SURVEY FOR AVIAN INFLUENZA, NEWCASTLE AND GUMBORO DISEASE ANTIBODIES AND BIOSECURITY PRACTICES IN LIVE BIRD MARKETS IN ABUJA

BY

Anthony Stephen, ADACHE
MSc/VET.MED/02812/2008-2009

DEPARTMENT OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA

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SEPTEMBER, 2012

DECLARATION

I declare that this research work titled "SURVEY FOR AVIAN INFLUENZA, NEWCASTLE, GUMBORO DISEASE ANTIBODIES AND BIOSECURITY PRACTICES IN LIVE BIRD MARKETS IN ABUJA" was carried out by me in the Department of Medicine, Ahmadu Bello University, Zaria, Nigeria. The work was done under the supervision of Professors P.A. Abdu, A.K.B. Sackey and. Lawal Sa'idu. The information derived from relevant literature and the works of other researchers were duly acknowledged. No part of this thesis has been previously presented elsewhere for the award of Degree or Diploma.

Anthony Stephen, ADACHE

Name of student	**Signature**	**Date**

CERTIFICATION

This thesis "**SURVEY FOR AVIAN INFLUENZA, NEWCASTLE, GUMBORO DISEASE ANTIBODIES AND BIOSECURITY PRACTICES IN LIVE BIRD MARKETS IN ABUJA**" by Anthony Stephen ADACHE meets the regulation governing the award of the degree of Master of Science (MSc) of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to God, my beloved family and friends who loved and supported me in the course of this study.

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My gratitude goes to God for His loving kindness and to my able supervisors, Professors P.A. Abdu, A.K.B. Sackey and Saidu Lawal for their invaluable contributions to this work.

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ABSTRACT

Poultry is a major source of protein in most parts of the world. Trade in poultry and poultry product is a source of livelihood of millions of Nigerians. However, the outbreak of Avian Influenza (AI) in Nigeria increased disease constraint in addition to Newcastle and Gumboro diseases. Live bird markets (LBMs) have been attributed as a mixing point of poultry from different households with increased likelihood of cross infection. The study assessed Avian Influenza, Newcastle and Gumboro diseases antibodies status of poultry in Abuja LBMs by Haemagglutination Inhibition (HI) and Agar Gel Immunodiffusion (AGID) tests respectively. Biosecurity practices were also assessed through administration of standard questionnaires. Prevalence and mean titre for avian influenza were 65.3% and 10.40±0.94 $\log_2$, respectively. Newcastle disease mean antibody titre was 5.91±0.27 $\log_2$ with prevalence of 64.3%. Gumboro disease prevalence was 69.8%. Among species tested, duck had the highest avian influenza prevalence of 84 % and chicken prevalence was 63.3%. Pigeons and turkeys have been exposed to Gumboro disease virus with chickens having Newcastle disease prevalence of 63.8 %. All the species had avian influenza antibodies titre $\geq 7\log_2$ except guinea fowl. Garki Area I LBM had the highest avian influenza prevalence of 87.9 % with Garki II new extension market having the lowest prevalence of 22.2 %. Fowl sellers engaged in risky practices such as stacking of cages. Sixty four percent (64.6%) of fowl sellers use sick poultry for food, 33.3% sell sick poultry, 29.2% throw away offal and only 20.8% bury dead poultry. Furthermore, 37.4% knew none of avian influenza signs though 69.8% were knowledgeable on its mode of transmission. Nonetheless, 90.0% poultry sellers would report avian influenza outbreak to Veterinarians. Poultry in Abuja LBMs are mainly local birds and have been exposed to avian influenza virus and are not protected

from Gumboro disease and Newcastle disease outbreaks which might be confused with highly pathogenic avian influenza. The exposure of poultry to avian influenza antigen is recent with the likelihood of them shedding the virus at high levels. The risk of ducks spreading infection to household local chickens is high as local poultry farmers get breeding stock from LBMs. Fowl sellers engage in risky practices likely to expose them and poultry to avian influenza virus. There is need to undertake avian influenza viral surveillance in all LBMs in Abuja and better articulate avian influenza risks to fowl sellers. There are no significant biosecurity measures employed by the LBMs in Abuja and this could be a source of disease outbreak in poultry, poultry sellers and handlers as well as the general public.

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LIST OF ABBREVIATIONS

µL	-	microlitre
ABUTH	-	Ahmadu Bello University Teaching Hospital
AGID	-	Agar Gel Immunodifusion Test
AI	-	Avian Influenza
AICP	-	Avian Influenza Control Programme
AIV	-	Avian Influenza Virus
AMAC	-	Abuja Municipal Area Council
CZC	-	Country Zone or Compartment
DEFRA	-	Department for Environment, Food and Rural Affairs
DNA	-	Deoxyribonucleic Acid
EDS	-	Egg Drop Syndrome
ELISA	-	Enzyme Linked Immunosorbent Assay
FAO	-	Food and Agricultural Organization
FCT	-	Federal Capital Territory
g	-	Gramme
GD	-	Gumboro Disease
HA	-	Haemaglutination
HAU	-	Haemaglutination Area Unit
HI	-	Haemaglutination Inhibition
HIT	-	Haemaglutination Inhibition Test
HPAI	-	Highly Pathogenic Avian Influenza
HPNAI	-	Highly Pathogenic Notifiable Avian Influenza
IBD	-	Infectious Bursal Disease

IVPI	-	Intravenous Pathogenicity Index
LBM	-	Live bird Market
LP	-	Local Poultry
LPAI	-	Low Pathogenic Avian Influenza
LPNAI	-	Low Pathogenic Notifiable Avian Influenza
ml	-	Millilitre
NA	-	Neuraminidase
NADIS	-	National Animal Disease Information Service
NBS	-	National Bureau of Statistics
ND	-	Newcastle disease
OIE	-	Organization Internationale de Epizootics
P	-	Prevalence
PBS	-	Phosphate buffered saline
RBC	-	Red Blood Cell
RNA	-	Ribonucleic Acid
SPSS	-	Statistical Package for Social Sciences
tRT-PCR	-	Triplex Real-time polymerase chain reaction
US	-	United States
USDA	-	United States Development Agency
UV	-	Ultra Violet
v/v	-	Volume over volume
WHO	-	World Health Organization

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

Avian influenza is a highly contagious acute viral infection or disease syndrome primarily of domestic and wild free-flying avian species (Abdu, 2007). It is caused by viruses that are members of the Family *Orthomyxoviridae* and are placed in the Genus influenza virus A (OIE, 2009). There are three influenza genera, A, B and C. Only influenza A viruses are known to cause natural infection in birds (Gunter ,2007).

An important feature of avian influenza epidemic and virus is the wide range of species of bird infected (Sa'idu *et al.,* 2008). The disease also affects mammals, such man, pigs, horses, cats, seals and whales (Alexander, 2000).

Highly Pathogenic avian influenza viruses have no natural reservoir. Instead they emerge by mutation when a virus, carried in its mild form by a wild bird is introduced to poultry (WHO, 2005). Once in poultry, the previously stable virus begins to evolve rapidly and can mutate, over an unpredictable period of time, into a highly lethal version of the same initially mild strain (WHO, 2005).

