates of illness and death worldwide (CDC, 2008).

Only AI A viruses, which can be maintained in a nonhuman reservoir and consisting of many serologically distinct subtypes, have the potential to cause human influenza pandemic (Katz, 2004).

2.13 **GOVERNMENT'S EFFORT AT CONTROLING HPAI IN NIGERIA**

Prior to January, 2006, there was no report of HPAI in Nigeria and there was no evidence that suggested the presence of the disease in the country (NADIS, 2006).

A Federal Avian Influenza Management Center was set up in Abuja following the outbreak in 2006. The center was based at the presidential villa under the supervision of the Honourable Ministers of Health, Agriculture, Information and National Orientation. The center provided strategic decision making with efficient communications and information dissemination to ensure a timely operational response and effective management across all sectors (Brandenburg, 2008).

A technical committee of experts on the prevention and control of HPAI in Nigeria was inaugurated on the 12th of December, 2005. The committee deliberated extensively on the nature, the global spread of the disease and its potential risks to Nigeria .The strategies

for the prevention and control of the disease were discussed as well as its surveillance network (FDLPCS, 2005).

Based on the result of risk analyses of HPAI in Nigeria, it was recommended that our overall policy should be modified stamping out involving slaughter of clinically affected poultry with full compensation, safe disposal of dead carcasses, adequate disinfection and decontamination and appropriate disease surveillance to determine the origin and extent of the disease (FDLPCS, 2005).

An action plan dealing with HPAI emergency which defines the command chain from the rural setting, the state veterinary services, to the National veterinary services was proposed. In addition, public awareness campaigns would be emphasized in the program (FDLPCS, 2005).

Following the first outbreak of HPAI in 2006, the government requested assistance from the World Bank. A S50 million project, entitled the Nigerian Avian Influenza Control and Pandemic Preparedness and Human Response Project (AICP) was activated as an emergency operation under the GPAI initiative in June 2006 (Brandenburg, 2008).

The project addresses the animal and human side of avian influenza and has 4 components: Animal health, Human Health, communication and public awareness and project management (Brandenburg, 2008).

2.14 **KNOWLEDGE AND AWARENESS OF HPAI**

Avian influenza is an emerging threat to public health, but little is known about how the public perceives this threat (Ganglia *et al.,* 2008).

A key factor in reducing the risk of an influenza pandemic is adequate preparedness, including providing prospective, accurate information to the public (Marinos *et al., 2007*).

In order to develop a communication campaign that advocates practices that has been shown to protect a person's poultry and family from avian influenza, it is important to understand the knowledge, attitudes and beliefs the target population holds related to avian influenza, as well as understand how and if AI fits into their life in general (Anon, 2008a).

Most people are not knowledgeable about AI and the danger it is likely to pose to the community. Rather, such an outbreak presents an opportunity for them to eat cheap poultry (Anon, 2008a).

At the community level, most people do not know the symptoms or prevention of bird flu. Veterinary and health officials argue that the extent of knowledge in the community is dependent on its socio-economic and educational levels (Anon, 2008a).

Farmers are believed to have some knowledge of the disease and even have a name for it and are also aware of symptoms in poultry. People are more likely to be aware of AI if: They receive the media communication about AI during the outbreak of 2006 or if poultry is a person's main source of income as they were the most affected when the outbreak was announced in 2006. For this reason, their level of concern is high, as they want to protect their source of income (Anon, 2008a).

Government officials have organized several campaigns aimed at teaching the symptoms and preventive measures of avian influenza. Television, radio and print media have been the communication medium. In addition, there have been seminars targeting farms and health officials (Anon, 2008a).

Government officials also advise farmers to fumigate the poultry sheds and to follow basic hygiene rules, including wearing personal protective gear (PPE) and respirators when being exposed to infected birds. Movement of birds within the country is also restricted. Overall, the government officials believe that awareness levels have increased since 2006 (Anon, 2008a).

Some stake holders believe there are still gaps in communication with the masses in a style that resonates with their way of life, language and literacy level (Anon, 2008a).

Others believe there is still a need for better communication strategies/campaigns and better use of channels for communication such as billboards, handbills/fliers. Pictorial and visual messages seem to be preferred especially for illiterate masses (Anon, 2008a).

The use of mass media as opposed to alternative media like popular theater or village rallies may slow down or halt the message to the people. This is especially true for rural people who may not have access to television, radio or electricity (Anon, 2008a).

There is high awareness of AI (amongst poultry farmers and community "experts" such as medical doctors and veterinary officers), pegged to the 2006 outbreak that led to a national emergency and several media campaigns (Anon, 2008a).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 AREA OF STUDY

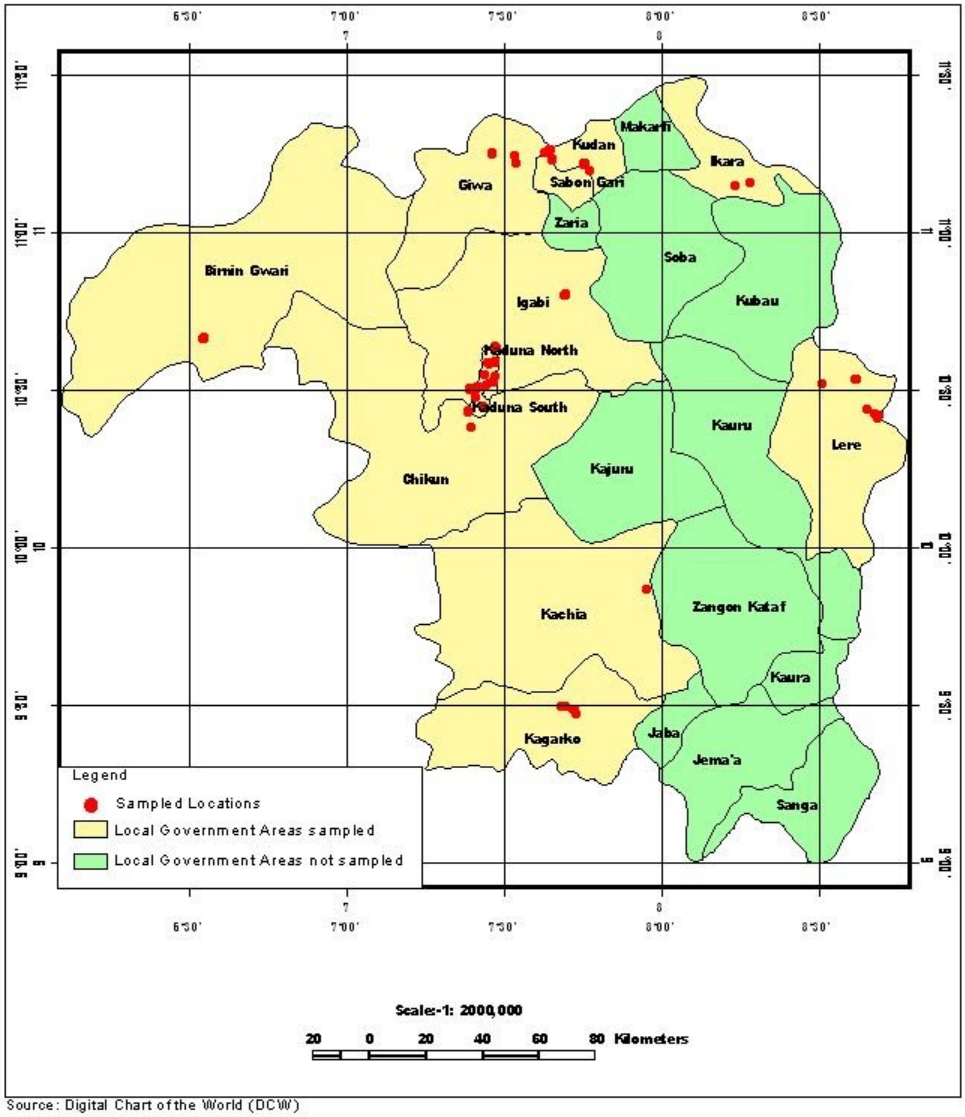
Kaduna state is located in Northwestern Nigeria and was created from the present Kaduna and Katsina states in 1989. The state has an estimated population of 6 million people based on the 2006 population census by the national population commission (Nigerian News, 2008). The state lies between latitude 8^{o} 45'' and 11^{o}30''N and longitude 6^{o}10''and 9^{o}E and shares borders with Katsina, Kano, Plateau, Niger , Zamfara, Bauchi and Nasarawa states as well as the Federal Capital territory. The state has 23 LGA's that are inhabited by ethnic groups including Hausa Fulani, Kaje, Bajju, Ham, Atyap Adara and Kataf among others. The occupation of the people living in Kaduna state include crop farming, livestock rearing, trading and fishing (Anon, 2006).

