STUDIES ON PREVALENCE OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) IN NORTHERN PART OF KADUNA STATE

BY

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(MSc/VET-MED/01182/2006-2007)

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APRIL, 2010

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN VETERINARY MEDICINE

DEPARTMENT OF VETERINARY SURGERY AND MEDICINE,
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APRIL, 2010

DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Surgery and Medicine under the supervison of Professor P.A. Abdu, Drs M. Bello and L. Sa'idu. 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.

_____ _____

Aye Langzad Adamu Date

CERTIFICATION

This thesis titled "**STUDIES ON PREVALENCE OF HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) IN NORTHERN PART OF KADUNA STATE "** by Aye, Langzad Adamu meets the regulations governing the award of the degree of Maser of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literacy presentation.

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DEDICATION

This work is dedicated to my parents, Madaki Aye Bargo and Matta Iko Aye, my wife, Sarah and our children, Victoria, Victor, Tabat and Rita.

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ABSTRACT

The study assessed the incidence, mortality and financial losses during the 2006 – 2007 Highly Pathogenic Aavian Influenza (H5N1) poultry outbreaks in Kaduna State; determined H5 antibodies prevalence among poultry in live bird markets, biosecurity practices and infrastructure in these markets through the use of outbreak data, haemagglutination inhibition test and structured questionnaires. Of the 128 farms involved in HPAI (H5N1) outbreaks, 85% were commercial farms with a mortality rate of 53.18 % and 113,151 (48.04%) poultry were depopulated. Sixty-six per cent of outbreak cases were reported after five days of onset with 92 % reported after all the birds were dead. Eighty-eight per cent of the farms did not seek veterinary advice, with 7.8 % of the farms were keeping multi-aged or multi-species poultry together. Farmers practiced borrowing of equipment and egg crates from other farms. The total financial losses during the epidemics was 984,500,272**.**00 Naira. Six of the seven live bird markets sampled had positive for H5 antibodies with Makarfi having the highest prevalence of 18.18 %. Poultry sampled had an overall prevalence of 7.84 % with pigeons having the highest prevalence of 18.18 %. Only 15.19 % of the live bird market studied had pipe borne water and all markets were located around residential areas and were not fenced. Among marketers, 98.73% and 88.73% respectively, do not seprate poultry by age or species with 41.77 % keeping poultry with rabbits. Most poultry processors do not wear protective clothing and engaged in risky behaviours. Poultry offal was eaten by 97.5 % of respondents. Most marketers trade in sick birds and throw away dead poultry.About seventy respondents (69.62 %) reported that the markets were not decontaminated with 63.29 % of respondents willing to disclose HPAI (H5N1) outbreak. There is the need for

a virological investigation of avian influenza virus in these live bird markets. Farmers and live bird marketers need education on the importance of enforcing biosecurity measures in farms and markets. Government should improve on the infrastructure in live bird markets in the State.

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LIST OF ABBREVIATIONS

AI	Avian Influenza
AGID	Agar Gel Immuno Diffusion
AICP	Avian Influenza Control Programme
ANOVA	Analysis of Variance
BBC	British Broadcasting Corporation
CDC	Centers for Disease Control and Prevention
CERDA	Center for Extension Research and Development
DIVA	Differenciating Infected from Vaccinated Animals
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organisation
FDLPC	Federal Department of Livestock and Pests Control Services
H	Haemagglutinin-
HAU	Haemagglutination Unit
HPAI	Highly Pathogenic Avian Influenza
HIT	Haemagglutination Inhibition Test
KDSG	Kaduna State Government
LBM	Live Bird Market
LBMs	Live Bird Markets
LBMS	Live Bird Marketing System
LGA	Local Government Area
LPAI	Low Pathogenic Avian Influenza
M	Matrix

N	Neuraminidase
NADIS	National Animal Disease Information System
NDV	Newcastle Disease Vaccine
NS	Non Structural
NVRI	National Veterinary Research Institute
OIE	Office Internationale des Epizooties
P	Prevalence
PACE	Pan African Programme for the Control of Epizootics
PBS	Phosphate Buffered Saline
RNA	Ribonucleic Acid
RBC	Red Blood Cells
RT-LAMP	Reversre Transcriptase Loop Mediated Isothermal Amplification
RRT-PCR	Reverse Transcriptase Polymarase Chain Reaction
SPSS	Statistical Package for Social Science
UNDP	United Nations Development Programme
USA	United States of America
USAID	United States Agency for International Development
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Avian influenza (AI) is a viral disease that is caused by type A influenza viruses which are members of the *Orthomyxoviridae* family. These viruses could be transmitted by aerosol contaminated dust inhalation or the ingestion of contaminated food, water, infected carcasses and contact with open wounds (Adene *et al.,* 2006).

Almost all avian species of all ages are susceptible to AI. The disease is recognized in two forms, a highly pathogenic form (HPAI) and a low pathogenic avian influenza (LPAI). Depending on the virus isolate, viral dose, species and age of birds, AI has an incubation period of 3 to 7 days (Abdu *et al.,* 2005; Hansen, 2005). The disease was initially reported in Italy in 1878 by Perroncito (Bankowski and Samadieh, 1981; Easterday *et al.,* 1997). It came into public health attention when a severe disease in poultry caused clinical disease in 18 people with six deaths in Hong Kong in 1997 (Morris and Jackson, 2005).

Human deaths associated with HPAI appear to have resulted from direct exposure to infected birds on poultry farms and live bird markets (Mc Mullin, 2004). Movement of poultry around has greatly aided in the spread of H5N1 infection, therefore LBMs play a crucial role in the maintainance and disemination of avian influenza virus in countries where birds are sold live to consumers (Morris and Jackson, 2005).

Poultry farmers often manage their financial risk when faced with deaths in birds by employing delayed reporting and disporsing the birds before they die to marketers where they end up in LBMs and aiding in the wide spread of diseases before control measures can be implemented(Morris and Jackson, 2005).

Both the virulence and infectivity of the H5N1 virus strains have varied ovtime as a sequence new genotypes has emerged and then each dominant subtype has been replaced by others with diffrent characteristics (Morris and Jackson, 2005). Since the emergence of H5N1, all strains have exhibited high virulence for chickens and some recent strains have shown high virulence for humans. Early strains had shown variable, but generally higher virulence (Morris and Jackson, 2005).

Infectivity of early strains appears to have been low to modrate, but more recent infectivity has been higher as measured by the rate of transmission between hosts. This is likely due to changes in the relative importance of different excretion routes and the total quantity of virus excreted by an individual, but may also be influenced by factorvs like greater involvement of some species in the infection process than previously and by undergoing evolution and adaptation (Choi *et al*., 2004).

Following the initial emergence of H5N1, the virus then began to evolve into a range of genotypes within the H5N1 group, which differed in some of their characteristics. Influenza viruses evolve much more rapidly in spill over hosts such chikens and turkeys than in reservoir hosts such as wild water birds (Suarez, 2000).

The evolutionary process for H5N1 involved geese and most likely domestic ducks, quails and possibly some other species as well, with exchange of infection between the species and reassortment of parts of the virus genome to produce various new genotypes (Webster and Hulse, 2004).

1.2 Statement of the Research Problem

Kaduna State has an estimated poultry population of 2,821,092 with about 90% being local poultry (FDLPCS, 2003). The HPAI outbreaks in Kaduna state devastated the poultry Industry through mass deaths, mass slaughter and the destruction of poultry and poultry products.

The disease reduces growth rate and egg production, has high morbidity and mortality with a high cost of control and eradication (FAO, 2006). Production losses from HPAI results in scarcities of poultry and poultry products, prompting exhorbitant costs of poultry products. There are economic losses to poultry farmers, farm staff, livestock health workers, households, government and society in general (Capua and Marangon, 2003). Besides threats to livelihood, societal economic growth and sustainable development, HPAI has serous zoonotic implication. The possibility of reassortment and rapid spread of the H5N1 virus posses great concerns of endemicity and developing into human pandemic with the possibility of human-to-human transmission and destroying millions of human lives as many Nigerians are in close contact with poultry and poultry products (Bello *et al.*, 2008).

Wetlands, lakes and ponds that attract migratory birds abound in Kaduna State where direct contact with domestic poultry could take place. Kaduna state shares common boundaries with Kano, Katsina, Zamfara, Niger, Nasarawa, Plateau, Bauchi States and the Federal Capital Territory where the disease has been reported and where illegal inter state trade in poultry and poultry products thrives with a high potential of the H5N1 virus spread.

The last HPAI outbreaks in Nigeria occurred in Katsina and Kano States, bordering Kaduna state in July 2008, necessitating the need for continued active surveillance and assessment of other epidemiological features of HPAI (H5N1) in Kaduna state (AICP, 2009b).

1.3 Justification of Research

The first reported HPAI (H5N1) outbreak in Nigeria occurred in Kaduna State (Adene *et al.,* 2006; NADIS INFO, 2006). The virus can cross species barriers to replicate in mammals and cause severe disease and is zoonotic (Subbrao *et al.,* 1998). The HPAI (H5N1) viruses could circulate in reservoirs, become endemic and reassort for better transmissibility even among human beings (Easterday *et al.,* 1997; Alexander, 1999). The HPAI outbreaks in Kaduna State had a negative impact on the State's economy (Abdu *et al.*, 2006; Ahmed, 2006; Ago, 2007). There is therefore, the need to restore poultry farmer's confidence for increased production to reactivate the poultry industry, create jobs, increase sources of income and much needed animal protein, considering the high human population growth rate of 2.87% per year relative to the slow growth rate of 1.6% per year for livestock (Ocholi *et al.,* 2006; UNDP, 2006). Live bird markets (LBMs) are

known to have played an important role in the spread of HPAI (H5N1) (Broor, 2005) and the virus has been shown to circulate among chickens and other poultry species in LBM (Katz, 2004; Bello *et al.*, 2008).

1.4 Aim and Obje4ctives of Research

The aim of the study was to determine the incidence and sero-prevalence of HPAI in poultry in live bird markets in Kaduna State.