The avian flu virus is an RNA virus and is thought to have 8 segments of single strands. It is spherical in shape with a diameter of 80-120 nm (Agarwal *et al.,* 2004). The virus is capable of antigenic drift or antigenic shift and there are many subtypes, H5N1 being one of the three known to pass from bird to humans (Agarwal *et al.,* 2004). On the surface of the virus are different spike proteins namely, haemaglutinin (HA) and Neuraminidase (NA) glycoproteins.

The HA and NA are the most important surface antigens for the host's immune response (Ardan, 1999). There are 16 HA (H1-H16) and 9 NA (N1-N9) subtypes which can be present in any combination (Alexander *et al.,* 2004). There can be about 144 possible combinations (Capua and Moragon, 2003). The segmentation of avian influenza genome facilitates genetic recombination by segment reassortment in hosts infected with two different influenza viruses at the same time (WHO, 2005). The highly pathogenic viruses have been restricted to subtypes H5 and H7 although not all viruses of these subtypes cause HPAI (Alexander, 2000).

Avian influenza viruses generally replicate in the gastrointestinal tract of wild ducks and are shed at high levels through the faeces and transmitted horizontally through the faeco-oral route (Sturm-Ramirez *et al*., 2004). Until recently, the viruses were in a state of evolutionary equilibrium in wild aquatic birds and the hosts usually showed no symptoms (Sturm-Ramirez *et al.,* 2004). Nevertheless, the H5N1 viruses remained antigenically homogenous until 2002 when considerable antigenic drift occurred resulting in high pathogenicity for ducks and other aquatic bird species (Sturm- Ramirez *et al.,* 2004).

The incubation period vary from 3-7 days and clinical signs of avian influenza depend on the isolate, the dose of the virus, age, species, sex, health status of the bird and route of virus entry (Wakawa *et al.,* 2008).

Based on the degree of virulence of the influenza virus in poultry, two forms of the disease are recognized:

a) Low pathogenic avian influenza (LPAI) with silence as the most notable sign of the illness and lack of normal sounds (Halvorson *et al.,* 1980). The most common

gross lesions of LPAI are catarrhal inflammation of sinuses, egg yolk peritonitis and ovarian follicle regression (Abdu, 2007).

b) Highly pathogenic avian influenza (HPAI) is more devastating and spreads very fast within flocks and mortality can approach 100 % within 48 hours (Easterday *et al.,* 1997). Common clinical signs of HPAI include the following- bluish wattles and combs, shell less and broken eggs, discolored legs, high mortality, dyspnoea, swollen head and face, cloudy and bloody eyes (Easterday *et al.,* 1997).

The common gross lesions are oedema of the head, cyanosis and hemorrhages of the comb and wattles, hemorrhages in proventriculus, intestines and caecal tonsils (Abdu, 2007).

Avian influenza viruses are spread through aerosols, fomites, migratory birds, close proximity between infected and susceptible hosts in live bird markets, trade in wild birds and in poultry products, contaminated clothes and equipment (Ocholi *et al.,* 2006). Inter species infection with avian influenza viruses occur through the reshaping of the HA protein receptor binding units (Robert *et al,* 2007*)*.

Until 1997, avian influenza viruses have not been transmitted directly from birds to humans (Abdu, 2007), but there is increasing evidence for the presence of both α-2-6 sialic acid (human-like influenza receptor) and α-2-3 sialic acid receptor (avian-like influenza receptor) in the human lungs, making the direct transmission of avian influenza virus to humans possible (Shinya *et al.,* 2006; Abdu, 2007).

In Nigeria, avian influenza was first reported at a commercial poultry farm in Kaduna State on the 14th January 2006 and confirmed by the OIE, Reference Laboratory at Padova, Italy, on the 7th of February, 2006 (Adene *et al.,* 2006; NADIS, 2006). This

outbreak in Nigeria was the first reported outbreak of the H5N1 Asian Strain on the African continent (NADIS, 2006).

The disease devastated the poultry sector of the economy, especially in the north-central region of Nigeria (OIE, 2009). Several control measures including culling, movement restrictions, compensation and improved biosecurity were implemented by the authorities to limit the spread of the disease. The disease was confirmed in 25 States and Federal Capital Territory (NADIS, 2006). As at January 2010, avian influenza has infected human beings worldwide with total death of 282 people out of 467 reported cases (WHO, 2010).

Ducatez (2006) previously identified three HPAI H5N1 virus strains circulating in Nigeria, which had close genetic relationships with viruses from Kurgan, Astrakhan and Egypt; while Salsberg (2007), demonstrated that African isolates have common ancestry with viruses from Europe and the Middle East. They suggested the possibility of wild migratory birds as being responsible for the introduction of these viruses into Nigeria.

Nigeria recorded almost 200 HPAI H5N1 outbreaks at different locations between January 2006 and January 2007, making it necessary to evaluate the role of trade and other risk factors in addition to those from migratory birds (Joannis *et al.,* 2008).

Newcastle disease is believed to be the most devastating disease in the local poultry and the main cause of high mortality (Bell, 1992; Nwanta *et al.,* 2006). Local fowls are reported to be more resistant than exotic breeds, while young domestic fowls and guinea fowls are more susceptible than adults (Oluyemi *et al.,* 1979; Nwanta *et al.,* 2006). Turkey and pigeon may develop generalized disease but clinical signs are rare in ducks

and geese (Nwanta *et al.,* 2006). Wild and domestic birds sometimes harbour Newcastle disease virus while showing no detectable clinical signs of the disease (Alders and Spradbrow, 2001). As village poultry have more contacts with these birds, they may play a part in amplifying the virulence of field viruses. Therefore, village chickens are probably very important for survival of ND virus in the environment and may also play an important role in the spread of the virus among commercial poultry flocks (Abdu *et al.,* 1999).

Infectious bursal disease also known as Gumboro disease is an infectious acute, mild or subclinical viral disease of 3-7 weeks old chickens characterized by trembling, incoordination, inflammation followed by necrosis and atrophy of bursa of Fabricius and immunosuppression and was first reported in Nigeria in 1969 (Abdu, 2007).

Surveillance study on IBD in Zaria revealed large portion of local fowls been exposed to IBD virus although there are reports of natural IBD infection in local chickens (Abdu *et al*, 1999). Sa'idu *et al.* (1994) reported more than 50% of IBD cases in local fowls were concurrently infested with external parasites, habour helminthes and coccidia.

1.1 STATEMENT OF RESEARCH PROBLEM

Nigeria with an estimated human population of over 140 million, first reported poultry and humans cases of HPAI infection in 2006 and 2007, respectively (Joannis *et al.,* 2008; WHO, 2008). Thereafter, efforts to carry out surveillance for the influenza virus have been intensified by the national authority (WHO, 2008). Nonetheless, eight African countries namely Burkina Faso, Cameroon, Cote D'Ivoire Djibouti, Egypt, Niger, Nigeria and Sudan have reported HPAI outbreaks after the Nigerian HPAI outbreak (WHO, 2008). The world indeed was concerned that H5N1 could adapt to and become easily transmitted from human to human. Once this adaptation occurs, it will no longer be a bird

virus; it will become a human influenza virus, which could cause a pandemic (WHO, 2008).