The area of study was 12 Local Government Areas (LGA) in Kaduna state. Six of the LGA's had outbreaks of highly pathogenic avian influenza that were reported and confirmed between 2006 and 2007. The remaining six local government areas were randomly selected from the LGA's that did not report any outbreak. The LGA's that reported and had confirmed highly pathogenic avian influenza outbreaks were Kaduna North, Kaduna South, Igabi, Chikun, Giwa, and Sabon Gari while those that were randomly selected were Ikara, Birnin Gwari, Kudan, Katchia, Kagarko and Lere.

The mean annual temperature of Kaduna state is 34^{o}C with the hottest months being from March-April (40^{o}C) and the coldest period (13.2^{o}C) is between December and January during the severe harmattan. Rainfall varies between 1,000 mm and 1,500 mm and the rainy season lasts for 150-200 days (Mid April-end of October). The dry season occurs from late October to early April (RIM, 1993).

Households sampled were predetermined by guides in the LGA's after previous sensitization of the poultry farmers. Averages of 5 birds were bled per household and only apparently healthy growers and adult birds were bled.

The state has an estimated poultry population of about 2,821,092 with about 2,564,100 (90.8%) being local chickens. The remaining poultry populations are exotic chickens (FDLPCS, 2003).



Source: Digital Chart of the World (DCW)

FIGURE 3.1: MAP OF KADUNA STATE SHOWING STUDY AREA AND SAMPLED LOCATIONS

3.2 **SAMPLE SIZE**

A total of 960 blood samples were obtained from apparently healthy local chickens and ducks. Only 605 samples from local chickens were eventually analyzed for the presence of antibodies because of inadequate antigen.

The sample size was calculated using the formula outlined by Thrusfield (1997).

$$n = \frac{Z^2 Pq}{d^2}$$

Where q = 1-p

n = sample size

p = anticipated prevalence

d = desired precision

z = appropriate value from the normal for the desired confidence.

Using the prevalence of 26.5% obtained by El-Yuguda and Baba (2002) for Borno state of Nigeria,

$$n = \frac{(1.96^2 \times 0.265 \times 0.735)}{(0.03)^2}$$

$$= \frac{3.841 \times 0.265 \times 0.735}{(0.03)^2}$$

$$= \frac{0.7481}{0.0009} = 831$$

3.3 **SAMPLE COLLECTION**

About 2.0–3.0 ml of blood was collected from the brachial vein of each local bird using a 5 ml syringe after carefully observing asepsis to avoid contamination of the blood. The samples were collected during the months of December 2007 through February 2008 from consenting poultry owners. All the poultry sampled had no history of vaccination

against avian influenza. The blood was allowed to clot in a cool place after which the serum was separated and placed into sterile cryo tubes. The serum was stored at -20^{o}C until analyzed.

3.4 **DATA COLLECTION**

A structured questionnaire was administered to consenting poultry farmers after the bleeding of their birds (Appendix I). Aspects covered by the questionnaire include demographic data of the respondents, flock size of various species of poultry kept as well as other livestock, management system such as housing, type of housing provided, handling of sick birds, methods of disposal of dead poultry, disposal of poultry feces, their knowledge and awareness of HPAI and their readiness to disclose outbreak of HPAI.

3.5 **LOW PATHOGENIC AI (H5N2) VIRUS ANTIGEN AND POSITIVE ANTI SERUM**

Low pathogenic AI H5N2 virus antigen and positive antiserum were obtained from the Friedrich-Loeffler Institute of Diagnostic Virology, Germany through the assistance of Dr Timm Harder, the Head of the AI Laboratory of the Institute.

3.6 **PREPARATION OF 1% RED BLOOD CELLS (RBC's)**

Two milliliters of blood was collected from five day old chicks and pooled in an equal volume of Alsever's anticoagulant solution. Cells were washed three times in PBS (PH 7.2) by centrifuging at 447.2 g for 5 minutes (Collee *et al.,* 1982). One per cent RBC (packed cell v/v) suspension was made by adding 99 ml of PBS to 1 ml of washed RBC.

3.7.1 **DETERMINATION OF TITRE OF LOW PATHOGENIC AI (H5N2) VIRUS ANTIGEN**

Hemagglutination test was carried out according to the method described by OIE (2005). Twenty five microlitre of PBS was dispensed into each well of V-bottom microtitre plates. Twenty five microlitre of the reconstituted antigen in PBS was placed into the first well. Two fold serial dilutions of 25 µl of the antigen suspension were made across the plate. A further 25 µl of PBS was dispensed into each well. Twenty five microlitre of 1% chicken RBC's was dispensed into each well. This was mixed by gently tapping the plates. The RBC's were allowed to settle for about 20 minutes at room temperature. Hemagglutination (HA) was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBC's. 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. The titre of the antigen was determined as 7.0 $\log_2$ and this was used in the hemagglutination inhibition test.