1.5 Objectives of Research

The objectives of the study were to:

1. Study outbreaks of HPAI (H5N1) in Kaduna State.
2. Estimate the economic impact of HPAI (H5N1) in poultry in Kaduna State.
3. Determine the prevalence of antibodies to H5 virus in poultry in LBMs in northern Kaduna State.
4. Determine the biosecurity practices and infrastructure in LBMs in northern Kaduna State.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

Avian influenza (bird flu), fowl plague or highly pathogenic avian influenza (HPAI), is a highly contagious viral infection, primarily of domestic avian species and free-flying birds. It is caused by viruses of the *Orthomyzoviridae* family of the influenza A genus H5N1 (Alexander, 1999; Perkins and Swayne, 2001). The *Orthomyxoviridae* family contains three genera A, B and C but only influenza A viruses are known to naturally infect birds. Influenza B and C viruses are almost, always exclusively isolated from humans (Lombin, 2006; Gunter, 2007).

Although all avian species are susceptible to infection with influenza A viruses, many wild species could carry the infection without portraying obvious clinical signs of disease (Abdu *et al.,* 2006). Chickens, turkeys and quails are highly susceptible; guinea fowls and pheasants are also susceptible while ducks are commonly asymptomatic carriers of the virus and serve as reservoirs of infection (Alexander, 2000; Hansen, 2005; Juthatip *et al*., 2008).

Avian influenza manifests in two forms, a mild form referred to as low pathogenic avian influenza (LPAI) and a rare, but highly fatal form described as highly pathogenic avian influenza (HPAI) (Alexander, 2000). The later form was first identified in Italy in 1878, though AI has worldwide occurrence (WHO, 2006a; World Bank, 2008).

Avian influenza drew public health interest worldwide with the first documented human case of infection with the virus in Hong Kong in 1997 during the HPAI (H5N1) epidemic in birds that also affected 18 humans out of which 6 died (WHO, 2005; Morris and Jackson, 2005). The virus was shown to have been transferred from birds to humans (WHO, 2006b). The H7N7 virus strain also caused mild disease in 83 people and the death of a veterinarian in the Netherlands in similar circumstances in 2003 (WHO, 2005).

The HPAI emerged in South East Asia in 2003 and has spread across Asia, Europe and Africa (Ocholi *et al.*, 2006). The disease killed over 220 million birds worldwide through both infection and culling to contain and prevent further spread of the H5N1 virus (USAID, 2008).

2.2 History of Highly Pathogenic Avian Influenza

Highly pathogenic avian influenza had spread across international borders by 1997 and was internationally recognized due to the deaths of 6 out of 18 clinically affected people in Hong Kong- China (Morris and Jackson, 2005).

Prior to the first outbreak in Nigeria, there has been no evidence to suggest the presence of HPAI (H5N1) virus in the country (Adene *et al*., 2006). However, a preliminary survey for antibodies against some selected viruses that affect rural chickens of different ages, conducted in Borno State, Nigeria reported influenza A antibodies (El-Yuguda and Baba, 2002).

Other African countries with confirmed cases of HPAI outbreaks include, Egypt, Sudan, Burkina Faso, Togo, Niger Republic, Cote de Voire, Djibouti, Cameroon, Ghana and Benin Republic (FAO, 2007).

2.3 Aetiology of Highly Pathogenic Avian Influenza

Highly pathogenic avian influenza is caused by a few of the type A influenza viruses (Hansen, 2005). The influenza A (H5N1) viruses are pleomorphic, filamentous, or spherical (80-100 nm in diameter), segmented, single – stranded RNA, enveloped and of negative polarity (Alexander, 1995; Abdu *et al*., 2005; Hansen, 2005). The HPAI (H5N1) viruses have multiple basic amino acids at the HAO cleavage sites, cleavable by ubiquitous proteases that occur through out host tissues (Abdu *et al.,* 2006; Kumbish *et al.,* 2006).

The classification of influenza A viruses into subtypes A, B and C is on the basis of differences in their antigenic nucleocapsid and matrix (M) proteins, haemagglutinin (HA) and neuraminidase (NA) (Fouchier *et al.,* 2005; Abdu *et al.,* 2006). These antigenic proteins are the basis for subtyping the influenza A viruses into the presently identified sixteen H (H1 – 16) and nine N (N1 – 9) subtypes. Each influenza A virus has one HA and one NA antigen in any combination (Fouchier *et al.,* 2005; Benedictis *et al.,* 2007).

The haemagglutinin surface glycoprotein projections (spikes) on the viral envelope aid in biding to oligosaccharide cellular receptors of neuraminic acid on host cell surfaces, for subsequent fusion and cell entry (Easterday *et al*., 1997). The matrix (M) protein is involved in assembly and budding while the neuraminidase exerts a sialolytic enzymatic action of cleaving the host sialic acid receptors, liberating virus progeny captured on

surfaces of infected cells (Metselaar and Simpson, 1982; Yuen and Wong, 2005). Neuraminidase also converts the mucus on cell surfaces into a less viscous fluid, preventing self aggregation of progeny virions and thereby promoting the spread of progeny virions released, over a larger area of the mucosa (Metselaar and Simpson, 1982). The influenza A (H5N1) virus codes for 11 structural and non-structural proteins (NSI) (Bjorn *et al.,* 2006; Malin *et al.,* 2007). The non-structural protein is a virulent factor that inhibits the synthesis of virus-induced type 1 interferon, an innate response of infected host cells (George *et al.,* 2007; Malin *et al.,* 2007).

The segmentation of the influenza A (H5N1) virus confers on it, a high capacity for reassortment by segment swapping (antigenic shift) as well as mutation within genome segments (antigenic drift) in concurrent viral infections (Swayne *et al.,* 1997; Trevor *et al.,* 2002; Suarez, 2003). These reassortments produce novel viruses with enhanced transmissibility (Alexander, 1999; Webster and Hulse, 2004; Hansen, 2005; Yuen and Wong, 2005).

2.4 Host Range of Highly Pathogenic Avian Influenza

Avian influenza A viruses are able to infect a wide range of hosts including humans, mammals and birds (Lombin, 2006). The HPAI viruses cause severe disease in humans (Alexander, 1995). All avian species of any age are susceptible to avian influenza A (H5N1) virus infection, though with varying degree of clinical disease (Suarez *et al*., 2003; Hansen, 2006). While domestic poultry and turkeys suffer severe, fulminating systemic clinical disease, duck and migratory sea birds could carry the infection and continue to shed the virus in faeces without portraying apparent signs of disease and

hereby serving as reservoirs of infection (Haydon *et al.,* 2002; Abdu *et al.,* 2006; Ezealor, 2006; Kumbish *et al.,* 2006; Juthatip *et al.,* 2008).

The H5N1 viruses infect dogs, horses, cats, swine and human beings and thus are zoonotic with serious consequences (Brown *et al.,* 1997; Trevor *et al.,* 2002). Interspecies infection with the H5N1 viruses occurs, through the re-shaping of the HA protein-binding units (Li *et al.,* 1990; Swayne *et al.,* 1997; Alexander, 1999; Banks *et al.,* 2001; Shane, 2002).

The cells of respiratory and gastrointestinal tract mucosa of swine, quails and human beings have, the alpha 2,6 and alpha 2,3-sialic acid receptors of the swine and avian influenza A (H5N1) virus strains (Beard and Webster, 1991). These species therefore, serve as potential "mixing vehicle" for virus reassortment in concurrent viral infections (Banks *et al.,* 2001). The AI virus is reported to prefer human cells (Beare and Webster, 1991; Skehel, 2006). Virus reassortment and possible transmission to humans, favours a potential human influenza pandemic (Larson, 1998; Katz *et al*., 1999; Nguyen *et al.,* 2004).

2.5 Geographical Distribution of Avian Influenza

Avian influenza viruses may probably be ubiquitous in wild aquatic birds. Pathogenic strains could emerge and cause disease in domestic poultry at anytime, in any country, without prior warning. Outbreaks have occurred at irregular intervals in all continents. The most serious outbreaks of recent, have been reported in Hong Kong 1997-1998 and 2003, Chile 2002, The Netherlands 2003 and South East Asia 2004 – 2006, Nigeria 2006 - 2008 (FAO, 2008), Nigeria, 2006 – 2008 (AICP, 2009a).

2.6 Epidemiology of Avian Influenza

The immediate source of infection for domestic poultry is hardly ascertained, however, most outbreaks probably start from direct or indirect contact of domestic poultry with aquatic birds (FAO, 2008). Many of the strains of the virus circulating in wild birds are either non pathogenic or mildly pathogenic for poultry (FAO, 2008). A virulent strain may however, emerge either by genetic mutation or by reassortment of less virulent strains. The former mechanism is supported by scientific evidence to have occurred in the Eastern part of the United States of America in 1983 – 1987 (FAO, 2008).

Only domestic poultry are known to have played a role in the AI transmission cycle from animals to humans (Alexander, 2007). Wild birds are primarily natural reservoir for influenza A viruses and are often the vector that introduces new outbreaks into domestic flocks. The AI virus can be highly contagious in domestic poultry which lack the resistance that exists in wild birds. Once present in domestic flocks, human activity becomes a risk for virus transmission (Obayelu, 2007).

Air-borne transmission could occur where birds are in close proximity and with appropriate air movement (FAO, 2008). Birds get readily infected via instillation of the virus into the conjunctival sac, nares or the trachea (FAO, 2007). Preliminary field and laboratory evidence indicates the 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 of the virus is however, unresolved, though it is unlikely that infected embryos could survive and hatch. Attempts to hatch eggs in disease isolation cabinets

from a broiler breeder flock at the height of disease was unsuccessful in yielding AI-infected chicks (FAO, 2008). This however, does not mean that broken contaminated eggs could not be the source of virus to infect chicks after hatching in the same incubator (FAO, 2008).

Humans are infected with AI virus through direct contact with bird feaces, respiratory droplets and by mechanical transfer through contact with contaminated formites, but certainly not through eating of chicken cooked at high temperature (WHO, 2004a). Birds infected with avian flu shed the flu virus in feaces, saliva and mucus and thus other birds become infected by eating, drinking or inhaling the virus. Wild migratory aquatic fowl can acquire HPAI infection without signs of clinical disease and spread this to domestic flocks (Abdu *et al*., 2005).