Highly pathogenic avian influenza outbreaks have led to trade bans and restrictions on poultry and poultry by products which negatively affected the poultry industry and subsequently that of the Federal Capital Territory and Nigeria at large (Abdu, 2007; Ahmed , 2006). Three area councils in the Federal Capital Territory reported outbreaks of the disease with attendant capital flight from the poultry industry. As a result, many jobs were lost and a lot of birds were also destroyed as a containment measure.

1.2 JUSTIFICATION OF STUDY

The expanding geography (infection of new location), biology (acquisition of new biological properties) and ecology (adaptation of new host range) of H5 influenza virus necessitates that every country should actually search for H5 viruses within its territories.

Poultry production is a key economic activity in Abuja and Nigeria in general. It contributes significantly to the family income especially in peri-urban and poor rural communities.

From one State in February 2006, the virus continued its spread to other parts of the country and has affected 97 local government areas including Bwari, Municipal and Kuje Area Councils of the Federal Capital Territory (Appendix 1).

Due to HPAI H5N1 in Nigeria, millions of poultry have been destroyed and one human death of a 22 year old female on the 17th of February, 2007.

1.3 AIM OF THE STUDY

The aim of the work was to determine AI, ND and GD antibodies prevalence in live poultry and asses fowl sellers biosecurity practices within LBMs in Abuja.

1.4 OBJECTIVES OF THE STUDY

The work specifically:

1. Determine the presence of AI, ND, and GD disease antibodies in poultry sold in live bird markets in the FCT

2. Determine the risks for avian influenza infection in the live bird markets by assessing fowl sellers biosecurity practices within selected LBMs in the area councils in the FCT.

CHAPTER 2

LITERATURE REVIEW

2.1. AVIAN INFLUENZA VIRUS INFECTION

2.1.1 Introduction

Notifiable avian influenza is defined by the World Organization for Animal Health as "an infection of poultry caused by any Influenza A virus of the H5 or H7 subtypes or by any avian Influenza virus with an intravenous pathogenicity index (IVPI) greater than 1.2 (or as alternative 75% mortality)"(OIE,2009).

2.1.2 History of Avian Influenza

A disease capable of causing extreme high mortality in infected fowls was first described in 1878 and was known as "fowl plague". The causative organism of this disease was known to be an ultra-filtrable agent, i.e, a "virus" as early as 1901, but it was not until 1955 that the relationship of this and other milder viruses isolated from birds with mammalian Influenza A virus was demonstrated (Easterday *et al.,* 1997).

The first reported isolation of an influenza virus from feral birds was the highly pathogenic avian Influenza (HPAI) H5N3 subtype virus obtained in 1961 from common sterns (*Sterna hirundo*) in South Africa (Becker, 1966).

Highly pathogenic avian influenza (HPAI) has been recognized for well over 100 years and for the first third of the 20th century, the virulent disease was endemic in some countries and occurred fairly regularly in others (Alexander, 1987). Since the first report of a highly pathogenic avian influenza outbreak caused by a virus of H5 subtype in 1959, primary outbreaks of HPAI in poultry have been reported 17 times (Easterday *et al.,* 1997). Most of the 17 outbreaks have shown limited spread, such as that in England in

1991 being self limiting to a single flock of birds (Alexander *et al*., 1993). However, in the United States of America in 1983, Mexico in 1994 and Pakistan in 1995, the disease became widespread infecting many flocks (Easterday *et al.,* 1997). An infection of ostriches caused by the H5N2 subtype was reported in Zimbabwe in 2005 (Alexander *et al.,* 2008). The first HPAI (H5N1) outbreak in Nigeria occurred in February, 2006 at Sambawa farms in Kaduna State (Adene *et al.,* 2006).

2.1.3 Distribution of Avian Influenza

Avian influenza has a worldwide distribution which includes Asia and Middle East, Europe, Africa, North and South America (Easterday *et al.,* 1997; NADIS, 2006). Durosinolorun (2008) reported sero-prevalence of H5N2 in local poultry in Kaduna state.

2.1.4 Incidence of Avian Influenza

The incidence of AI varies with geographic region, species, age of bird, time of year and environmental or agricultural system practiced (Swayne and Jackwood , 2008). Avian influenza is seasonal in high risk areas due to the migratory activities of waterfowls (Abdu *et al.,* 2005). In Nigeria, most outbreaks of avian influenza occur during the harmattan period though an outbreak in waterfowls was reported in the hot month of April in Zaria (Abdu e*t al*., 2005; Wakawa *et al.,* 2008).

Adeniji *et al.* (1993) revealed influenza virus antibodies in chicken sera though neither the genotype nor presence of the antigen was investigated though Owoade *et al*. (2002) reported prevalence of H1N1 in Ibadan of 35%, 57.1% and 93.4% for broilers, broiler breeders and point of lay pullets respectively.

A number of factors have been proposed for the increased incidence of avian influenza which comprise increase in the global poultry population, increase in intensification of

the poultry industry with a concurrent increase in free-range production and even climate change leading to alterations in wild bird migratory paths, improved diagnostic capability and surveillance (Capua and Alexander, 2004; Lee and Suarez, 2005).

2.1.5 Classification of Avian Influenza

Influenza A viruses can be divided into subtypes based on their haemagglutinin (H) and neuraminidase (N) antigens with 16 haemaggulutinin (H1-H16) subtypes and 9 neuraminidase (N1-N9) have been described to date (OIE, 2009).

Based on their pathotype, avian influenza viruses can be grouped into highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) (Abdu *et al.,* 2005). Low pathogenic avian influenza can be infection from any of the 16 haemaggulutinin (H1-H16) and 9 neuraminidase (N1-N9) subtypes with all H5 and H7 subtypes that are not HPAI have been classified as Low Pathogenic Notifiable Avian Influenza (LPNAI) (OIE, 2009). This classification is based on the potential of the LPAI to become HPAI after circulating in poultry. Currently, all avian influenza viruses that have double basic amino acid at the cleavage site are considered highly pathogenic and notifiable, denoted as Highly Pathogenic Notifiable Avian Influenza (HPNAI).

Also, HPAI viruses should have an intravenous pathogenicity index (IVPI) in 6-week-old chickens greater than 1.2 or caused at least 75% mortality in 4-to 8-week-old chicken infected intravenously (OIE, 2009).

Avian influenza has two surface glycoproteins, haemagglutinin and neuraminidase which give the virus its antigenic subtype specificity (Swayne and Jackwood , 2008; Abdu *et al.,* 2005). There are therefore 144 possible avian influenza subtypes with no cross protection between haemagglutinin subtypes (Swayne , 2008). The segmented nature and

the presence of different surface proteins ensures diversity in viral population in both random mutations within the genome segments (antigenic drift) as well as reassortment of gene segments (antigenic shift) between different avian influenza viruses infecting the same animal at a particular point in time (Beard, 1998).

The antigenic differences among avian influenza viruses have varied implications for vaccination as specific antibodies are produced against the haemagglutinin protein and to a lesser extent neuraminidase protein. The importance and interpretation of antigenic drift in poultry though complicated causes changes at antigenic sites affecting specificity of neutralizing antibodies and hence immune response (Suarez, 2008).