3.7.2 **DETERMINATION OF ANTIBODIES TO LOW PATHOGENIC AI (H5N2) VIRUS**

Hemagglutination inhibition test was conducted according to the method described by OIE (2005). Twenty five microlitre of PBS was dispensed into each well of microtitre plates. Twenty five microlitre of serum was placed into the first well of the microtitre plate after heat inactivating the sera at 56^{o}C for 30 minutes in a water bath. Two fold dilution of 25 µl of the serum were made across the plate. A 25 µl of four HAU of the virus/antigen was added to each well and left for a minimum of 30 minutes at room temperature. Twenty five microlitre of 1% RBC's was added to each well, mixed gently and allowed to settle for about 30 minutes at room temperature. The HI titre was the highest dilution of serum causing complete inhibition of 4 HAU of the antigen. The agglutination was assessed by tilting the plates. Only wells in which the RBC's stream at the same rate as the control wells (containing 50 µl PBS and 25 µl RBC's only) were considered to show inhibition. The validity of the test was assessed against a negative

control serum, which gave a titre >4.0 log_2 and a positive control serum for which the titre was >12.0 log_2.

3.8 **DATA ANALYSES**

Data generated on antibodies levels in sera were expressed as mean ± standard error of the mean (x ± S.E: M) and reduced into tables. Data obtained from questionnaires on poultry and other livestock population, management system, knowledge and readiness to disclose HPAI outbreak, type of housing provided for birds, methods of disposal of poultry waste, methods of disposal of excess flock, source of the breeding stock were coded. Statistical analyses and frequency of response to each question were carried out using the Statistical Package for Social Sciences (SPSS) version: 15 program. Odds ratio (OR) > 1 indicated association with infection, while OR < 1 was interpreted as lack of association (sparing or protective). For Chi square (χ^2), P value <0.05 was considered significant.

CHAPTER FOUR

4.0 **RESULTS**

4.1 **LOW PATHOGENIC AI (H5N2) VIRUS ANTIBODIES**

The overall prevalence to low pathogenic AI H5N2 virus antibodies in local chickens in the areas of the study was 18.1%.

Of the 280 samples collected from the HPAI outbreak reported LGA's, 21 (7.5%) were positive to low pathogenic AI H5N2 virus while the remaining 259 (92.5%) were negative (Table 4.1). Igabi, Chikun and Kaduna South had no positive samples (Figure 4.2).

In the LGA's with no report of HPAI outbreak, 325 samples were tested out of which 89 (27.3%) were positive while 236 (72.7%) were negative (Table 4.1).

The highest mean titer (7.4±1.0) was recorded in Birnin Gwari LGA (Table 4.2).

There was statistical significant difference ($p<0.05$) in the antibody prevalence in the LGA's that reported outbreak and those that did not report outbreak. The presence of antibodies to H5N2 tend to protect against outbreak of H5N1 (OR=0.22).

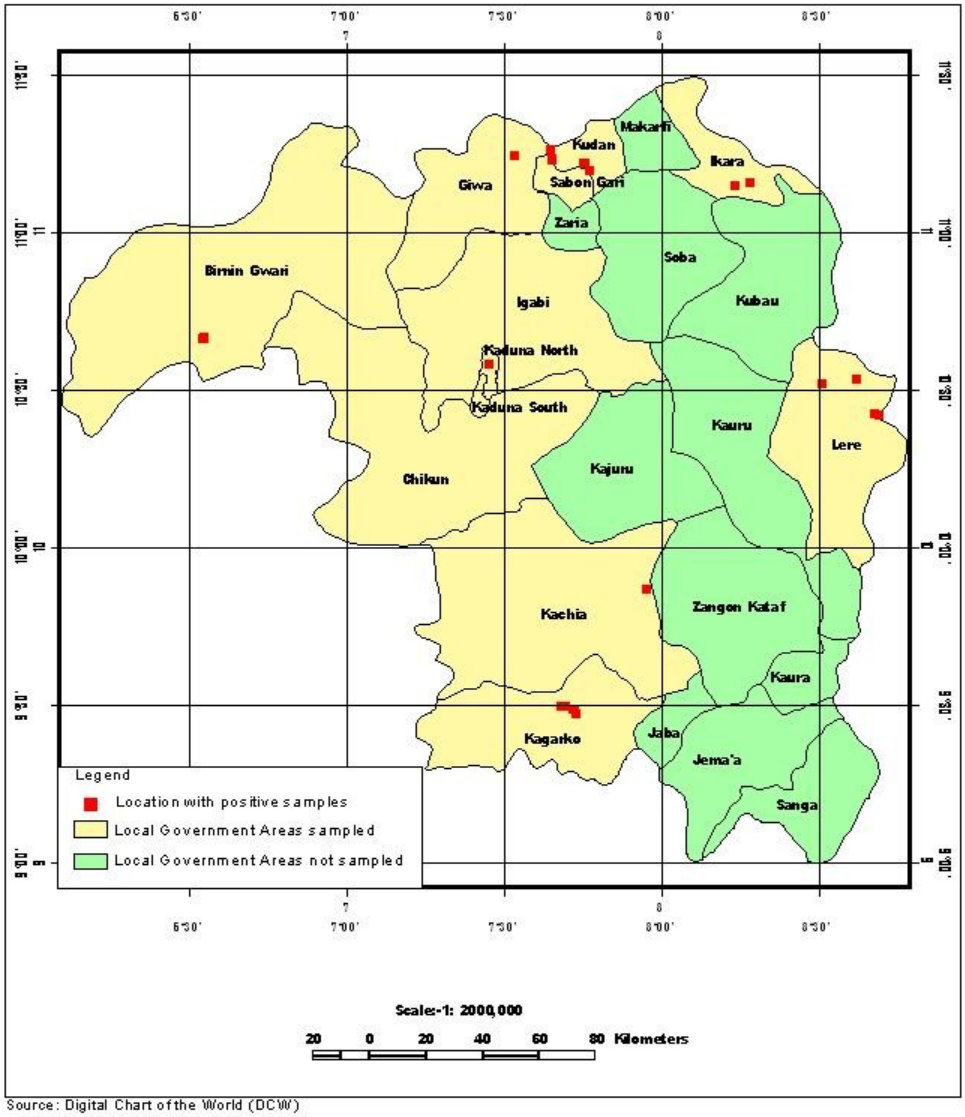
Table 4.1: Prevalence of low pathogenic H5N2 avian influenza virus antibodies in the LGA's that reported outbreak and those that did not report outbreak

Group	No. positive (%)	No. negative (%)	Total no. sampled
Outbreak reported	21 (7.5)	259 (92.5)	280
Outbreak not reported	89 (27.3)	236 (72.7)	325
Total	110 (18.1)	495 (81.9)	605