The virus can also be carried on the bodies and feet of animals such as rodents and reptiles (WHO, 2004b). In a food handling and preparation setting, the concern is that the AI virus could be transmitted from uncooked birds or bird products. Avian influenza can contaminate eggs and poultry meat (frozen and/or commercially packaged). The HPAI virus can survive in carcass and blood for as long as 3 weeks (WHO, 2004b). Broken contaminated eggs in incubators infect healthy chicks and garbage flies have also been implicated in the spread of the AI virus within and between flocks (Beard, 1998).

Trade in poultry appears to have been the predominant means of spread of the AI virus in Africa (FAO, 2006). Nigeria became the first African country to have experienced outbreaks of the H5N1 in poultry in February 2006. While some study tends to show that three sub-lineages of the virus were independently introduced into Nigeria through

migratory birds paths, some other study suggests that independent trade imports could have been the source of spread (Ducatez *et al.,* 2006). The H5N1 has been shown to move from poultry to migratory birds and back in a "relay transmission" thus may account for some of the continuing geographical spread (WHO, 2006a).

The natural reservoirs for influenza A viruses are known to be wild water fowls and shorebirds. Surveillance study in these birds in North America showed that the influenza A viruses are recovered repeatedly from them. The virus recovery is however, dependent on species of birds. Live-bird marketing system (LBMS) in some regions of the Untied States in addition to the natural reservoir, has been recognized as serving as a man-made reservoir of influenza viruses and has been linked to HPAI outbreaks in poultry (Senne *et al.,* 2006). Swine appear to have a very important role in the epidemiology of turkey's infection with the swine influenza virus when they are in close proximity (FAO, 2008).

2.7 Clinical Signs of Avian Influenza

The incubation period of AI varies from 3 – 7 days depending on the isolate, the dose of the inoculum, species and age of birds and concurrent infections, it may however, be up to 21 days (Abdu *et al.,* 2005; Hansen, 2005; FAO, 2008).

The clinical signs of AI vary from sudden onset with little or no apparent signs, to severe respiratory, enteric and nervous disease (Alexander, 2000). Other signs include swelling of the head and neck, cyanosis of the comb, wattles and non-feathered areas of the skin, a rapid spread and mass mortality that could be 100 % (Abdu *et al.,* 2006; Adene *et al*., 2006; Kumbish *et al.,* 2006).

In broilers, the signs of the disease are frequently less obvious, with severe depression, inappetance and a marked increase in mortality being the first abnormality to be observed. Oedema of the face and neck, torticolis and ataxia may also be seen. In turkeys, the disease is similar to that seen in layers, but it lasts 2 – 3 days longer and is occasionally accompanied by swollen sinuses. In the domestic geese, the signs of depression, inappetance and diarrhoea are similar to those in layers, though with frequent swelling of sinuses (FAO, 2008; Wakawa *et al*., 2008).

Generally, clinical signs observed affect the respiratory, enteric and reproductive systems. Some infected poultry died without apparent signs, or with minimal signs of depression, anorexia, abnormal flock silence, huddling, ruffled feathers and fever. Prominent clinical signs observed in most outbreaks were oedematous and cyanotic comb, wattles and non-feathered areas of the skin (Easterday *et al*., 1997). There is usually dypsnoea, sneezing, coughing with nasal discharges. Other signs include sinusitis with mucoid occulo-nasal discharges, in addition to profused, greenish diarrhoea and prominent discolouration of the shanks and feet. Soft –shelled eggs were initially layed by hens, followed by a sudden reduction in egg production, and then was severe difficulty in breathing, thus birds had stretched necks, or heads rested on litter with open mouths. Some of the birds at later days showed neurological signs of ataxia, torticolis and convulsion (Easterday *et al*., 1997; Adene *et al*., 2006; Wakawa *et al*., 2008).

2.8 Gross Lesions of Highly Pathogenic Avian Influenza

Gross lesions of HPAI at postmortem examination varied with systems and organs involved. Birds that died of per acute form of HPAI, showed minimum gross pathologic

changes, predominantly congestion of the viscera and muscles and signs of dehydration (Easterday *et al*., 1997). The larynx and trachea were haemorrhagic or congested with mucoid exudates with cloudy or darkened air sacs (Easterday *et al*., 1997; Adene *et al*., 2006; Wakawa *et al*., 2008). Ecchymotic or petechial haemorrhages of the epicardial fat and the proventricular junction, extensive subcutaneous haemorrhage around the entire breast muscles and some featherless parts of the body are other gross lesions (Easterday *et al*., 1997; Abdu *et al*., 2005; Adene et al., 2006).

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Petechial and ecchymotic haemorrhages of the abdominal fat and serosal surface of the intestines are usually present with enlarged, friable liver and spleen usually having grey or yellow necrotic foci (Easterday *et al*., 1997; Adene *et al*.,2006). Kidneys are usually congested and swollen, while the ovarian follicles are regressed and some ovaries are necrotic with presence of shell-less eggs usually in the oviduct (Easterday *et al*., 1997; Adene *et al*., 2006).

2.9 Diagnosis of Avian Influenza

2.9.1 Rapid test

Rapid tests have been developed for field detection of influenza type A antigen and antibodies (Edan *et al.*, 2003) and they include: Directigen, Flu Detect and ELISA.

2.9.1.1 *Directigen^{TM} flu A+B test to detect antigens*

Directigen test is a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral antigen from nasopharyngeal washes and aspirates. The test is highly sensitive and can be used to distinguish influenza A antigen from the

viral antigen of influenza B in one test. The kit is however, very expensive (Edan *et al.*, 2003).

2.9.1.2 *Flu Detect^{TM} avian influenza antigen test*

Flu Detect test is used for detecting all the 16 subtypes of influenza viral antigens in 15 minutes. It can be used both in the field or the laboratory. The test has high accuracy (Edan *et al.*, 2003).

2.9.1.3 *ELISA kit by IDEXX*

The ELISA test kit is used for detecting antibodies against the type A avian influenza viruses in suspected sera (Edan *et al.*, 2003). Viral antigens at the bottom of wells and incubated for a period during the antigen react with the antibodies against the avian influenza virus in the suspected sera. The sensitivity of this test however, is quite low and could show false negative results and therefore, results of this test should always be combined with epidemiological and clinical data and other laboratory tests for confirmation (Edan *et al.*, 2003).

2.9.2 Gene sequence detection and analysis

Specific primers for H and N types can be used for RT – PCR and rRT – PCR, though this does not provide fine details. Further genetic analysis requires access to a DNA sequencer. This procedure enables the 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 relationships of the virus to be established (FAO, 2004). New tests have been developed such as reverse

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

2.9.3 Serology

2.9.3.1 *Haemagglutination inhibition (HI) test*

The HI test is the subtype – specific test recommended for AI (OIE, 2009). It is sensitive and specific when an epidemiologically appropriate antigen is used. It can be used to monitor antibodies response to vaccination and where birds survive infection and to monitor circulation of influenza virus (FAO, 2004).

2.9.3.2 *Agar gel immunodiffusion (AGID) test*

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).

2.9.3.3 *Competitive ELISA using Group Antigen*

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

2.9.3.4 *Differentiating infected from vaccinated animals (DIVA) System*

Antibody detection using immunofluorescence

This test uses cells infected with a vaculovirus 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).

Antibody detection using inhibition of neuraminidase

The Neuraminidase test is essentially a biochemical assay to identify the AI Neuraminidase type of isolates and also to characterise antibody infected birds. The test requires expertise and usually conducted at OIE reference laboratory (FAO, 2004).

2.9.4 Virus isolation and characterisation

Avian influenza viruses can be readily isolated from the tracheal and cloacal swabs. They grow well in the allantoic sac of embryonating chicken eggs and agglutinate red blood cells. The haemagglutination is not inhibited by Newscatle 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).

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

Neuraminidase (N) typing is carried out on allantoic fluid when haemagglunating 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 of Highly Pathogenic Avian Influenza

Highly pathogenic avian influenza must be differentiated from other respiratory diseases or causes of decrease in egg production to enhance recognition and control of the disease. Diseases that need differentiating from HPAI include: infectious bronchitis, infectious laryngotrachitis, lentogenic Newcastle disease. Mycoplasmosis, infectious coryza, ornithobacteriosis, turkey coryza, the respiratory form of fowl cholera and aspergillosis (Swayne, 2005). Highly pathogenic avian influenza must also 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).

2.11 Prevention and Control of Highly Pathogenic Avian Influenza

Avian influenza virus is highly contagious and easily spread. The commonest method of control of AI is the culling and depopulation of the infected flocks, quarantining of the affected areas until the disease is no longer present (OIE, 2009). Vaccination is possible and has been tried, though it is not widely considered a viable control method (Obayelu, 2007).

After depopulation of infected flocks, the buildings and equipments are vigorously decontaminated by disinfection, the litter should be collected and buried in deep pits before new birds are brought in, this process takes several weeks to accomplish. The virus can be killed by common disinfectants or heat. It is recommended that heat of 76^0C be applied for cooking chicken, 82^0C for turkey dark meat, 74^0C for ground chicken and

turkey and 71^0C for eggs (WHO, 2004a). 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 need for implementation of biosecurity measures which reduce human exposure to potentially infective birds, litter, feathers, dust and equipment (Obayelu, 2007).

Vaccination prevents clinical signs and death, reduce viral replication and shedding from the gastrointestinal tract. Specific protection is achieved through the use of autogeneous virus vaccines or from vaccines prepared from AI virus of the same haemagglutinin subtype. Antibodies to the viral neuraminidase may provide some protection (Swayne, 2005). The three types of vaccines that have been used to control AI are inactivated homologous or heterologous vaccines or recombinant vaccines (Durosinlorun, 2008).

2.11.1 Inactivated homologous vaccines

The vaccines were originally prepared as "autogeneous" vaccines containing the same AI virus strain as the one causing the problem in the field. They have been used extensively in Mexico and Pakistan during AI epidemics (Swayne, 2005).

The efficacy of these vaccines in preventing clinical disease and in reducing the amount of virus shed in the environment has been proved in field and experimental trials (Swayne, 2005). The disadvantage of this is the inability to differentiate vaccinated from field–exposed birds, unless vaccinated sentinels are kept in the shed (Capua and Marangon, 2003).