2.1.6 Resistance of Avian Influenza Virus to Chemical and Physical Agents

The AI viruses remain viable for long in tissues, faeces and water and can survive low relative humidity and temperature in aerosol; low temperature and high moisture in faeces and remain infective in lake water for up to four days at 25 ^{0}C and over 30 days at 0 ^{0}C (Webster *et al.,*1992; Abdu *et al.,* 2005). However, avian influenza viruses are rapidly destroyed by both physical and chemical methods.

Physical methods include high temperature, extremes of pH, dryness, dry heat, steam and ultra violet (UV) lights (Abdu *et al.,* 2005; Birnbaum and O'Brien, 2008). The UV light is not effective at inactivating AI viruses in poultry houses as organic materials shield the virus from UV light. However, ultra violet light destroys influenza by nucleic acid damage and is most effective on clean surface while heat inactivates avian influenza virus (AIV) in manure when poultry house is heated to 32^{0}C for three hours or 38^{0}C for 30 minutes (Cardona, 2007). Though, treating surfaces with steam at 100 ^{0}C for a long time also inactivates AIV though the possibility of maintaining a constant temperature is being

questioned (AusVetPlan, 2000). Composting of poultry litter when done properly is reported to generate enough heat to inactivate the virus.

Numerous chemical agents are used to inactivate AIV has been reported ranging from simple soap and detergents to numerous disinfectants. Washing of hands with soap and water is the major way of disinfecting the hands with soap and detergents are important in removing the organic materials on surfaces as well as inactivating AIV by destroying the lipid outer membrane necessary for viral attachment (Birnbaum and O'Brien, 2008). The efficacy of soap and detergents in inactivating AIV are influenced by detergent type, specific chemical components, presence or absence of bleach and/or enzymes, water hardness, environmental pH, temperature and presence of organic matter (Birnbaum and O'Brien, 2008).

Disinfectants used against avian influenza are either inorganic or organic such as iodine and iodophors, peroxygen compounds, alcohols, phenols, cresols; quaternary compounds such as hydrogen peroxide and peracetic acid are strong oxidizers and produce free radicals which inactivate AIV. Peracetic acid remains active in the presence of organic matter unlike hydrogen peroxide (Birnbaum and O'Brien, 2008). Though 70% ethanol is effective for inactivation of AIV used especially in small hatchery equipments, its activity is limited in presence of organic materials.

2.1.7 The Disease Entity

2.1.7.1 *Susceptible hosts*

Contrary to previous reports that HPAI viruses are normally present in the wild birds, current evidence strongly suggests that HPAI only arise as a result of mutations after H5 or H7 LPAI viruses have been introduced to poultry from wild birds (Garcia *et al.,* 1996;

Perdue *et al*.,1998). However, findings of Nicolas *et al*. (2008) revealed H5N2 subtype of avian influenza virus with highly pathogenic genotype in healthy water fowls species sampled in Nigeria. It is speculated that interspecies infections with avian influenza do occur (Shane, 2002).

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

2.1.7.2 *Transmission*

Live bird markets, movement of infected birds, contaminated equipment, clothing, shoes, vehicles and eggs are means of AIV transmission (Mubiru, 1998). The spread in a flock is usually rapid by direct contact though on occasion, spread is erratic. The transmission of AI slows as the number of susceptible birds decrease in the population (Swayne, 2003).

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

birds may introduce the virus into poultry flocks (Easterday *et al.,* 1997; Abdu *et al*., 2005).

Direct transmission of virus may be by inhalation of these secretions or contaminated dust via aerosol if birds are in close proximity and with appropriate air movement (Abdu *et al.,* 2005).

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

2.1.7.3 *Incubation period*

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

2.1.7.4 *Clinical signs*

In highly pathogenic avian influenza infections, onset might be sudden with high morbidity (100%) and mortality (50-100%); ceasation to moderate drop in egg production and the laying of eggs with abnormal shell and pigmentation. Greenish diarrhoea, anorexia, depression, ruffled feathers, coughing, sneezing, rales, dyspnoea, sinusitis, mucoid ocular and nasal discharges; cyanosis and oedema of the eyes, head, comb, wattles; discoloration of the shanks and feet have been reported (Easterday *et al.,* 1997; Adene *et al.,* 2006). Poultry that survive the acute disease exhibit nervous signs such as excitation, convulsion, circling and ataxia (Swayne *,* 2003).

The low pathogenic avian influenza (LPAI) in chickens and turkeys causes an inapparent infection to drop in egg production with mild respiratory disease and low mortality. Secondary bacterial or viral infections may cause severe respiratory disease with high mortality (Swayne, 2003; Paul and Schrier, 2001).

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

2.1.7.5 *Morbidity and mortality*

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

2.1.7.6 *Pathology*

Gross pathology

Lesions are variable depending on the severity of disease and the species of poultry (Abdu *et al.,* 2005). In chickens, there is usually congested and swollen comb and wattles; oedema of the eyes. The comb tips are usually cyanotic with nasal and oral discharges. Legs are congested and haemorrhagic; severe congestion and oedema of the lungs; enlargement and congestion of the kidneys with urates. Necrotic foci are present on the wattles, skin, liver, spleen, kidneys and lungs (Easterday *et al.,* 1997). Petechial

haemorrhages of serosal and mucosal surfaces of the heart, proventricular junction, small intestines and abdominal fat (Adene *et al.,* 2006).

In other gallinaceous birds, HPAI virus infections result in oedmatous, haemorrhagic and necrotic lesions in visceral organs, cardiovascular and nervous systems (Swayne and Jackwood, 2008). In per-acute phase, there is usually no lesion unlike in the acute phase, chickens have ruffled feathers with swelling of the head, face, upper neck, legs and shanks accompanied by Petechial to ecchymotic subcutaneous haemorrhages especially in the un-feathered skin.

Low pathogenic avian influenza viruses usually produce catarrhal, fibrinous, serofibrinous, mucopurulent or caseous inflammation of the sinuses. Air sacs are thickened with caseous to fibrinous exudates. There may be catarrhal or fibrinous peritonitis and egg yolk peritonitis: catarrhal and fibrinous enteritis. There is usually ovarian regression with occasional fibrinous pericaditis (Abdu *et al.,* 2005).

Histopathology

In HPAI, histopathologic lesions consist of necrosis and inflammatory changes in multiple organs such as skin, brain, heart, pancreas, lungs, adrenal glands and lymphoid organs (Swayne and Jackwood, 2008). In per-acute phase, there is little or no microscopic lesions while in acute cases, there is multiple organ involvement with predominantly necrotic lesions and apoptotic cell death associated with inflammation, haemorrhages and oedema. There is oedema of dermis of comb with vesicles in epidermis, micro vesicles in the epithelium; larger vesicles or focal necrosis underneath the comb epithelium, invaded by heterophils and lymphocytes, accompanied by a marked lymphocytic peri vasculitis in the dermis (Easterday *et al.,* 1997). The lesions seen on the wattles, are oedema, hyperaemia, haemorrhages and foci of perivascular cuffing. There is severe myocarditis

and myocardial necrosis, oedema, hyperemia and haemorrhages in the heart (Adene *et al.,* 2006).