P=0.019 OR=0.22

Table 4.2: Low pathogenic H5N2 antibody titer of local chickens in Kaduna state

Local Government Area	1	2	3	4	5	6	7	8	9	10	11	12	Mean titer± SE	Min	Max
Ikara	0	0	2	0	0	0	3	1	5	1	1	8	2.6±0.5	3	12
Kachia	0	0	0	0	1	1	0	0	0	1	0	5	1.4±0.5	5	12
Lere	0	0	0	0	0	0	0	1	1	1	0	6	1.5±0.5	8	12
Birnin Gwari	0	0	0	0	0	0	1	2	2	1	3	9	7.4±1.0	7	12
Kagarko	0	0	0	0	0	0	1	2	3	1	4	17	4.7±0.6	7	12
Kudan	0	0	0	0	0	0	0	0	0	0	2	2	1.0±0.5	11	12
Giwa	0	0	0	0	0	0	0	0	2	0	0	1	0.8±0.4	9	12
Sabon Gari	0	0	0	0	0	0	3	3	1	3	0	7	2.8±0.5	7	12
Igabi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chikun	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kaduna South	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kaduna North	0	0	0	0	0	0	0	0	0	0	0	1	0.2±0.2	12	12
Total	0	0	2	0	1	1	8	9	14	8	10	56		3	12



Source: Digital Chart of the World (DCW)

FIGURE: 4.1: MAP OF KADUNA STATE SHOWING LOCATIONS OF POSITIVE SAMPLES

4.2 KNOWLEGDE, AWARENESS AND READINESS TO DISCLOSE OUTBREAK OF HPAI (H5N1).

The level of awareness of HPAI was very high among respondents. About 146 (84.9%) of the respondents said they have heard of the disease while 26 (15.1%) said they have not heard of the disease. About 34 (19.8%) knew some signs of HPAI while 138 (80.2%) did not know any signs of HPAI. The sign of HPAI known by most of the farmers was swollen wattles and comb which was known by 15 (34.9%) of the respondents. Eight respondents, representing (18.6%) knew high mortality, 8 (18.6%) knew CNS signs, 9 (30.9%) knew greenish diarrhea while 3 (6.9%) knew discoloration of shanks (Table 4.3).

Most of the respondents (78.5%), heard of HPAI through the radio, 3.5% heard from friends/relatives, 3% were grouped as others while the question was not applicable to 15.1% of the respondents (Table 4.4).

About 150 (87.2%) of those interviewed said they would report HPAI, 20 (11.6%) said they would not report while 2 (1.2%) said they do not know if they would report or not. Out of the 150 respondents who said they would report HPAI, 90 (52.3%) said they would report to help in the control and eradication of the disease, while 60 (34.9%) said they would report to seek veterinary care (Table 4.5). Of the 20 respondents who said they would not report HPAI, 2 (10%) said they would not report since they can not recognize the disease, 11 (55%) said they do not know where to report, 2 (10%) said nothing will be done even if they report, 3 (15%) said reporting of poultry disease was not important while 2 (10%) said they would not report for social and religious reasons (Table 4.6).

There was no association between gender and awareness of HPAI ($P<0.05$) ($\chi^2 =0.218$ df=1).

Awareness was however higher among males than females (68.5% and 31.5%) respectively. Knowledge and readiness to disclose outbreak of HPAI was also highest among males (73.5% and 70.7% respectively) but there was no association between gender and knowledge as well as gender and readiness to disclose outbreak of HPAI.

Educational status ($P<0.05$) ($\chi^2 = 16.635$ df = 5) and occupation ($P<0.05$) ($\chi^2 = 9.984$ df = 4) of respondents also had statistical association with knowledge of the HPAI (Table 4.11).

The age group of respondents did not have any significant association with the clinical signs of HPAI known by the respondents.

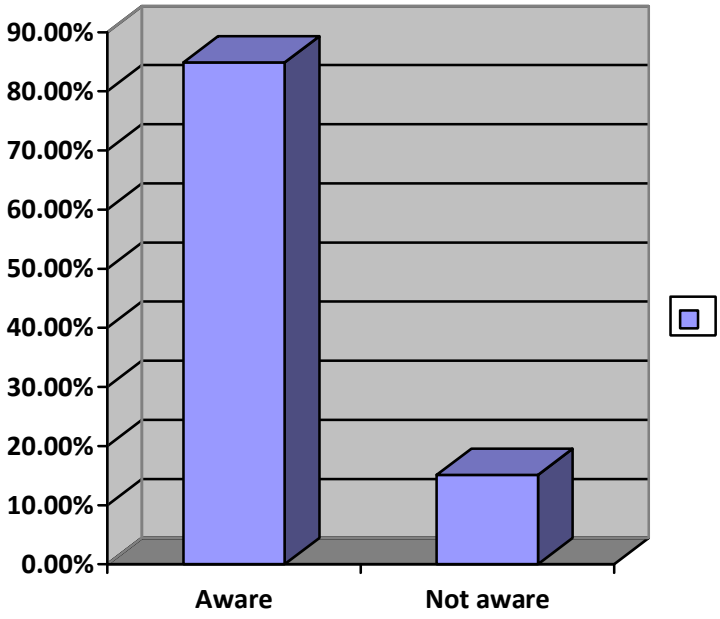


Figure 4.2: Level of awareness of highly pathogenic avian influenza among respondents in 12 government areas of Kaduna state.

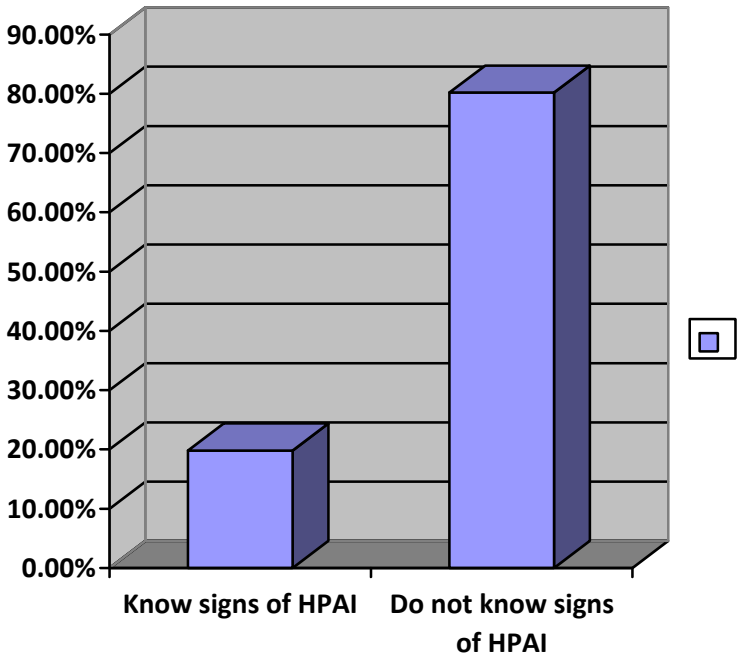


Figure 4.3: Knowledge of HPAI among respondents in 12 local government areas of Kaduna state.