2.11.2 Inactivated heterologous vaccines

The vaccines are manufactured similar to the inactivated heterologous vaccines. They differ in the fact that the virus strain used in the heterologous vaccine is the same haemagglutinin subtype as the field virus, but has heterologous neuraminidase (Capua *et al.,* 2000).

2.11.3 Recombinant vaccines

Several recombinant fowl pox viruses expressing the H antigen have been developed and one has been licensed and is in use in Mexico (Beard *et al.*,1992; Webster *et al.,* 1996; Swayne *et al.,* 1997; Swayne *et al*., 2000).

Experimental data for fowl pox recombinants expressing the H7 antigen has also been obtained (Boyle *et al.,* 2000). Other vectors have been used successfully to deliver the H5 and H7 antigens, using the infectious laryngotrachitis virus (Luschow *et al.,* 2001; WHO, 2006a). Only in Mexico, has field experience with a recombinant virus to control AI been obtained, where a LPAI H5N2 virus exist (Villarreal–Chavez and Rivera Cruz, 2003).

2.12 Highly Pathogenic Avian Influenza Control in Kaduna State

With the report of the first case of HPAI outbreak, a State Bird Flu Response Committee was formed with the Permanent Secretary, Ministry of Health as Chairman and the Director of Veterinary Services Ministry of Agriculture as Secretary. Similar Committees were formed at the zonal and LGAs level, comprising of the Zonal Veterinary Officer, all the Area Veterinary Officers, the Local Government Veterinary Unit Heads and all livestock Superintendents. The zonal team was supplied with personal protective equipment (PPE), disinfectants and sprayers and was the first to investigate any reported

suspected case of HPAI outbreak, in collaboration with the Kaduna State Desk Officer on Avian Influenza Control and a Federal Government team from the Federal Department of Livestock and Pest Control Services, Abuja.

When a case of suspected avian influenza outbreak was reported, the zonal team informed the Director of Veterinary Services and the Federal team. These teams mobilized with their PPE, disinfectants and other logistics, based on the WHO (2006b) recommendations and went to the site to investigate. All necessary precautions such as wearing protective equipments were taken on visit to each site of reported suspected HPAI outbreak. Each site was inspected for and questions asked on biosecurity like fencing, cleaning and disinfection, ownership of fumigation materials, footbaths, human traffic and general management practices.

Affected birds at each site were visually and physically inspected for signs of the disease. About five apparently sick birds and five dead birds were collected in leather bags into ice – packed coleman boxes and sent to the Viral Diagnostic Laboratory of the NVRI, Vom, Nigeria for postmortem examination and laboratory diagnosis. Meanwhile a 12 page HPAI epidemiological form was issued to and filled in by the owners of the affected farms for the collection of necessary data.

This epidemiological form required information on the type and location of farm, number of birds, breed and spieces, type and location of hatchery, source of birds, age, debeaking operations, housing system, other birds and animal species (free, domestic or captive). Other information required in the form included access of farm to other birds, movement

of birds, eggs, humans, vehicles, new additions, equipment borrowing and relationship of farm to other farms around.

Other necessary information required included records of history of disease, mortalities on the farm, vaccinations and treatments. The decision to depopulate a poultry farm was based on several factors that included the sudden onset, high morbidity, rapid spread, mortality exceeding 50% within hours or two days, deaths of other birds on the farm, cases of mortality in chickens in nearby farms within the area and within the period. Other indicators or factors considered before depopulation of a poultry farm, were observed clinical signs, the HPAI disease having been declared in the state and a positive diagnosis report received from the Viral Diagnostic Laboratory, NVRI, Vom (FDLPCS, 2006).

Whenever three or more of the above factors like sudden onset of disease, high morbidity, rapid spread, mortality exceeding 50% within hours and observed clinical signs were true, a tentative diagnosis of HPAI H5N1 was made and a decision to depopulate the stock, decontaminate a farm and pay compensation was taken. Some of the suspected farms were quarantined till laboratory results were obtained, or until more of the above mentioned factors were “true”, that such farms were depopulated and treated accordingly.

Depopulation procedures took place between two and seven days after reporting, while fumigation and decontamination of premises and sites were conducted immediately or after up to five days of depopulation. Dead and culled birds were disposed in pits at burial site of each outbreak. The burial pits were three meters wide and three meters deep

for between 300 – 2,000 birds. Dead and euthanized birds were packed in polythene bags, placed into the pits and burnt first before burial. Surfaces and floors were cleared of organic material and litter and buried in deep pits.

There was constant monitoring of all people in contact with the affected birds or farms for evidence of any respiratory disease. Blood samples from Veterinary Doctors and other personnel involved in the containment of the HPAI in the State were screened for antibodies against the H5N1 virus by a team of Veterinary and Medical experts from CDC, Atlanta, USA, who visited the State during the period of the outbreaks.

No human case was recorded in the screening exercise, apparently because of the enlightenment campaign in the State on the dangers of HPAI, the use of PPE by those involved in the handling of the outbreaks and the prompt and constant decontamination of the sites of outbreaks. Depopulation of birds was mainly by slaughter and packaging in polythene bags. Burning in deep pits covering with soil, followed by fumigation of the farm, equipment, materials, clothes and vehicle were conducted before leaving the site.

Further fumigation with DISKOL® (containing Benzalkonium chloride 5%, Glutaraldehyde 7.5%, Formaldehyde 7.5%, stabilizers and antioxidants) of the affected sites was conducted on a weekly interval after depopulation for four weeks.. The dilution of the disinfectant was at the rate of 4 ml per litre of water for empty sheds, using backpacked napsack sprayers.

The control measures taken were as recommended by the "HPAI Standard Procedure" of February 2006, issued by the Federal Ministry of Agriculture and Rural Development, in conjunction with the National Animal Disease Information Systems (NADIS) and the Pan African Programme for the Control of Epizootics (PACE), Abuja, Nigeria.

2.13 Public Health Significance of Highly Pathogenic Avian Influenza

Avian influenza viruses exhibit host adaptation and rarely infect humans, usually as isolated individual cases of human infection do occur without human – to human transmission. Risk factor for human infection in the 1997 HPAI outbreak in Hong Kong was direct contact with infected poultry and not handling, cooking or consumption of poultry meat (CDC, 2008). The HPAI (H5N1) strain infected poultry and wild birds in nine countries in Asia in 2004 in which 37 human cases were confirmed with a case fatality rate of 68% in Thailand and Vietnam (Swayne, 2005).

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

There is little pre-existing natural immunity to the H5N1 virus in human population. Should the H5N1 viruses gain the ability for efficient and a sustained transmission among humans, an influenza pandemic could result with a potential of high rates of ill health and death world wide (Katz, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area were seven Local Government Areas (LGAs) of Kaduna State; Giwa, Makarfi, Sabon gari, Soba, Ikara, Kubau and Lere. Kaduna State is made up of 23 LGAs and is about 5,100 square kilometers in size, with a land area of about 4.4 million hectares out of which about 2.0 million hectares are arable land. Kaduna State is located in the North Western region of Nigeria and lies between latitudes $8^0$2'and $11^0$32' North of the Equator and Longitudes $6^0$15'and 8^0 6' East of the Greenwich Meridian. The State is in the Northern Guinea Savannah zone of Nigeria and has two marked seasons of a hot (wet) rainy season (April to early November) with the rain peaking between June and October. The dry season begins from November, with a cold spell in December through January and extends to April, the hottest month (KDSG, 2008).

There is great variation in the wet season as from the North to the South, with an average of five months of rainfall. The southern part has a heavier rainfall than the North. The mean annual temperature is 34^0C, the hottest month being March – April (40^0C) and the coldest period is between December and January (13.2^0C). Rainfall varies between 1,000 mm and 1,500 mm and lasts for about 150-200 days (KDSG, 2008).

The major occupation of the people of Kaduna State is agriculture, producing food and cash crops and raring livestock. The estimated human population of Kaduna state according to the 2006 census is over 6,066,562 (KDSG, 2008).

3.2 Sample Size

The sample size of 238 was obtained using the prevalence of 19.2% earlier determined by Obi and Ahmed (2008) in a previous study in LBMs in states that reported AI outbreaks in Nigeria using the following formular N = $\frac{Z^2PQ}{D^2}$

Where N = Sample size

Z = The desired precision (1.96)

P = Prevalence (19.2%)

Q = 1 – prevalence

D = Allowable error (5%)

3.3 Data Collection

Records of HPAI (H5N1) outbreaks in Kaduna State were obtained from the State Bird Flu Response Committee, the Federal Department of Livestock and Pest Control Services, the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria and the National Veterinary Research Institute, Vom. The data included the poultry species, number affected, number that died and the number depopulated.

Structured questionnaires were administered to consenting poultry marketers at LBMs. The questionnaire demanded to know the biosecurity measures employed in the LBMs, sources and destinations of live birds, knowledge of poultry diseases among poultry marketers and their readiness to disclose poultry disease outbreaks, with particular emphasis on AI. Other information obtained were prices of poultry and poultry products, trade or otherwise in sick birds, major species marketed and the means by which dead birds, offals, feathers and other poultry wastes are disposed (Appendix I).

3.4 Sample Collection from Live Bird Markets

During sample collection between April and May 2009, sample forms were used to record vital information such as serial number, date of sample collection, the LGA, LBM, town/village, owner of poultry, breed, species, type and sample collected (Appendix II).

Sera (306) from various poultry species from LBMs at Giwa (45), Makarfi (55), Sabon-gari (53), Tudun Saibu (57), Ikara (29), Anchau (33) and Yan kaji (34) were tested by the HI test. These included sera of 41 commercial chickens, 180 local chickens, 40 guinea fowls, 34 ducks and 11 pigeons (Table 4.4).

About 2 ml of blood was aseptically collected from the brachial vein of each bird, using a 21 gauge needle and a 5 ml syringe each, for each bird, after restrain by an assistant. The blood was carefully transferred into sterile test tubes, labeled with an acronym number, the place and date of collection were indicated. The blood samples were kept in a shade at room temperature to allow for clotting and then transported in a cold man box packed with ice packs to the Nutrition Laboratory of the Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria.

The blood samples were centrifuged at 447 g for 5 minutes, the sera obtained were transferred into sample bottles, kept at -20^0C until examined for H5 antibodies by the HI test.