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

2.1.7.7 ***Differential Diagnosis***

Avian influenza should be differentiated from diseases exhibiting similar clinical signs and gross lesions such as acute fowl cholera, velogenic Newcastle disease, infectious bronchitis, infectious coryza, chlamydiosis, infectious laryngotracheitis, adenoviruses causing respiratory disease or drop in egg production (e.g. EDS '76) and mycoplasmosis.

A tentative and final diagnosis can be arrived at through detection of viral antigen or antibodies in infected poultry through any of the diagnostic method recommended by OIE (OIE, 2009). Classical method for AIV diagnosis is viral isolation in embryonated eggs. Other methods include agar gel Immunodiffusion (AGID) test, haemagglutination inhibition (HI) test, ELISA and reverse transcriptase polymerase chain reaction (RT-

PCR) or real-time reverse transcriptase-polymerase chain reaction (rRT-PCR) test. RT-PCR is more sensitive than viral isolation since it is able to detect nucleic acids of AIV even when the viruses are no longer viable (Spackman *et al.,* 2008).

2.1.7.8 *Control*

There are three possible outcomes for any control strategy which include prevention, management and eradication (Swayne, 2008). The goals are achieved only through development of a broad strategy involving any of the following features: education, biosecurity, diagnosis and surveillance, removal of infected source and reducing host susceptibility to the virus (Swayne, 2008).

The Nigerian strategy is swift detection and control of AI outbreaks and close monitoring of the situation (AICP, 2009). The features of the control strategy involves establishing a viable epidemio-surveillance and laboratory network for proactive surveillance, ensuring swift disease outbreak reporting within 12 hours; stamping- out, disinfecting farms and restriction of movement of poultry, animals and personnel (AICP, 2009).

Education

The general public should be involved in the education process by communicating information on risk and dispelling incorrect information and rumors (Swayne, 2008). Education which is the provision of knowledge on AI transmission and role of individuals in the prevention, management and eradication is a critical aspect in any AI control program (Swayne, 2008).

Avian influenza education involves training of all stake holders in the poultry industry on the control process regarding how the AIV are introduced, spread and how such events

could be prevented. There is also need for educating these individuals on the control of risky behaviors and actions that would reduce the spread of the viruses.

In Nigeria, amongst other communication strategies is the educating and identifying risky behaviors, attitudes, perceptions and believes among community members (AICP, 2009). This is achieved through the initiation of a community dialogue system (AICP, 2009).

Biosecurity

Biosecurity involves prevention of contamination, controlling movement of birds or products, people and equipments or reducing the amount of virus through cleaning and disinfection (Easterday *et al.,* 1997). Biosecurity consist of 2 categories- inclusion biosecurity involving quarantine and other measures designed to keep the virus on infected farm of CZC and exclusion biosecurity aimed at keeping the virus out of AI-free farms or CZC (Swayne, 2008). Cleaning and disinfection of equipments and clothing or footwear or farm workers are essential to prevent introduction and spread from farm to farm.

In Nigeria, the structure of the commercial poultry industry consist of mostly sector 2 and 3 farms with little or no biosecurity and constant introduction of new birds from relatively unknown source which worsen the biosecurity problems (AICP, 2009).

Diagnosis and surveillance

Diagnosis is usually achieved through serological detection of AI antigen or specific antibodies by rRT-PCR, antigen-capture ELISA, AGID test, viral isolation in embryonated eggs or HI test. Accurate and rapid diagnosis is essential for swift and successful control of AI (Easterday *et al.,* 1997).

The poor veterinary infrastructure in Nigeria poses problems with surveillance though measures have been undertaken to improve surveillance. These include developing a traceability mechanism for monitoring of poultry and poultry product movements; revitalizing veterinary quarantine infrastructure and control post; promoting training of backyard poultry farmers on HPAI recognition and control including their role in animal disease surveillance (AICP, 2009).

Passive surveillance is critical for identification of LPAI due to the mild nature of the infection. On the contrary, HPAI with high mortality requires active surveillance to identify where AIV infection is located within a CZC or certifying a CZC free from the infection (Swayne, 2008).

Surveillance is vital for evaluating success of a control strategy and for use in decision making as a prelude to improve control strategies.

Elimination of source of virus

In HPAI, elimination involves humane depopulation and disposal of carcasses, eggs and manure through composing, incineration, rendering or landfill burial though in LPAI infection, controlled marketing of birds 2-3 weeks following recovery from infection and washing of eggs prior to marketing is practiced in some developed economies (Swayne, 2008). The OIE recommends stamping-out policy in notifiable LPAI infection (OIE, 2009).

Stamping-out would only be successful if compensation is paid by governments in a timely manner (Swayne, 2008). Nigeria practices a modified stamping-out policy where birds on infected farms are depopulated and the farms disinfected. This is followed by compensation which is aimed at encouraging reporting of disease outbreak (AICP, 2009).

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

Reducing host susceptibility

Reducing the susceptibility of AIV hosts is compatible with modern rearing systems (Capua and Marangon, 2003). Vaccination when incorporated with other features of AI control such as controlled marketing and surveillance is thought to be cost-effective and utilizes the industry infrastructures, regulatory power, requires neither ethical destruction of healthy birds nor costly disposal (Halvorson, 2005). This method is said to put more control cost on producer with infected birds (Halvorson, 2005). Vaccinated birds are protected from clinical disease; though they shed the virus at reduced amount into the environment making it impossible to differentiate infected from vaccinated birds though inactivated heterologous vaccines allows vaccinated and naturally infected birds to be differentiated using the "DIVA" strategy (Swayne, 2008). The advocates for vaccination believes the ability to differentiate vaccinated/infected from vaccinated/non-infected and ethical question of destruction of healthy birds calls for review of stamping-out as first option in an outbreak of AI (Capua and Marangon, 2003). Increasing host resistance to AIV reduces the negative effects of the disease or prevents infection if exposed to virus. This is achieved through using breeds genetically resistance to AIV or producing passive or active immunity to susceptible host (Swayne, 2008).

There are reports of Thailand native chicken breed as being more frequently present in survivors after H5N1 HPAI village outbreak (Booynawat *et al.,* 2006). Small interfering RNA (siRNA) has been used to silence expression of some AI viral genes in avian and mammalian cells thereby conferring resistance to AI viral replication (Li *et al*., 2005). Currently, Nigeria does not vaccinate as an official policy though there is possibly of adopting the vaccination option (AICP, 2008).

2.1.7.9 ***Public health significance of avian influenza***

Until 1996 there were only three reported isolation of AIV from humans (Capua and Alexander, 2004).

In the 20th century, antigenic shift in human influenza A virus occurred on four occasions, 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1) resulting in pandemic. The H7N7 virus was isolated from a woman with conjunctivitis in England who kept ducks (Banks *et.al*.,2000). There were two independent isolations of H9N2 in March, 1999 from girls aged one and four who recovered from flu-like illness in hung Kong. In May, 1997, H5N1 subtype was isolated from a young child who died and by December, 1997, the same strain infected 18 people of which 6 died (Shortridge *et al.,* 1998). There were also evidences of three cases of human-to-human transmission within families (Capua and Alexander, 2004). In Nigeria, only one human death has being reported in 2007 in Lagos.