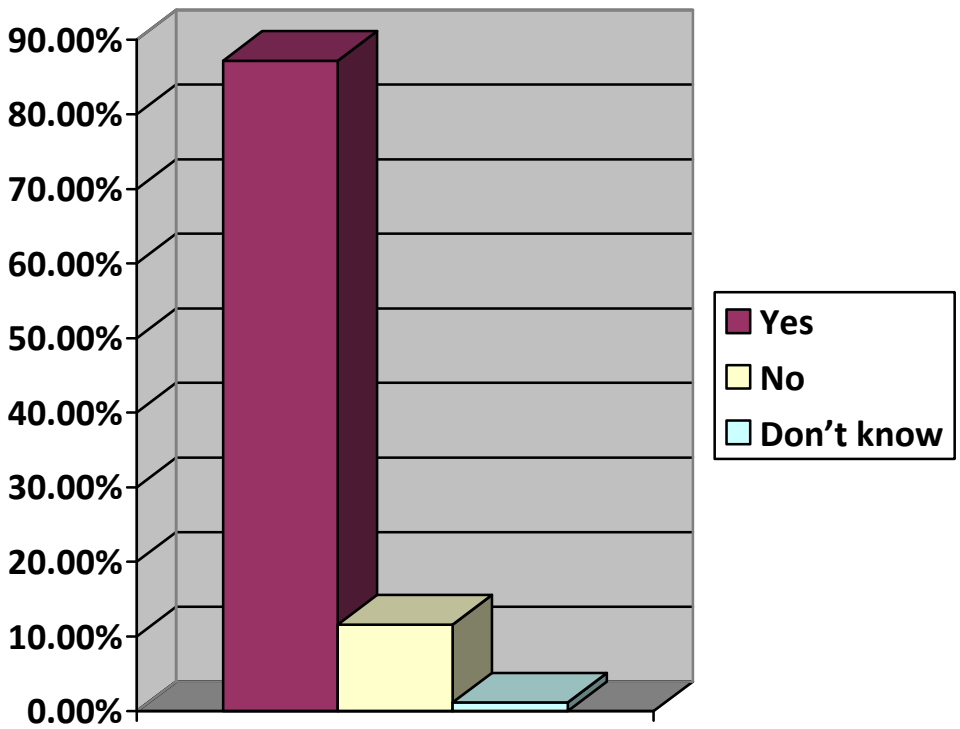


Figure 4.4: Readiness to disclose outbreak of HPAI among respondents in 12 local government areas of Kaduna state.

Table 4.3: Signs of HPAI known by respondents.

Signs	Number and percentage of respondents
Swollen wattles and comb	15 (34.9)
Very high mortality	8 (18.6)
CNS signs	8 (18.6)
Greenish diarrhea	9 (20.9)
Discoloration of shank	3 (6.9)

Table 4.4: Medium through which respondents heard of HPAI.

Medium	Number and percentage of respondents
Radio	135 (78.5)
Friends and relatives	6 (3.5)
Others	5 (3.0)
Not applicable	26 (15.1)

Table 4.5: Reasons why respondents would report HPAI.

Reason	Number and percentage of respondents
To help in eradication and control of HPAI	90 (60.0)
To seek veterinary care	60 (40.0)

Table 4.6: Reasons why respondents would not report HPAI.

Reason	Number and percentage of respondents
Can not recognize disease	2 (10.0)
Do not know where to report	11 (55.0)
Nothing will be done if they report	2 (10.0)
Reporting poultry disease is not important	3 (15.0)
Social/religious reasons	2 (10.0)

4.3 DEMOGRAGHIC CHARACTERISTICS OF RESPONDENTS

A total of one hundred and seventy two (172) questionnaires were administered in thirty three (33) villages of the twelve (12) LGA's covered in this study (Table 4.7). One hundred and nineteen (69.2%) of the respondents were males while 53 (30.8%) were females (Table 4.8). The highest frequency of respondents (19.2%) was from 25-34 age-group while the lowest frequency (1.2%) was from 75-84 age-group (Table 4.9).

Most respondents had a form of education ranging from Islamic (27.3%), primary (23.3%), secondary (24.4%) and adult education (1.7%), while those with no form of education were 9.3% (Table 4.10).

Regarding occupation, 45 (26.2%) were farmers, 44 (25.6%) students, 36 (20.9%) housewives, 21 (12.2%) civil servants, while the remaining (15.1%) were grouped as others (Table 4.11).

Table 4.7: Distribution of the number of villages and chickens sampled per local government area.

Local Government Area sampled	No. of villages sampled	No. of questionnaires administered	No. of blood samples obtained
Ikara	2	27	74
Kachia	1	30	55
Lere	4	9	62
Birnin Gwari	1	11	23
Kagarko	4	13	67
Kudan	3	9	44
Giwa	3	12	36
Sabon Gari	3	9	60
Igabi	2	14	53
Chikun	4	9	42
Kaduna South	2	15	43
Kaduna North	4	14	46
Total	33	172	605

Table 4.8: Sex of respondents in the 12 local government areas sampled.

Sex	No of respondents	Percentage of respondents
Male	119	69.2
Female	53	30.8
Total	172	100.0

$\chi^2 = 0.218$ df=1 P = 0.641

Table 4.9: Age distribution of respondents in the 12 local government areas sampled.

Age group	No of respondents	Percentage of respondents
5-14	20	11.6
15-24	26	15.1
25-34	33	19.2
35-44	32	18.6
45-54	26	15.1
55-64	20	11.6
65-74	13	7.6
75-84	2	1.2
Total	172	100.0

$\chi^2 = 102.773$ df = 7 P=0.000

Table 4.10: Educational status of respondents in the 12 local government areas sampled.

Type of education	No of respondents	Percentage of respondents
Islamic education	47	27.3
Primary education	40	23.3
Secondary education	42	24.4
Tertiary education	24	14.0
Adult education	3	1.7
None	16	9.3
Total	172	100.0

$\chi^2 = 16.635$ df = 5 p=0.000

Table 4.11: Occupation of respondents in the 12 local government areas sampled.

Occupation	No of respondents	Percentage of respondents
Farmer	45	26.2
Civil servant	21	12.2
Student	44	25.6
House wife	36	20.9
Others	26	15.1
Total	172	100.0

χ^2 =9.984 df = 4 P=0.000

4.4 **MANAGEMENT PRACTICES AMONG RESPONDENTS.**

The most commonly practiced management system by the respondents was the extensive which was practiced by 170 (98.8%) of the farmers interviewed, while 2 (1.2%) practiced the intensive management system (Table 4.12).