3.5 Haemagglutination Inhibition Test

3.5.1 Avian influenza antigen and positive serum

Avian influenza H5 antigen and H5 positive serum were obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria.

3.5.2 Preparation of 1% red blood cells (RBCs) suspension

Blood was collected from five day old chicks and pooled in an equal volume of Alserver's anticoagulant solution. Cells were washed three times in Phosphate Buffered Saline (PBS) by centrifuging at 447 g for 5 minutes (Collee *et al*., 1982). Exactly 99 ml of PBS was added to 1 ml of the RBCs to make a 1% suspension.

3.5.3 Determination of type A H5 antigen titre

Haemagglutination (HA) test was carried out according to the method described by OIE (2009). About 50 ul of antigen suspension was placed into the first well of a microtitre plate. A further 25 ul of PBS was dispensed into the second to the ninth wells of the plates. A 25 ul of 1% chicken red blood cells was dispensed into each well. These were mixed by gently tapping the plate and then allowing the RBCs to settle for 40 minutes at room temperature. The HA was determined by tilting the plate and observing for the presence or absence of tear- shaped streaming of the RBC. The titration was read to the highest dilution giving complete HA (no streaming); this represented 1HA unit (HAU) and was calculated accurately from initial range of dilutions.

3.5.4 Haemagglutination inhibition test

The antibody titre in sera was determined by haemagglutination inhibition (HI) test (OIE, 2009). Fifty microlitre of sera was despenced into first well of a row on a microtitre plate.

Twenty five micro litres of PBS was dispensed into each well of the microtitre plate. Twenty five microlitre of serum was taken from the first well into the second well and mixed. Two-fold dilutions of the 25 ul volumes of the serum were made across the plate. Four HAU of the antigen suspension in 25 ul was added to each well and left for 30 minutes at room temperature. A 25 ul of chicken RBC was added to each well and after gentle mixing was allowed to settle for about 40 minutes at room temperature. The HI titre is the highest dilution of the serum causing complete inhibition of RBCs agglutination by the 4 HAU antigen. Wells considered positive to HI were those in which RBCs stream at the same rate as positive control wells and the results were expressed in $\log_2$.

3.6 Statistical Analysis

Data from records were summarized in a table while data generated on antibodies titres in sera were analyzed using analysis of variance (ANOVA) and expressed as mean, standard errors of the means ($X \pm SE$). Values of $P<0.05$ were considered significant. Data generated from questionnaire on LBMs were coded and frequency of response to each question were analysed by Chi square using the statistical package for social science (SPSS) version 15.

CHAPTER FOUR

4.0 RESULTS

4.1 Study of Highly Pathogenic Avian Influenza (H5N1) Outbreaks in Kaduna State

Reported HPAI (H5N1) outbreaks in Kaduna State were confirmed by NVRI, Vom, in Giwa, Sabon garin, Igabi, Kaduna North, Kaduna South and Chukun Local Government Areas.

One hundred and twenty eight farms were affected in the outbreaks. In these outbreaks, 121, 653 (51.7 %) of the 235, 487 birds died naturally of HPAI, while 113,151 (48.04 %) were depopulated by the HPAI control team (Table 4.1). Though about 85% of the farms were commercial, records of farm activities were either defective or lacking.

Fifty six (44%) of the cases were reported between one to five days after HPAI outbreak in the farm, 118 (92%), after birds have either started dying or have all died in the farms. The onset of the disease was sudden in 80% of the affected farms, recording a mortality rate of 53% within three days. Only 12% of the 128 farms involved in the outbreaks had Veterinarians attached to them for professional care and advice on poultry health management. The remaining 88% of the farms were self-managed with no veterinary consultation.

Ten (7.8 %) farms had multi-species, multi-aged birds housed together on the same premises, while three poultry farms had poultry with other animal species such as sheep, pigs or cattle on the same farm.

Four of the affected farms were owned by the same farmer, who used the same vehicles, feed stores, poultry houses, equipment and farm attendants to service all the four farms, despite the long distances (up to 4 km) between the farms.

Nine farms located several kilometers apart, recorded HPAI outbreak within an interval of one day. Farm attendants admitted to have visited friends at different poultry farms and exchanged gifts in poultry and poultry products. Egg dealers borrowed and used egg crates from different farms within and across state borders.

4.2. Estimated Financial and Economic Implications of Highly Pathogenic Avian Influenza Outbreaks in Kaduna State.

The financial and economic losses caused by the 2006 – 2007 HPAI H5N1 outbreaks in Kaduna State were enormous (Table 4.2).

Table 4.1: Incidence, mortality and depopulation of poultry during the 2006 and 2007 HPAI (H5N1) outbreaks in Kaduna State, Nigeria. †

Species	No. affected (%)	No. died (%)	No. depopulated (%)	Specie mortality rate (%)
Commercial chickens	232,390 (98.69	121,251 (99.67)	111,139 (98.22)	52.18
Local chickens*	808 (0.34)	258 (0.21)	350 (0.49)	31.93
Ostriches*	153 (0.07)	0 (0)	30 (0.03)	0.00
Geese*	115 (0.05)	34 (0.03)	61 (0.05)	29.57
Ducks*	717 (0.31)	0 (0)	264 (0.23)	0.00
Guinea fowls*	147 (0.06)	0 (0)	67 (0.06)	0.00
Pigeons	175 (0.07)	0 (0)	175 (0.16)	0.00
Parrots	44 (0.02)	0 (0)	44 (0.04)	0.00
Pheasants	12 (0.04)	0 (0)	5 (0.004)	0.00
Turkeys	882 (0.38)	110 (0.90)	772 (0.68)	12.47
Peacocks	3 (0.001)	0 (0)	3 (0.003)	0.00
Pigs	41 (0.02)	0 (0)	41 (0.04)	0.00
Total	**235,487(100%)**	**121,653(51.66%)**	**112,951(47.96%)**	**51.66**

*Some of the birds were hastily sold out before the investigating team visited the farms.
†Source of data: Kaduna State Government; Veterinary Teaching Hospital, ABU, Zaria; NVRI, Vom and FDLPCS.

Table 4.2: Financial losses during the 2006 and 2007 HPAI H5N1 outbreaks in Kaduna State.

Species	No. bird loss	No. bird sold	Cost/bird (N)	Losses due to death (N)	Cost/egg (N)	Annual egg production†	Losses due to eggs (N)	Total loss (N)
Commercial chicken	232,390	0	1,300	302,107,000	20	280	650,692,000	952,799,000
Local chickens	608	200	700	425,600	20	68	27,472	453,072
Ostriches	30	123	70,000	2,100,000	2,000	70	140,000	2,240,000
Geese	95	20	12,000	1,140,000	1,000	55	5,225,000	6,365,000
Ducks	264	453	850	224,400	15	35	138,600	363,000
Guinea fowls	67	80	675	45,225	20	115	154,100	199,325
Pigeons	175	0	175	30,625	NA	NA	NA	30,625
Parrots	44	0	12,000	528,000	NA	NA	NA	528,000
Pheasants	5	7	150	750	NA	NA	NA	750
Turkeys	882	0	3,500	3,087,000	150	250	16,537,500	19,624,500
Peacocks	3	0	13,000	39,000	500	80	80,000	119,000
Pigs	41	0	18,000	738,000	4,000	13	1,040,000	1,778,000
Loss	**234,604**	**883**		**310,465,600**			**674,034,672**	**984,500,272**

NA- Not applicable to the species; †- Assumed that half of affected birds were females.

4.3 Prevalence of H5 Subtype Antibodies among Poultry species in Live Bird Market in Kaduna State

The overall sero-prevalence rate of H5 antibodies in poultry in the area of study was 7.84 % (Table 4.3). Of the 98 sera from LGAs where HPAI has been reported, 10 (10.20 %) were positive to antibodies against the H5N1 virus while the remaining 88 (89.80 %) were negative. The highest prevalence of 18.18% was recorded in Makarfi LBM (Table 4.3), while all samples from Ikara LBM were negative to H5 antibodies. However, among poultry species, pigeon's prevalence rate was the highest (18.2 %) (Table 4.4). In LGAs with no HPAI reported outbreak (Table 4.4), 208 sera were tested, out of which 14 (4.81 %) positive.

Table 4.3: H5 antibodies prevalence of live poultry from live bird markets in Northern Part of Kaduna State and history of highly pathogenic avian influenza outbreak in the local government areas.

Reported	LGA	LBM	Species of birds sampled							
			Local chickens	Commercial chickens	Guinea fowls	Pigeons	Ducks	Total tested	Total no positive	Prevalence (%)
Yes	Sabon gari	Sabon gari	37	9	4	0	3	53	7	13.21
Yes	Giwa	Giwa	35	0	5	0	5	45	3	6.67
No	Makarfi	Makarfi	30	0	17	6	2	55	10	18.18
No	Kubau	Anchau	25	1	2	0	5	33	2	6.06
No	Ikara	Ikara	17	0	5	0	7	29	0	0.00
No	Soba	Tudun Saibu	31	3	7	5	11	57	1	1.75
No	Lere	Yankaji	5	28	0	0	1	34	1	12.94
Total	**7**	**7**	**180**	**41**	**40**	**11**	**34**	**306**	**24**	**7.84**

Table 4.4: Distribution of the number of samples positive for H5 antibodies by species in live bird markets in northern part of Kaduna State.

Species	No. tested	No. positive	Prevalence (%)
Local chickens	180	15	8.33
Commercial chickens	41	2	4.88
Guinea fowls	40	4	10.00
Pigeons	11	2	18.18
Ducks	34	1	2.94
Total	**306**	**24**	**7.84**

4.4 Biosecurity Practices and Infrastructure in Live Bird Markets in Northern Part of Kaduna State

Only one (15.19 %) LBM out of the seven surveyed had either pipe borne water or a well, two (30. 4 %) LBMs bought water from vendors whose source is not known. Three (45.6 %) LBMs had no known source of water supply and all the LBMs have human settlements around them (Table 4.5).

About 23 (29.11 %) of respondents said HPAI (H5N1) has been reported in the LGAs. Only 1 (12.66 %) and 9 (11.39 %) respondent (s) kept poultry separated by age and types respectively. Thirty-three (41.77 %) of the poultry marketers sell and keep other animals such as rabbits with poultry in the same cages (Table 4.6).