2.1.7.10 ***Economic significance of avian influenza***

Since its emergence in Africa in 2006, the highly pathogenic avian influenza (HPAI) virus of the H5N1 subtype has spread rapidly to poultry farms in several countries, including Burkina Faso, Cameroon, Ghana, Niger and Nigeria (OIE, 2009). In February

2006, Kaduna State in Nigeria was the first of 36 States to report the infection of poultry by H5N1 (Monne *et al*., 2008). By April 2006, more than 325,000 chickens in Nigeria alone were identified as having H5N1 virus; of these, 223,000 died of H5N1 infection and the rest were slaughtered as a control measure (Ahmed, 2006). Since 2006, the infection has spread to 25 States and FCT in Nigeria (AICP, 2009). Though the last outbreak occurred in October 2007, the spread of HPAI poses a challenge to the poultry industry in Nigeria (Ahmed, 2006).

Based on estimates from the National Bureau of Statistics (NBS) in Nigeria, since 2000, the poultry subsector in Nigeria grew at 5.9 percent per year, reaching a population of 150 million in 2005 until the appearance of HPAI in 2006 (Obi an*d* Ahmed, 2008). Since then, a significant reduction in the poultry trading activities (imports and exports) has been observed in Nigeria (Obi an*d* Ahmed, 2008).

Outbreaks of HPAI undoubtedly pose serious consequences on the livelihood of many poor households, disrupting an important source of cash income for producers and a source of protein for consumers' diets. Moreover, HPAI can threaten the advancement of women, as raising poultry has become an important channel for women in developing countries to develop assets or income because entry costs are low and the work can be integrated with their domestic responsibilities (Ahmed, 2006)

2.2 LOCAL POULTRY

Local poultry production and consumption fits especially well with the sustenance economy imposed on hundreds of millions by circumstances of large family size and intractable poverty as they provide sustainable income at minimal investment as well as convenient, cheap protein. As egg producers, chickens offer the additional advantage of

being the ideal size to accommodate instant preparation for total consumption as a single family meal. The advantage of avoiding waste adds to the importance of local poultry in rural communities.

Local poultry are products of their environment and have survived hash conditions for many generations. These local poultry are late maturing with a unique genetic make up enabling them to become broody, hatch their own offspring and rear them in low input systems (Abdu *et al.,* 1992). Their meat taste very different from commercial broiler meat and is highly demanded in rural communities.

2.2.1 Population of Local Poultry in Nigeria

Local birds are kept in villages in small numbers where disease control is a major constraint limiting their production. The husbandry system of managing local birds is usually either extensive or semi extensive where birds are provided some food and left to supplement their diet through scavenging with provision of housing (Abdu *et al.,* 1999). These management systems expose the birds to continuous challenge by pathogens and other environmental hazards, such as extremes of temperature. Studies in Kaduna State revealed that local chickens were kept by 98.3% of farmers, ducks by 32.7%, turkeys by 13.3%, guinea fowls by 11.5% and pigeons by 14.2% (Abdu *et.al.,* 1999). The mean number of birds per household was 28.9 for chickens, 5.8 for ducks, 3.2 for turkeys, 10.8 for guinea fowls and 31.7 for pigeons (Abdu *et al.,* 1999).

Local poultry population in Nigeria was estimated at about 150 million of which 102.8 million are local chickens (RIM report, 1993); 44 million guinea fowls (Akinwumi *et al.,* 1979); 1 million turkeys and 1 million ducks (Nawaethe and Lamorde, 1982).

2.2.2 Diseases of Local Poultry

Diseases and predators are main causes of mortality in local poultry thereby hindering the development of the industry (Ibrahim and Abdu, 1992). Kekeocha (1978) identify the harsh climatic condition to which local poultry are exposed due to the extensive system of management as a contributing cause of mortality though this is contrary to reports that local fowl are adapted to stressful tropical environment (Nwosu, 1997). Ayivor and Hellings (1982) reported 70% mortality of local fowl hatched between hatching and brooding. Viral, bacterial and fungal diseases have been reported together with ectoparasites and endoparasites (Sa'idu *et al.,* 1994).

2.2.2.1. *Viral diseases*

Newcastle Disease (ND)

Studies revealed that ND occurs between October and March during the harmattan period with suggestions that the incidence within these months are influenced by wind velocity on transmission and the outcome is being worsened by the cold stress (Sa'idu *et al.,*1994, Manchang *et al.,*2004). Newcastle disease is often reported as concurrent infection with fowl pox, external parasites or helminths (Sa'idu *et al.,* 1994).

Newcastle disease is believed to be the most devastating disease in the local poultry and the main cause of the high mortality (Bell, 1992; Nwanta *et al.,* 2006). Local fowls are reported to be more resistant than exotic breeds (Oluyemi *et al.,* 1979), while young domestic fowls and guinea fowls are more susceptible than adults (Nwanta *et al.,* 2006). Turkey and pigeon may develop generalized disease but clinical signs are rare in ducks and geese (Nwanta *et al.,* 2006). Wild and domestic birds sometimes harbor Newcastle disease virus while showing no detectable clinical signs of the disease (Alders and Spradbrow, 2001). These strains require several passages in chickens before becoming

highly virulent for chickens. As village poultry have more contacts with these birds, they may play a part in amplifying the virulence of field viruses. Therefore, village chickens are probably very important for survival of ND virus in the environment and may also play an important role in the spread of the virus among commercial poultry flocks (Abdu *et al.,* 1999).

Effective control of ND in rural poultry would require effective application of suitable vaccines and rigorous biosecurity which is lacking in Nigeria (Nwanta *et al.,* 2006), and a monitoring system be put for surveillance of common disease of LP (Nwanta *et al.,* 2008).

Previous Nigerian governments in the 1950's and 60's organized vaccination campaigns for ND prevention in local poultry but was discontinued notwithstanding its successes. Rural poultry farmers reduce their losses by reducing their flock size through sales before or during the period of greatest risk though some slaughter sick birds for consumption or sell cheaply to butchers (Abdu *et al.,*1999) which assist in the spread of the disease (Nwanta *et al.,* 2006).

Infectious Bursa Disease (IBD)

Infectious bursa disease also known as Gumboro is an acute, mild or subclinical viral infections of 3-7 weeks old chickens characterized by trembling, incoordination, inflammation followed by necrosis and atrophy of bursa of Fabricius and immunosupression (Abdu, 2007). It was first reported in Nigeria in 1969 (Ojo *et al.,* 1973).. Sa'idu *et al.,* (1994) reported more than 50% of IBD cases in local fowls were concurrently infested with external parasites and harbor helminthes and coccidia.

The average mortality rate of IBD in local poultry was reported as 16% being second to ND as cause of mortality in local chickens (Abdu *et al.,* 1992). Sero-pervalence studies of local poultry in Tanzania revealed IBD prevalence of 36% in the dry season and 42.3% in the rainy season (Permin;1997).