Majority of the respondents 165(95.9%), provided a form of housing for their birds while only 7(4.1%) did not provide any form of housing. The types of housing provided included baskets which was used by 2 (1.2%) of the respondents, 43 (25.0%) provided cages, 3 (1.7%) used a combination of cage and hut, 1 (0.6%) used a combination of cage and room, 66 (38.4%) used hut, 1 (0.6%) used a combination of hut and basket, 4 (2.3%) used a combination of hut and room, 1 (0.6%) used kitchen, 43 (25.0%) used rooms, 1 (0.6%) used store while the question was not applicable to 7 (4.1%) of the respondents (Table 4.13).

From the interview, 119 (69.2%) of the respondents obtained their breeding stock from village markets, 27 (15.7%) from neighbors, 14 (8.1%) through gifts, 6(3.5%) through inheritance, 3 (1.7%) bought from city markets, 2 (1.2%) hatched at home while 1 (0.6%) acquired his breeding stock from another state (Table 4.14).

With regards to the disposal of excess birds, 2 (1.2%) of the respondents dispose them as gifts, 6 (3.5%) sell to their neighbors, 30 (17.4%) slaughter for consumption, 2 (1.2%) slaughter for consumption and sell at the village market and to neighbors, 1 (.0.6%) sell at the village market and to poultry dealers, 30 (17.4%) slaughter for consumption and sell at the village market, 88 (51.2%) sell at the village market, 5 (2.9%) sell at the village market and to neighbors. The question was not applicable to 8 (4.7%) of the respondents (Table 4.15).

Out of the 172 respondents interviewed, 84 (48.8%) reported visiting live bird markets while 88 (50.6%) did not visit live bird markets.

Regarding the handling of sick birds, 3 (1.7%) reported they leave sick birds to die, 27 (15.7%) sell off sick birds, 124 (72.1%) slaughter, 7 (4.1%) slaughter and sell while the question was not applicable to 11 (6.4%) of the respondents (Table 4.16).

The commonest means of disposal of poultry feces was by spreading on the farm as a source of manure. This was the case with 137 (79.7%) of the respondents, 16 (9.3%) bury the poultry feces, 15 (8.7%) dispose into the dustbin, 2 (1.2%) burn, while 2 (1.2%) sell the feces (Table 4.17).

Apart from local chickens, other types of poultry encountered during the study were ducks, turkeys, guinea fowls, pigeons, canaries, commercial chickens, geese and owl. Local chickens were mostly reared (93.6%) compared to other species of poultry. This was followed by ducks and pigeons which were reared by 69 (40.1%) and 31 (18%) of the respondents respectively. Other species of poultry encountered were few in number and reared by few respondents (Table 4.18).

Other animals encountered during the survey included sheep, goats, dogs, cattle, pigs, rabbits, guinea pig, fish, cats and horse. Goats, sheep and cattle were the mostly reared animals compared to the other animals (Table 4.19).

There was an association between the presence of ducks and detection of antibodies to low pathogenic H5N2 influenza A virus ($P<0.05$) ($\chi^2 = 24.257$ df =1) (Appendix II).

There was no association between visit to live bird market and detection of antibodies ($\chi^2 = 0.092$ df = 1).

Table 4.12: Management systems practiced by respondents.

Management system	Number and percentage of respondents
Intensive	2 (1.2)
Extensive	170 (98.8)

χ^2 =0.85 df=1 P=0.356

Table 4.13: Types of housing provided for poultry by respondents.

Type of housing	Number and percentage of respondents
Basket	2 (1.2)
Cages	43 (25.0)
Cage and hut	3 (1.7)
Cage and room	1 (0.6)
Hut	66 (38.4)
Hut and basket	1 (0.6)
Hut and room	4 (2.3)
Kitchen	1(0.6)
Room	43 (25.0)
Store	1 (0.6)
Not applicable	7 (4.1)

Table 4.14: Source of breeding stock in the 12 local government areas sampled.

Source of stock	No. of respondents	Percentage of respondents
Village market	119	69.2
City market	3	1.7
Neighbor	27	15.7
Gift	14	8.1
Inheritance	6	3.5
Others	3	1.8
Total	172	100.0

Table 4.15: How excess birds were disposed by respondents.

Methods of disposal	Number and percentage of respondents
Gift	2 (1.2)
Sell to neighbor	6 (3.5)
Slaughter for consumption	30 (17.4)
Sell at village market and to poultry dealers	1 (0.6)
Slaughter for consumption and sell at village market	30 (17.4)
Sell at village market	88 (51.2)
Sell at village market and to neighbor	5 (2.9)
Slaughter, sell at village market and to neighbor	2 (1.2)
Not applicable	8 (4.7)

Table 4.16: Methods of disposal of sick birds by respondents.

Methods of disposal	Number and percentage of respondents
Left to die	3 (1.7)
Sell off	27 (15.7)
Slaughter for consumption	124 (72.1)
Slaughter and sell	7 (4.1)
Not applicable	11 (6.4)

Table 4.17: Methods of disposal of poultry feces by respondents.

Method of disposal	Number and percentage of respondents
Spread on farm as manure	137 (79.7)
Bury	16 (9.3)
Dustbin	15 (8.7)
Burn	2 (1.2)
Sell	2 (1.2)

Table 4.18: Number (Mean ± SE) of poultry per household and percentage of respondents.

Type of poultry	Minimum	Maximum	Mean ±Standard Error
Local chicken	1	200	21.51±2.64 (93.6%)
Duck	1	50	3.91±0.57 (40.1)
Guinea fowl	2	30	0.48±0.22 (4.1%)
Turkey	1	11	0.37±0.12 (7.6%)
Pigeon	1	100	5.49±1.18 (18.0)
Canary	2	100	0.62±0.58 (1.7%)
Owl	1	1	0.01±0.11 (0.6%)
Commercial poultry	10	1000	10.65±6.24 (5.2%)
Geese	7	7	0.04±0.04 (0.6%)

Table 4.19: Number (Mean ±SE) of other animals reared and percentage of respondents.

Type of animal	Minimum	Maximum	Mean ±Standard Error
Sheep	1	50	4.78±0.80 (43.0%)
Dog	1	8	0.58±0.11 (20.9%)
Goat	1	150	6.22±1.15 (54.1%)
Cattle	1	1040	9.23±6.16 (29.1%)
Pig	1	10	0.08±0.06 (1.7%)
Rabbit	6	10	0.20±0.11 (2.3%)
Guinea pig	8	20	0.27±0.14 (2.3%)
Fish	20	20	0.12±0.12 (0.6%)
Cat	1	15	0.23±0.09 (9.9%)
Horse	1	1	0.01±0.01 (0.6%)