The poultry marketers and processors do not wear coveralls, eye goggles, boots, face masks, hand gloves or washed their hands with 97.47 % of respondents using poultry offal for food while 2.53 % throw away the poultry offal (Table 4.7).

About 87 (98.73 %) and 79 (100 %) respondents threw away dead poultry and poultry feathers respectively. However, none would bury poultry feathers and only 1 (12.66 %) bury dead poultry (Table 4.8)

Fifty nine (74.68 %) of the respondents would trade in sick birds, 67 (84.81 %) processed poultry within the LBMs while 55 (69.62 %) reported that there was no decontamination at the LBMs (Table 4.9).

Forty-eight (60.76 %) reported government intervention in the form of fumigation. All the LBMs have poultry marketers association with 71 (89.87 %) respondents reported knoweldge of HPAI though only 35 (44.30 %) knew clinical signs of HPAI (Table 4.9). Only 19 (24.05 %) of the respondents knew similar diseases to HPAI. Most 57 (72.15 %) respondents did not believe HPAI is zoonotic. Fifty (63.29 %) respondents reported willingness to disclose HPAI (H5N1) outbreak (Table 4.9). Biosecurity was not observed in the live bird markets since non of the poultry marketers and processors wore personal protective equipment or properly washed their hands after handling poultry.

4.5 Sources and Destinations of Live Poultry Traded in Live Bird Markets in Northern Kaduna State

Trade in live poultry in Northern Kaduna State reveal that all live bird markets studied serve as feeder markets for the Sabon gari and Kaduna live bird markets (Figure 4.1-4.7).

Table 4.5: Live bird market type, water source and closeness to human settlements in northern part of Kaduna State.

LBM type	Daily		Weekly		Source of water								Human settlement			
	No.	%	No.	%	Well		Vendors		None		Tap		Yes		No	
					No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sabon gari	12	44.15	0	0	12	15.19	0	0	0	0	0	0	12	15.19	0	0
Giwa	0	0	11	20.75	0	0	0	0	11	13.93	0	0	11	13.92	0	0
Makarfi	0	0	10	18.87	0	0	10	12.66	0	0	0	0	10	12.66	0	0
Anchau	0	0	12	22.64	0	0	0	0	12	15.19	0	0	12	15.19	0	0
Ikara	0	0	10	18.87	0	0	0	0	10	12.66	0	0	10	12.66	0	0
Tudun Saibu	0	0	10	18.87	0	0	10	12.66	0	0	0	0	10	12.66	0	0
Yankaji	14	58.33	0	0	0	0	0	0	0	0	14	17.72	14	17.72	0	0
Total	**26**	**32.91**	**53**	**63.09**	**12**	**15.19**	**20**	**25.19**	**33**	**41.77**	**14**	**17.72**	**79**	**100**	**0**	**0**

Table 4.6: Biosecurity measures in live bird markets in northern part of Kaduna State.

Report	Report HPAI in LGA		LBM fenced		Poultry kept by age		Poultry kept by type		Other animals in LBM	
Response	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Yes	23	29.11	0	0	1	2.66	9	11.39	33	41.77
No	56	70.87	79	100	78	98.73	70	88.73	46	58.23
Total	**79**	**100**	**79**	**100**	**79**	**100**	**79**	**100**	**79**	**100**

Table 4.7: Biosecurity practices in live bird markets in northern part of Kaduna State.

Methods of disposal	Sick birds		Dead birds		Poultry offals		Poultry feathers	
Response	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Use as food	79	100	0	0	77	97.47	0	0
Throw away	0	0	78	97.33	2	2.53	79	100
Bury	0	0	1	12.66	0	0	0	0
Total	**79**	**100**	**79**	**100**	**79**	**100**	**79**	**100**

Table 4.8: Trade in sick birds, poultry processing, decontamination and government intervention in live bird markets in northern part Kaduna State.

Response	Buy sick birds		Poultry processing in LBM		Decontamination of LBM		Government intervention at LBM		Fumigation of LBM	
	No	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Yes	59	74.68	67	84.81	24	39.38	48	60.76	48	60.76
No	20	25.32	12	15.19	55	69.62	31	39.24	31	39.24
Total	**79**	**100**	**79**	**100**	**79**	**100**	**79**	**100**	**79**	**100**

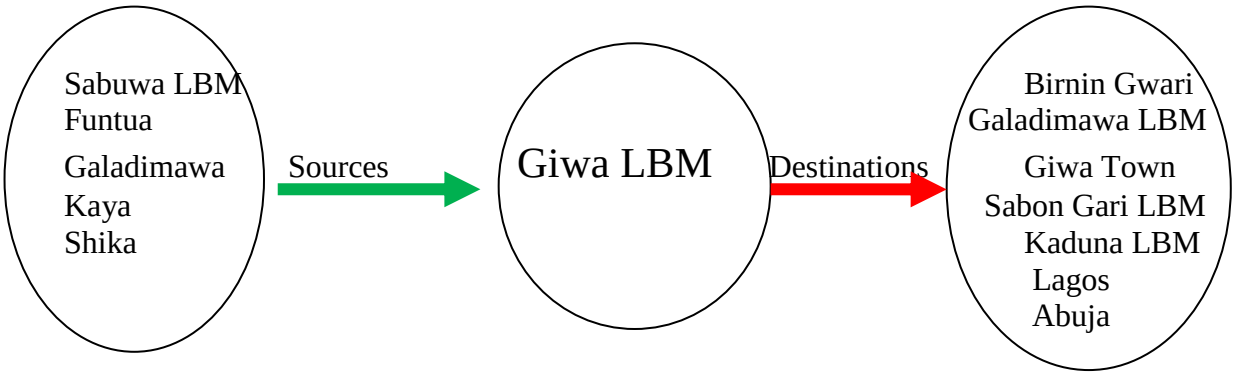

Figure 4.1: Sources and destinations of live poultry traded at Giwa live bird market.

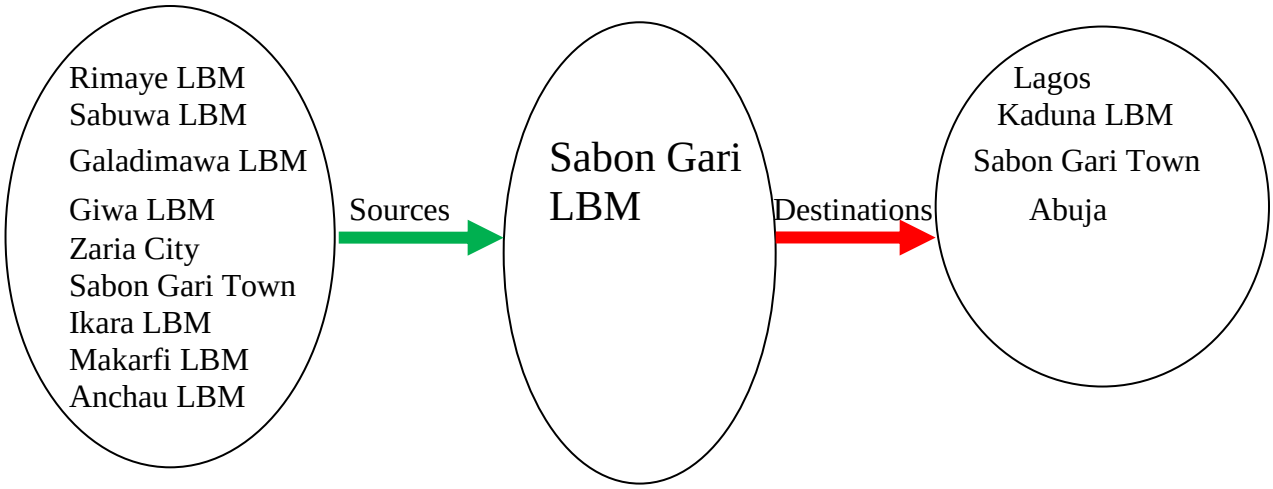

Figure 4.2: Sources and destinations of live poultry traded in Sabon Gari live bird market.

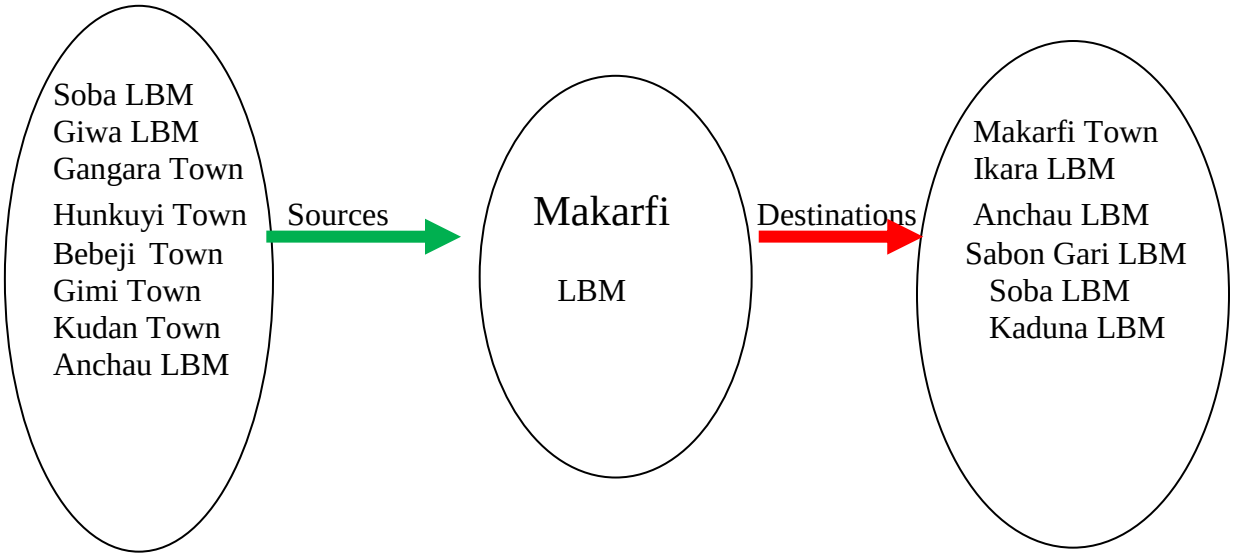

Figure 4.3: Sources and destinations of live poultry traded at Makarfi live bird market.