A retrospective study of IBD in the Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH) revealed that IBD occur throughout the year with high incidence from April to July and November though another study reported no seasonality for IBD with highest incidence occurring in May and December (Abdu *et al.,* 1992; Tong *et al.,* 1992). Local chickens on free range were 3.5 times more likely to be infected with IBD virus compared to confined exotic breeds (Tong *et al.,* 1992).

Fowl Pox

Fowl pox is a common viral disease in local poultry frequently seen in young birds less than 10 weeks old which is often severe (Sa'idu *et al.,* 1994). It is usually prevalent in the rainy season from April to September (Sa idu *et al.,* 1994) and reported as the third most important causes of mortality in local chickens (Abdu *et al.,* 1999). Fowl pox is controlled by vaccination through intradermal route. Fowl pox together with ND and IBD account for 76% of mortality in LP (Abdu *et al.,* 1999)

2.2.2.2 *Bacterial, ricketsial and fungal diseases*

There is serological evidence of *Brucella abortus, Salmonella gallinarium* and *Salmonella pullorum* infection in local poultry (Abdu *et al.,* 1992; Assam, 1997). *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies were also found in local chickens (Abdu *et al.,* 1999). Tuberculosis is present in local poultry but seldom cause troubles as it is a disease of older birds and nowadays fowls are known slaughtered while

still comparatively young. Fowl typhoid, colibacillosis and fowl cholera are common bacterial disease in local chickens (Abdu *et al.,* 1999). Other bacteria and fungi isolated from local poultry include *Streptococcus faecalis*, staphylococcus, *Campylobacter fetus sub sp. jejuni, Trichopyton gallinae, Aspergillus fumigatus* and *Candida albicans* (Abdullahi *et al.,* 1992). Occular aspergillosis characterized by conjunctivitis and serous ocular discharge; and pulmonary aspergillosis have been reported (Abdu *et al.,* 1992).

2.2.2.3 *External parasites*

There are reports of severe parasitism in rural poultry among which nematode, lice and cestodes impairing their production potential. Although it was suggested that better nutrition would reduce adverse effects of parasitism including rate of infestation, parasitism is reported to be problem to optimum production performance of poultry (Rahman *et al.,* 2003). Studies have been undertaken to detect type and intensity of parasites in the local poultry which revealed various types of poultry parasites with severe to mild load (Rahman *et al.,* 2003). It had been reported that parasitism had hampered up to 17% of weight gain in growing chicks and 12.5% of egg production in laying hens (Bhowmic *et al.,* 1982)

Lice are common though seldom responsible for pathology in especially free range birds although under intensive management lice infestation may result in feather damage, reduced feed intake, abandonment of eggs and death (Fabiyi,1988). The lice encountered in Nigeria were *Menacanthus Stramineus*; Menacanthus palliclulus in forest area, *Menopon gallinae* in forest and parts of savannah areas, *Numidilipeurius tropicalis* and *Goinocotes gallinae* through out the country; *Liperurus caponis, Amyrsidea powelli and Gonoides gigas* (Fabiyi, 1988; Abdu *et al.,*1992; Assam, 1997). Prevalence of lice was

higher during the hot season than in the cold season (Fabiyi, 1988). However, Adene *et al.* (1975) reported the presence of *Culogaster heterographus* in local chickens which was absent in the study carried out by Fabiyi (1988) who rather reported presence of *Culogaster occidentalis.*

Ticks, fleas and flies reported in rural poultry were *Argas persicus, Haemophysalis hoodi hoodi, Amblyomma variagatum, Hyolomma sp., Echidnosphaga gallinae and pseudolynchia canariensis* respectively (Abdu *et al.,* 1992).

Mites infestation when heavy, results in death of local hens sitting on egg due to blood loss or abandonment of eggs. The mites reported in local poultry include *Bdellonyssus bursa, Dermanyssus gallinae, Meganinia cubitales, Cnemidocoptes sp, Syringophilus spps and Cytolocchus nudus* (Abdu *et al.,* 1992).

2.2.2.4 *Helminthiasis*

Reported helminths of LP in Nigeria are *Tetrameres* spp*., Syngamus trachea, Capillaria sp., Gongglonema* spp*., Dispharynx spiralis, Acuria* spp*., Cheolospirura hamulosa, Heterakis* spp. *Subulura* spp. *Strongyloides avium and Ascaridia* spp*,* (Sa'idu *et al.,* 1994; Abdu *et al.,* 1999). Others include *Ameobatarenia* spp*, Choanotaenia infundibulum, Davainea proglottina, Hymenolopis* spp*, Raillietina* spp *and Prostharmostomum* spp (Abdu *et al.,* 1999; Assam, 1997).

The most common helminths of local chicken are *Syngamus trachea, cestodes, Tetramere* spp*. and Ascaridia galli* in which over one third of infested local poultry had more than one specie of helminths (Abdu *et al.,* 1992). Helminth infestation is common during the rainy season though Schillhorn Van Veen *et al.* (1975) reported no seasonal difference in helminth infestation except those with indirect life cycle (Spiruroids and tape worms)

which are common during dry season. Young birds have higher incidence of helminthiasis (Abdu *et al.,* 1992; Sa'idu *et al.,* 1994).

Helminthes are among the common endoparasites in LP as they become infested during scavenging for food by picking up helminthes eggs and feeding on insects and earthworms which are intermediate hosts of helminthes. Studies on incidence of infection among local poultry in Bangladesh revealed helminthes incidence rates of 7.82%, 62.69%, 54.61%, 4.48%, 58.71%, 4.10% and 0.512% for *Acuaria hamulosa, Ascaridia galli, Heterakis gallinae, Capillaria sp., Raillietia sp., Echinostoma revolutum and Monostom sp.* respectively (Rahman *et al.,* 2003).

2.2.2.5 *Coccidiosis*

Coccidiosis is a rare clinical entity in village poultry though Eimeria spp. have been reported in rural poultry (Assam, 1997). Coccidiosis results in death of chickens (51%) but with concurrent infection of IBD in local chickens, mortality can be up to 75% (Schillhorn Van Veen *et al.,* 1975).

2.2.2.6 *Hemoparasitism*

Frequently encountered hemoparasites of rural poultry were *Plasmodium relictum* and *Borrelia anserina* though *Plasmodium gallinaceaum, Aegyptianella pullorum* and *Leucocytozoon sp.* have been reported in local poultry (Abdullahi *et al.,* 1992; Assam, 1997).

2.2.2.7 *Miscellaneous conditions*

Other conditions reported in rural poultry were unabsorbed yolk sac, bumble foot and egg yolk peritonitis (Abdu *et al.,* 1992). The non infectious conditions were fracture of the leg, femur and shank; subcutaneous emphysema, ventral and abdominal hernia, torsion of

the intestine; prolaspe of rectum, dog bite, kerosene and rodenticide poisoning. Tumors of the wings, eyes, lungs, proventriculus, subcutaneous tissue of the neck; metacarpus, ceacal tonsils and ball of the foot (Abdu *et al*.,1992).