CHAPTER FIVE

5.0 DISCUSSION

The result of this study revealed the presence of antibodies to low pathogenic H5N2 influenza A virus in apparently healthy local chickens in Kaduna state. The antibodies detected were as a result of natural infection since vaccination of the village poultry is rarely undertaken in Nigeria (Abdu *et al.,* 1987; Dipeolu *et al.,* 1998). The results of the antibody prevalence against low pathogenic H5N2 influenza A virus adds to previously existing knowledge and findings about influenza A virus in Nigeria. Previous findings about influenza A virus in Nigeria include the findings of Adeniji *et al.* (1993), who reported the prevalence of antibodies to influenza A virus among chickens at Ibadan, Nigeria, El-Yuguda and Baba (2002) who also reported the presence of antibodies to Influenza A virus in village chickens in Borno State, Nigeria, and Wakawa (2007) who reported antibodies to influenza A virus in clinically sick and apparently healthy village chickens in Kaduna, Katsina, and Jigawa states of Nigeria. Other findings include that of Gaidet *et al.* (2008), who reported H5N2 highly pathogenic avian influenza viruses in two healthy wild water fowl in Northern Nigeria. The HA and NA sequences of the viruses isolated revealed no poultry adaptive mutations, suggesting these viruses may have evolved from H5N2 low pathogenic to high pathogenic avian influenza in wild bird host. Other findings about avian influenza in Nigeria include the result of FAO-FDL HPAI active disease surveillance conducted in 36 states and the Federal Capital Territory which showed a prevalence of less than 20%, the active targeted surveillance in live bird markets in 25 states of Nigeria and the Federal Capital Territory that recorded outbreaks which showed the evidence of HPAI infection in 8 live bird markets including the isolation of four H5N1 virus in 4 states, one RT-PCR positive bird in a fifth state and evidence of AI virus in the form of antibodies in 5 states and finally the LBM survey in 11 states without outbreak which showed the detection of HPAI in LBMs in 2 states (Obi and Ahmed,2008). Outside Nigeria, this study is in agreement with the findings of Masatoshi *et al.* (2007) who reported the isolation of low pathogenic H5N2 avian influenza virus from chickens in Japan during the year 2005-2006.

The implication of the presence of antibodies to low pathogenic H5N2 virus in apparently healthy local chickens is that the virus is most probably circulating in local chicken population in Kaduna state. It may also be an indication that the country is not completely free of HPAI virus infection since it has become increasingly evident that some (LPAI) H5 or H7 have the capacity to mutate into more virulent strains that cause extensive losses and high mortality (Hall, 2004). Also, HPAI viruses are generally considered to emerge from LPAI precursors once introduced and adapted to gallinaceous poultry population (Alexander, 2000; Stallknecht *et al.,* 2007).

Also, the introduction of H5 or H7 subtypes of LPAI viruses to susceptible poultry is the basis of the de novo development of highly pathogenic biotypes (Harder and Werner, 2006).

The absence of antibodies to low pathogenic H5N2 AI in most of the outbreak reported local government areas may be due to non exposure to this strain and lack of previous vaccination (Wunderwald and Hoop, 2002). The culling of infected poultry, improved biosecurity measures and decontamination exercises that have taken place in major live bird markets in most of these local government areas might also have contributed to low levels of infection recorded. Biosecurity is considered the most important tool to prevent and control avian influenza (De Benedictis *et al.,* 2007).

Again, control of H5 and H7 subtypes of LPAI in poultry, by testing and culling infected flocks, may be advisable in non- endemic areas in order to reduce the risk of a de novo development of HPAIV from poultry holdings (Harder and Werner, 2006).

Based on the administered questionnaire, there seems to be some statistical association between the presence of ducks and the detection of antibodies to low pathogenic H5N2 avian influenza virus. However, this association cannot be confirmed until the analyses of the collected duck samples for antibodies.

The highest mean titer (7.4±1.0) recorded in Birnin Gwari LGA may be attributed to the high duck population reported in the LGA during this study. The role of domestic and wild ducks in the epidemiology of AI is documented (Hanson, 2005).

The high level of awareness to HPAI may be due to the success of the efforts of the Federal Government of Nigeria in the control and eradication of the disease through various methods of public enlightenment.

The high percentage (80.2%) of the respondents that did not know any signs of HPAI is significant and shows that though farmers are aware of HPAI, there is still more to be done to improve their knowledge of the disease. Other means of enlightenment such as the use of handbills with pictures need to be intensified. Low knowledge of the disease may also be attributed to the fact that majority of the farmers (78.5%) heard of HPAI through the radio.

The high percentage of respondents (87%) who said they would report AI may be an indication that owners of local poultry are now aware that reporting disease outbreak helps in the control and eradication of HPAI. This is evident in the fact that 52.3% of the respondents gave this reason for why they would report AI.

This study did not find any association between the presence of cats and dogs and the detection of antibodies to low pathogenic H5N2 avian influenza virus even though some avian influenza viruses have been isolated from these species of animals (Songserm *et al.,* 2006a, Songserm *et al.,* 2006b).

The statistical association ($P<0.05$) between the method of disposal of excess birds and the presence of antibodies to low pathogenic H5N2 avian influenza virus is also in agreement with the view of previous workers that the live bird markets play a significant role in the epidemiology of AI (Suarez *et al.,* 2003; Landman and Schrier, 2004). This is further explained by the fact that 51.2% of the respondent reported that excess birds were sold in the village live bird market.

The statistical association ($P<0.05$) between educational status of respondents with the knowledge of HPAI was also expected as individuals with higher educational status would have access to other sources of information that can improve knowledge of HPAI other than governments public enlightenment campaign. As for occupation, it was expected that the occupation of respondents would also have an impact on their

knowledge of HPAI. For example students and farmers who make up majority of the respondents (25.6% and 26.2% respectively) should be in the forefront of those with knowledge of HPAI; students because of their presence in knowledge settings and because farmers are stakeholders in the fight against HPAI.

Local chickens were the commonest type of poultry reared by the respondents. This is in agreement with the findings of Nwanta (2002) who found that 100% of the households sampled in 13 LGA's of Kaduna state had local chickens. However the average number of local chicken per household in this study was 21.51±2.64. This is higher than the average of 18.4±8.1 birds per house hold recorded by the same author in 2002.

This study also revealed that commercial poultry are now being reared in some of the villages sampled. This differs with the finding of Nwanta, (2002) who found no commercial poultry in all the households he sampled.

Goats and sheep were the most common other animals kept by the respondents. This differs from the finding of Nwanta, (2002) who found that cattle were the most common other livestock kept by local poultry farmers. It is possible that more farmers are now shifting to the rearing of small ruminants now because of their relatively low cost when compared to cattle.

CONCLUSIONS

Based on the result of the serosurvey:

The study showed an overall prevalence rate of 18.1% for low pathogenic H5N2 avian influenza virus antibodies.

The presence of antibodies to low pathogenic H5N2 avian influenza in this study suggests that natural infection with this virus subtype occurs in Kaduna state.

There was a higher prevalence of low pathogenic H5N2 avian influenza virus antibodies in LGA's that reported outbreak than those that did not.

Based on the result from administered questionnaire:

The live bird market could play a vital role in the epidemiology of low pathogenic H5N2 influenza virus.