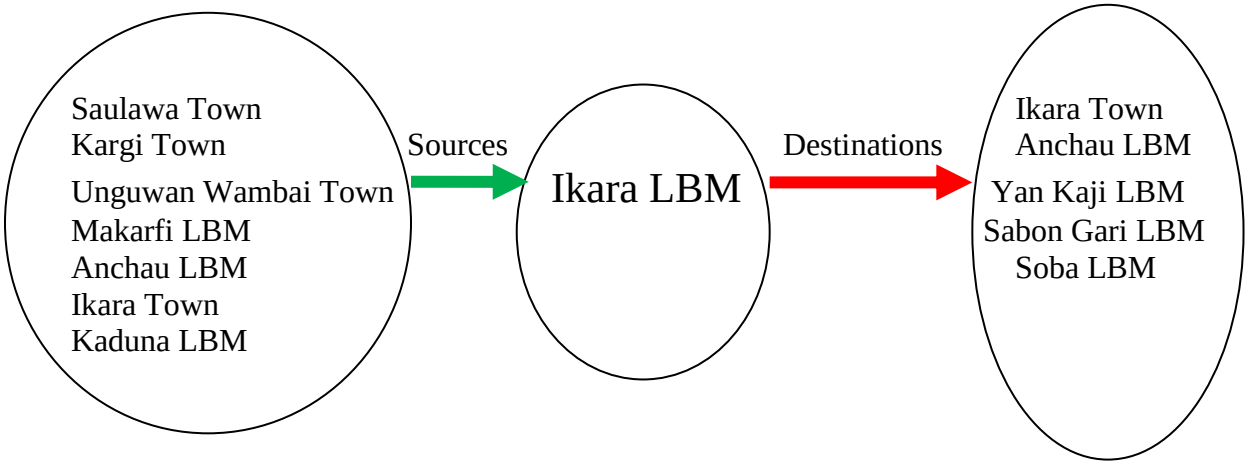

Figure 4.4: Sources and destinations of live poultry traded at Ikara live bird market.

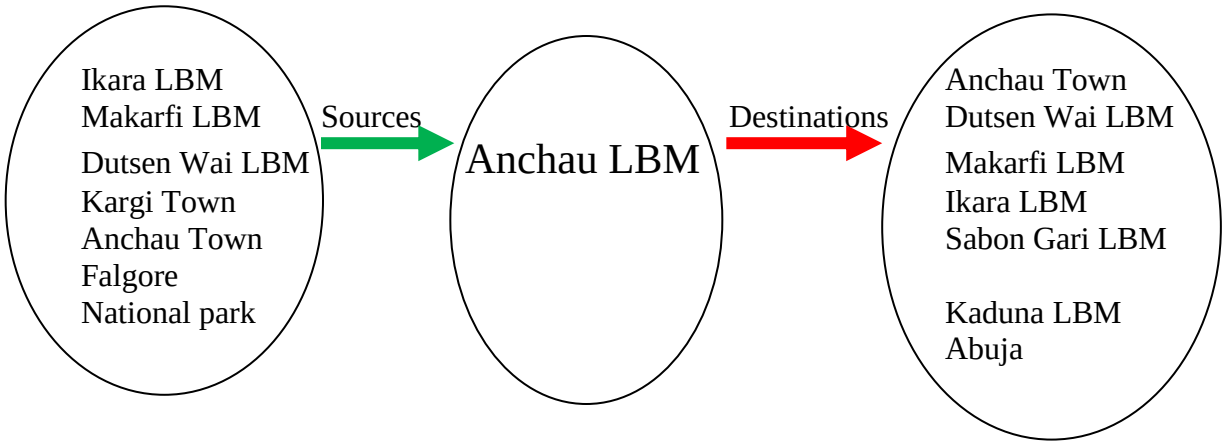

Figure 4.5: Source and destination of live poultry traded at Anchau live bird market.

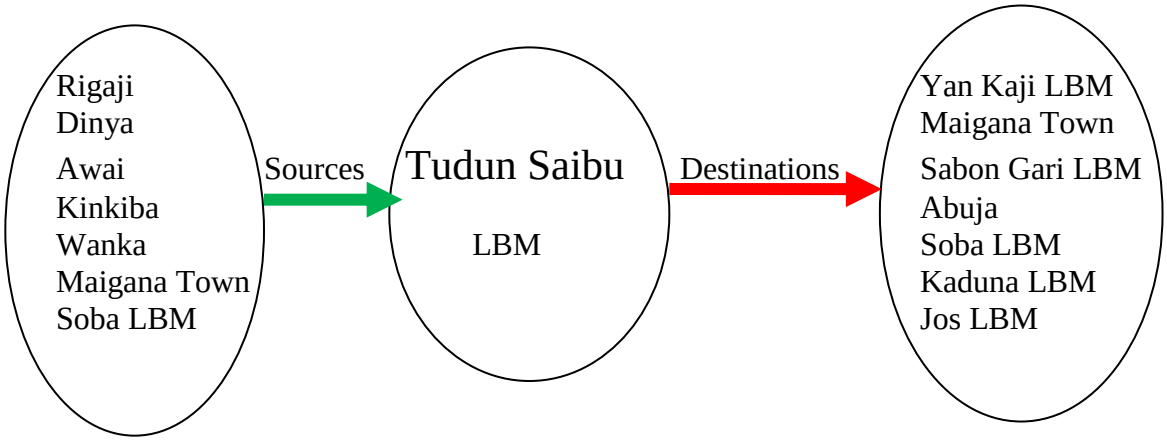

Figure 4.6: Source and destination of live poultry traded at Tudun Saibu live bird market.

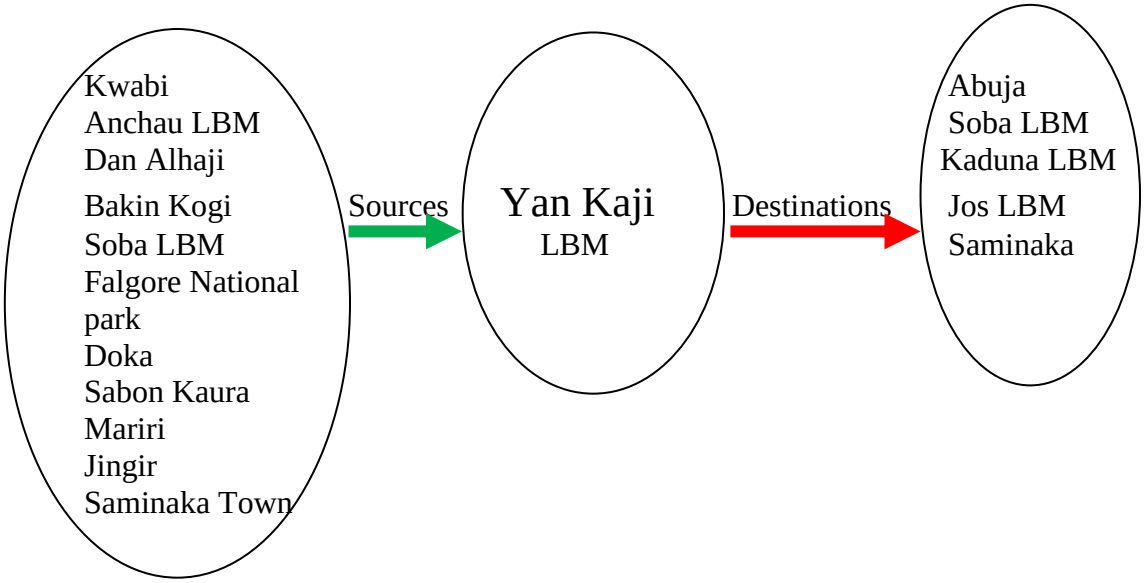

Figure 4.7: Source and destination of live poultry traded at Yan Kaji live bird market.

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The mortality rate (52.1%) of chicken in the study is simillar to reports of Kaduna State Government of 54.50% though lack of or poor record in most farms shows that these mortality rates might not be accurate (KDSG, 2008). It is not a surprise that more chickens, geese and turkeys died of the HPAI because they are known to be highly susceptible to the H5N1 virus (Easterday *et al*., 1997). A lot of poultry farms raised both commercial chickens and local chickens together, it therefore appears the former were the source of infection to the later. It is documented that pigs serve to mix influenza viruses in multiple virus infections, reassorting for better and wider transmissibility (Swayne and Jackwood, 2008). The loss of large number of poultry, either due to HPAI or culling for control purposes, deprived Nigerians of an important source of dietary protein.

The delayed reporting of outbreaks of more than five days interval would result in delayed institution of control measures and futher spread of infection increasing both human and poultry exposure to H5N1 virus (Easterday *et al*., 1997). Late reporting of outbreaks would increase the likelihood that the H5N1 virus will become endemic (WHO, 2005). The late reporting might be due to inadequate or lack of compensation paid to farmers which might have discouraged them from reporting outbreaks or lack of logistics or inadequate veterinary manpower in the state (WHO, 2005). The sudden onset of the disease during outbreaks in Kaduna state is in conformity with previous findings (Abdu *et al*., 2005; Wakawa *et al*., 2008).

The poor biosecurity reported in most of the 128 farms might be as a result of low or minimal involvement of animal health services providers or extension workers as most of the farms were managed by owners with inadequate knoweledge of poultry production. This poor biosecurity will increase the risk of introduction of HPAI (H5N1) virus into poultry operations with resultant economic loss (Swayne, 2008). The biosecurity risk is further hightened by keeping multi-age and multi-species poultry in the same farm by 55 of the farms as suceptibility to H5N1 between species and ages differ (Easterday *et al*., 1997). Species, such as duck serves as reseviors while poultry of different age could ensure maintainace of virus within the farm (Swayne *et al*., 1997). The practice of using the same equipment and egg crates by 8 different farms would enhance the spread of H5N1 virus between the farms and might likely be the cause of HPAI outbreaks in nine farms on the same day within the same area as reported in this study (Easterday *et al*., 1997).