There was association between the presence of domestic ducks and detection of low pathogenic H5N2 virus antibodies.

There is high level of awareness and readiness to disclose the presence of HPAI but low knowledge of the disease amongst the poultry farmers interviewed.

There was association between educational status and occupation of respondents with the knowledge of avian influenza.

Majority of respondents sell and consume sick birds and also buy their birds from live bird markets.

RECOMMENDATIONS

It is recommended:

That there should be enhanced and sustained surveillance to establish the presence or absence of LPAI (H5N2) and other LPAI

Biosecurity measures in live bird markets are introduced also in HPAI outbreak not reported local governments areas of the country

More efforts should be made to improve the knowledge and ability of local poultry farmers to recognize HPAI.

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Appendix I: Questionnaire on Farmer's awareness and their readiness to disclose outbreak of HPAI in Kaduna State.

Personal information

1 Serial No……

2 Address……

3 GPS; Latitude…… Longitude……

4 Sex ……5 Age ……

6 Location of farm… ……..

7 L.G.A ……

8 Educational Status of Farmer

8.1 Primary……8.2 Secondary……8.3 Tertiary…….
8.4 Others (Specify)……. 8.5 None……

9 Occupation of Farmer

9.1 Full time Farmer…… 9.2. Business/Trading……..9.3 Civil Servant…….9.4Student……..9.5 Housewife……. 9.6 Others (Specify)…….

10 Type of poultry kept and number

10.1 Local chickens……10.2 Commercial poultry…….

10.3Turkeys……..10.4 Ducks……..

10.5 Guinea fowls……10.6. Others…….

11 Source of breeding stock

11.1 Village market……. 11.2 City market…… 11.3 Neighbors……..11.4 Gift…….. 11.5 Others (Specify)……

12 How excess flock is disposed

12.1 Village market…… 12.2 City market…… 12.3 Neighbors……

12.4 Others (Specify)……

13 Other animals available and their number

13.1 Goats…….. 13.2 Sheep…… 13.3 Cattle…….. 13.4 Pigs……

13.5 Dog…… 13.6 Others (Specify)……

14 Do you visit live bird market?

14.1 Yes……14.2 No…….

15 Housing provided for birds?

15.1 Yes…… 15.2 No…….

16 If yes, what type?

16.1 Cage……. 16.2 Baskets…… 16.3 Hut…….. 16.4 Others (Specify)…….

17 Management systems

17.1 Intensive……. 17.2 Extensive…….

18 Handling of sick birds

18.1 Slaughter for consumption…… 18.2 Sell off…… 18.3 Left to die…….

18.4 Others (Specify)……

19 Handling of dead birds

19.1 Bury…….. 19.2 Burn…….. 19.3 Dust Bin……. 19.4 Fed to dogs…….

19.5 Consume……. 19.6 Others (Specify)……

20 How do you dispose of poultry feces?

20.1 Burn……. 20.2 Bury…….. 20.3 Compose…….. 20.4 Spread on farm…….20.5 Sell……

21Have you ever heard of avian influenza/ Bird Flu

21.1Yes…….. b. No……..

22 If yes, through which medium

22.1 Books/Magazines/other print media…….
22.2 Electronic media (Specify)……
22.3 Relatives/Friends……
22.4 Fellow Farmers……...
22.5 Others (Specify)…….

23 Do you know some symptom/signs of avian influenza?

23.1Yes…….. 23.2 No……

24 If yes to question no 23 which of the symptoms/signs do you know

24.1) Very high mortality…….24.2) swollen wattles/comb……..

24.3) Discoloration of Shanks……24.4) CNS signs…… 24.5 Greenish diarrhea…….. 24.6) Others (Specify)…….

25 If you suspect your birds have avian influenza, would you report?

25.1 Yes…… 25.2) No……

26 If yes to question no 25 why would you report?

26.1 To get compensation…… 26.2 To help in the control/eradication…… 26.3 To seek Vet Care…….26.4 obliged to report…….26.5 Others (specify)……

27 If no, to question 25, why

27.1 Not obliged to report…….27.2 Reporting is not essential……. 27.3 social/religious reasons……27.4 Others (Specify)……

Appendix II: Association between other poultry and detection of H5N2 antibodies.

Type of poultry		Result negative	Result positive	Total
Duck	Yes	63	6	69
	No	58	45	103
	Total	121	51	172
χ^2 =24.25 df =1 P= 0.000				
Guinea fowl	Yes	3	4	7
	No	118	47	165
	Total	121	51	172
χ^2 =2.644 df =1 P= 0.104				
Turkey	Yes	12	1	13
	No	109	50	159
	Total	121	51	172
χ^2 =3.251 df =1 P= 0.071				
Pigeon	Yes	23	8	31
	No	98	43	141
	Total	121	43	172
χ^2 =0.268 df =1 P= 0.605				
Canary	Yes	3	0	3
	No	118	51	169
	Total	121	51	172
χ^2 =1.287 df =1 P= 0.257				
Owl	Yes	1	0	1
	No	120	51	171
	Total	121	51	172
χ^2 =0.424 df =1 P= 0.515				
Commercial poultry	Yes	6	3	9
	No	115	48	163
	Total	121	51	172
χ^2 =0.062 df =1 P= 0.804				

Geese	Yes	1	0	1
	No	120	51	171
	Total	121	51	172

χ^2 =0.424 df =1 P= 0.515

Appendix III: Association between other animals and detection of H5N2 antibodies

Animal		Result negative	Result positive	Total
Sheep	Yes	47	27	74
	No	74	24	98
	Total	121	51	172
χ^2 =2.909 df =1 P= 0.088				
Dog	Yes	26	10	36
	No	95	41	136
	Total	121	51	172
χ^2 =0.077 df =1 P= 0.782				
Goat	Yes	65	28	93
	No	56	23	79
	Total	121	51	172
χ^2 =0.020 df =1 P= 0.887				
Cattle	Yes	33	17	50
	No	88	34	122
	Total	121	51	172
χ^2 =0.639 df =1 P= 0.424				
Pig	Yes	3	0	3
	No	118	51	169
	Total	121	51	172
χ^2 =1.287 df =1 P= 0.257				
Rabbit	Yes	4	0	4
	No	117	51	168
	Total	121	51	172
χ^2 =1.726 df =1 P= 0.189				
Guinea pig	Yes	3	1	4
	No	118	50	168
	Total	121	51	172
χ^2 =0.042 df =1 P= 0.837				

Fish	Yes	1	0	1
	No	120	51	171
	Total	121	51	172
χ^2 =0.424 df =1 P= 0.515				
Cat	Yes	13	4	17
	No	108	47	155
	Total	121	51	172
χ^2 =0.339 df =1 P= 0.560				
Horse	Yes	1	0	1
	No	120	51	171
	Total	121	51	172
χ^2 =0.424 df =1 P= 0.515				