The finanacial loss due to HPAI was enormous amounting to almost 984,500,272.00 Naira considering that majority of the poultry farms were backyard farms. This finanacial loss does not include the loss of livelihood of the poultry workers and the emotional stress undegone by farmers. Additional cost of N69 million was incured by government paid as compensation to farmers to control HPAI outbreaks (AICP, 2009). The resultant consequence of funds used for payment of compensation would be shelving other developmental projects which would have benefited the general public (AICP, 2010).

The presence of H5 antibodies in all LBMs except Ikara indicates that the poultry in the LBMs have had contact with AIV of the H5 subtype and the virus is probably still

circulating in Kaduna State. There is apparently no association between LGAs with reported HPAI (H5N1) outbreaks and those without reports. The high prevalence in LBMs in LGAs where HPAI outbreaks have not been reported implies AIV might be circulating in areas belived to be AIV free or the presence of wetlands might incease the mingling of poultry and wild birds (Easterday *et al.,* 1997). The high prevalence of H5N1 antibodies in pigeons and guinea fowls which have unrestricted movement increases the risk of viral spread and they may act as a bridge species as they mingle with wild birds in wetlands and ponds (Whitworth *et al.*, 2007).

The lack of potable water supply in most of the LBMs increase the biosecurity risk in the LBMs as poor hygine would result in contamination of poultry carcass thereby increasing human exposure to virus when slaughtering HPAI H5N1 contaminated poultry. Provision of water contaminated with faeces from infected birds is an important source of infection for poultry in the LBMs (FAO, 2008). The presence of human setlements near LBMs further incresaes the risk of human exposure.

The practice of rabbits sharing the same cage with poultry might aid in the spread of the H5N1 virus as rabbits are known to be infected with H5NI virus sporadically and the infection may cause disease with high morbidity and high mortality (Etienne *et al.,* 2006).

Live bird marketers might be exposed to HPAI H5N1 virus through constant contact with live birds as they do not wear protective clothings neither do they take other protective measures, such as washing of hands and not eating with bare hands. Other risky behaviours engaged by live bird marketers, such as processing of poultry in the market, throwing away dead birds and feathers; consumption of poultry offals and engaging in

trade in sick poultry would result in contamination of the market and spread of the virus with increased human exposure. Marketers engage in these practice because of poor risk awareness or perception of HPAI, and improper education on HPAI risk in poultry and humans necessary for proper control of infection (Durosinlorin, 2008).

The education of the live bird marketers would be made easier by partnering with the live bird marketers' associations. These associations can play a positive role in the control of HPAI H5N1 by assisting in information, gathering and dissemination (FAO, 2008). The live bird marketers association could assist in gaining cooperation of their members during active virus surveillance. The willingness of the marketers to report outbreaks would reduce the time lapse for intervention ensuring swift control of outbreak thereby preventing virus becoming endemic in the LBMs (FAO, 2008).

The LBMs at Sabon gari and Yan kaji are operated daily, thus making effective hygienic and decontamination practices difficult to maintain. This posses serous danger of H5N1 infection to poultry marketers, processors and consumers. None of the seven LBMs was fenced off from residential areas, shops, public high ways or other parts of the main markets where trade and poultry processing were carried out with other businesses. This exposes the public to the risk of infection with the H5N1 virus.

In conclusion, the study highlights the practical experience encountered in handling the HPAI outbreaks in Kaduna State of Nigeria, the enormous finanacial losses incured, the prevalence of H5 antibodies in LBMs and different poultry together with the bisecurity

lapses currently found in live bird markets. Moreover, poultry is being recycled among live bird markets in the study area with the risk of spreading infection (AICP, 2010).

5.2 Conclusions

1. The 2006-2007 HPAI (H5N1) outbreaks in Kaduna State affected mostly commercial poultry farms.
2. The total financial loss during the 2006–2007 HPAI (H5N1) outbreak was almost one billion Naira.
3. Antibodies against the H5 influenza virus were detected in chickens, pigeons, ducks and guinea fowls while all the LBMs except Ikara LBM had poultry positive for H5 antibodies.
4. Biosecurity measures and practices in LBMs were very poor together with the infrastructure.
5. There is strong suggestion that the live bird markets play a role in the epidemiology of HPAI in the northern part of Kaduna State.

5.3 Recommendations

1. Poultry farmers should be educated on the need of enforcing proper biosecurity measures in poultry farms.
2. Live bird marketers should be educated on HPAI (H5N1) risks and preventive measures.
3. Kaduna State Government should improve on the infrastructures especially water supply to all live bird markets in the State.

4. Government should either fence off or relocate live bird markets from main markets, human settlements, high ways and other livestock markets.
5. Active surveillance and close monitoring of guinea fowls and pigeons should be carried out as they may have special role in the epidemiology of HPAI in the state.

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APPENDICES
APPENDIX I

QUESTIONNAIRE ON AVIAN INFLUENZA IN LIVE BIRD MARKETS IN SEVEN NORTHERN LOCAL GOVERNMENT AREAS OF KADUNA STATE – NIGERIA

PLEASE ANSWER YES OR NO, WHERE APPLICABLE

SECTION A: **Market identity**

1. Serial Number _____ Date _____
2. State _____
3. Local Government Area _____
4. GPS Coordinates – Latitude _____Longitude _____
5. Has there been any report of Bird flu in the Local Government?
   Yes ☐ No ☐
6. Town/Village _____
7. Name of live bird market _____
8. Market type: a). Daily ☐ b). Weekly ☐ c). Informal ☐ d). Others specify _____
9. Market days. M ☐ T ☐ W ☐ Th ☐ F ☐ S ☐ Sun ☐

SECTION B. Biosecurity in the Market

10. Is the market fenced off? Yes ☐ No ☐
11. Which is the nearest big Town/Village? _____
12. Are birds kept in cages? Yes ☐ No ☐
13. Are the birds kept separately by
    1. Age? Yes ☐ No ☐
    2. Bread? Yes ☐ No ☐
    3. Species? Yes ☐ No ☐
    4. Type? Yes ☐ No ☐
14. Are other animals sold in the market? Yes ☐ No ☐
15. If yes, list the other animals sold. 1). Cattle ☐ 2). Sheep ☐ 3). Goats ☐ 4. Pigs ☐ 5). Donkeys ☐ 6). Horses ☐ 7. Rabits ☐
    8). Others (specify) _____

16. What do you do with.

		Prepare for food	Throw away	Gift	Bury	Burn
1.	Sick birds?	☐	☐	☐	☐	☐
2.	Dead birds?	☐	☐	☐	☐	☐
3.	Offals?	☐	☐	☐	☐	☐
4.	Feathers?	☐	☐	☐	☐	☐

17. Any biosecurity practices observed in the market like

a. Wearing over alls? Yes ☐ No ☐
b. Wearing boots? Yes ☐ No ☐
c. Wearing facemasks? Yes ☐ No ☐
d. Wearing hand gloves? Yes ☐ No ☐
e. Wearing goggles? Yes ☐ No ☐
f. Washing of hands? Yes ☐ No ☐

18 Has the market a poultry processing area? Yes ☐ No ☐

19. Source of water. a). Well ☐ b). River/stream ☐ c). Tap ☐ d). Gutter ☐
e. Shallow pit ☐

20. Is the processing area always cleaned and disinfected? Yes ☐ No ☐

21. By who? _____

22. How far (in meters or kilometers) is the market from a residential area?

_____

23. Where does the water run of from the processing area flow to? _____

24. Do you buy sick birds? Yes ☐ No ☐

25. If yes, why? _____

26. If no, why? _____

SECTION C. Trade in the Market

27. Who is incharge of the market? _____

28. Is there, or has there been government intervention in the market?

Yes ☐ No ☐

29. If yes, specify _____

30. Who brings poultry to the market? a). Adult b). Children c). Males d). Females

31. From which Town? _____ Village? _____ Country _____

32. Who buys poultry? a). Vendors ☐ b). Consumers ☐ c). Restaurants ☐ d). Dealers ☐

33. Where are poultry taken to from the market? _____

34. Which are the periods of high sales?_____

a). Edil fitri Yes ☐ No ☐

b). Edil kabir Yes ☐ No ☐

c). Maulud Na bi Yes ☐ No ☐

d). Christmas Yes ☐ No ☐

e). Easter Yes ☐ No ☐

f). Mew year Yes ☐ No ☐

35. Is there an association of the Poultry sellers? Yes ☐ No ☐

36. If there is, who is a). The Chairman? _____

b). The Secretary? _____

37. What is the number of a). Owners? Male ☐ Female ☐

b). Sellers? Male ☐ Female ☐

c). Buyers? Male ☐ Female ☐

38. What are the prices of the following?

	From the owners?	From the sellers?	From the buyers?
a.	Chickens _____	_____	_____
b.	Guinea fowls _____	_____	_____
c.	Turkeys _____	_____	_____
d.	Ducks _____	_____	_____
e.	Geese _____	_____	_____
f.	Pigeons _____	_____	_____

g. others specify _____ _____ _____

i. Chicken Eggs _____ _____ _____

j. Turkey Eggs _____ _____ _____

k. Ducks Eggs _____ _____ _____

l. Geese Eggs _____ _____ _____

m. Pigeons eggs _____ _____ _____

n. Others (specify) _____ _____ _____

Section D. Knowledge of Poultry Diseases by the Marketer

39. Do you know any poultry disease (es)? Yes ☐ No ☐

40. If yes, which one(s)? _____

41. Do you know of bird flu? Yes ☐ No ☐

42. Do you know the signs of bird flu? Yes ☐ No ☐

43. If yes, list some signs of bird flu? _____

44. Do you know bird flu affects human beings? Yes ☐ No ☐

45. Do you believe bird flu affects human beings? Yes ☐ No ☐

46. Do you know of any disease(s)? Similar to bird flu? Yes ☐ No ☐

47. If yes, which one(s)? _____

48. Incase of bird flu outbreak, will you report? Yes ☐ No ☐

49. If no, why? _____

50. If yes to Who?_____

Sign ----------

AYE L. ADAMU

APPENDIX II

AVIAN INFLUENZA SAMPLE COLLECTION FORM

S/No	Date of Sample Collection	State	Local Gov. Area	Name of live bird market	Town/Village	Owner of poultry	Breed	Species	Type	Sample collected	Sign

Name:----------

Sign: ----------