2.2.3 Local Poultry and Avian Influenza

Local poultry classified within sector 4 of the FAO classification has minimal or no biosecurity due to its scavenging feature. The low biosecurity is frequently cited by some Asian countries as one of primary risk factors for continuous outbreaks and the persistence of the virus in domestic poultry populations (Otte *et al.,* 2006). Based on this assumption, some governments are considering the prohibition of unconfined poultry flocks in order to increase 'biosecurity' in LP production (Otte *et al.,* 2006). These measures may be prohibitively expensive for resource-poor local poultry producers and thus could force them to abandon poultry keeping, thereby depriving them of small but valuable amounts of protein in their diets, much needed petty cash and, mostly importantly, an investment opportunity for escaping poverty (Otte *et al.,* 2006).

The control of highly pathogenic avian influenza (HPAI) is priority for all poultry production systems including LP. Local poultry roam freely in villages with increased chances of contacting wild birds and other AIV contaminated formites. In Nigeria, outbreak of HPAI in LP has been reported in Jigawa State (Wakawa *et al.,* 2009).

A reduction of overall mortality in LP by vaccination against endemic killer diseases such as ND, fowl cholera and duck plague, with rapid and effective disposal of dead birds, points the way to a cost-effective approach to the long-term prevention and control of HPAI (Alders *et al.,* 2007). This awareness is critical for the survival of the LP producers themselves in the events of a HPAI pandemic due to their management systems (Rushton *et al.,* 2005).

2.2.4 Role of Live Bird Markets in the Spread of Highly Pathogenic Avian Influenza

Terrestrial birds, such as quail and chicken, two species commonly found at live bird markets, are not natural hosts for influenza, yet may be recognizable enough by the virus to become infected (Suarez *et al.,* 1999). Despite this, the influenza virus, finely attuned to ducks and other waterfowl and having remained in total evolutionary stasis in aquatic birds, must mutate to survive and effectively spread in, for example, the gut of a chicken (Webster, 2004). The influenza can no longer remain a strictly waterborne virus although it can still spread through feces when chickens peck at each other's droppings, the virus must and presumably adapt to the novel body temperature and pH of its new environment (Dronamraju, 2004; Hollenbeck, 2005).

In such foreign environments as land-based poultry, influenza is highly effective at mutating and quickly begins to try to adapt to the new host in order to survive (Shortridge, 1992; Suarez *et al.,* 1999; Suarez, 2000). Given adequate time and number of hosts within which to mutate, some bird flu viruses are able to learn how to invade other organs, such as the lungs, through which to travel.

Researchers have shown that H5N1 seemed to enter chicken populations as an intestinal virus but leave as more of a respiratory virus. (Sturm-Ramirez *et al.,* 2005). The heamagglutinin spike of H5 and H7 viruses can mutate over time to be activated by enzymes in any organ in the body, allowing the virus to essentially liquefy the bird from the inside- the "flubola" phenomenon of highly pathogenic avian influenza. (Marwick, 1998). While influenza remains harmless to its waterfowl hosts in its natural state, the virus can be forced into what evolutionary biologists call an "acute life strategy" in which

its only choice may be to rapidly overwhelm the host to gain entry and survive in new environments. (Van-Blerkom, 2003). Natural selection mediates this evolutionary process, choosing over time the virus with the perfect balance of lethality and contagion (Dimmock *et al.,* 2001). However, transitions are not always towards lesser virulence (Davis, 2005). Infecting an unfamiliar host such as a chicken, the influenza virus may become as virulent as possible to overpower the bird's defenses (Suarez, 2000).

2.2.5 Mutation of Avian influenza Virus Facilitation by Industrial Poultry Production

The world Organization for Animal Health (OIE) and the Food and Agriculture Organization of the United Nations (FAO) agree that it has been proven that once LPAI viruses gain access to poultry facilities, they can "progressively gain pathogenicity in domestic birds through a series of infection cycles until they become HPAI (Capua and Marangon., 2003; Morris and Jackson, 2005). However, deadly bird flu viruses do not tend to arise in merely any poultry operation as according to U.S. Department of Agriculture researchers, it is the "high density confinement rearing" method that gives bird flu "a unique chance to adapt to the new species" (Suarez *et al.,* 2003).

Hafez (2003) has noted how intensification in farm animal production practices often allows pathogens to enter the food chain at its source and to flourish, largely because of stress-related factors. The physiological stress created by crowded confinement can have a "profound" impact on immunity, predisposing animals to infection through diminished immune function thereby reducing protective responses to vaccinations (Maes *et al.,*

2000; André and Tuyttens, 2005). As vaccine immunity is compromised by factors such as immunosuppressive stress, mutant clones have an increased opportunity to selectively multiply and to be seeded in the environment (Witter, 1998; Quattro, 1999). Studies exposing birds to stressful housing conditions provide solid evidence in support of the concept that stress impairs adaptive immunity in chicken (El-Lethey *et al.,* 2003).

Moist fecal dust and ammonia in the air irritate the birds' respiratory passages, further increasing susceptibility in chickens already compromised by the stress of confinement. Feces decomposition generates several irritating chemicals, including hydrogen sulfide, methane, and ammonia which in a poultry house is nauseating to the caretaker, irritates the eyes, and affects the chickens (Cole *et al.,* 1999).

According to the WHO, the birds are often slaughtered on the spot, normally with very little regard for hygiene (Macan-Markar, 2005). Birds that remain unsold at the end of the day may go back to nearby farms, taking whatever new viruses they picked up with them (Appenzeller, 2005). Highly concentrated poultry and pig farming, in conjunction with traditional live animal or 'wet' markets, provide optimal conditions for increased mutation, reassortment and recombination of influenza viruses (Webster, 2004). Considering the Hong Kong outbreak and other outbreaks around the world traced to live poultry markets, live bird markets are described as the missing link in the epidemiology of avian influenza.

Efforts to purge bird flu viruses from live poultry markets over the years have been unsuccessful despite periodic quarantine, depopulation, cleaning, and disinfection (Lee *et al.,* 2004). In 2004, an outbreak of highly pathogenic H5N2 was discovered in a 7,000-chicken broiler facility in Texas after the owner introduced a chicken from a live poultry

market in Houston into his flock leading to the description of live-bird markets in large metropolitan areas as a man-made reservoir of influenza virus (Senne *et al.,* 2006).

The rampant reassortment of AIVs in LMBs could increase the risk of species crossover because it would increase the chances of the occurrence of the correct constellation of genes to create a virus that replicates efficiently in mammals (Suarez *et al.,* 1999).

When an outbreak of avian influenza occurs in an area with a high poultry population density, the application of rigorous biosecurity measures might not be possible (Capua and Marangon, 2003). Implying biosecurity measures may be simply incompatible with modern high-density rearing systems (Capua and Marangon, 2003). Similarly, when sheds and facilities are situated in close enough proximity to another, once seeded, the virus may be able to spread through the wind (Alexander, 1993).

Though the US poultry industry claims to have the best biosecurity systems in the world there are reports of widespread disregard for biosecurity precautions among both large and small domestic producers (Canning, 2005; Schmit, 2005).

2.2.6 Evidence of Highly pathogenic Avian influenza in Nigeria and its Genetic Characterization

The prolonged continuous circulation of HPAI H5N1 virus in domestic poultry in Asia is unprecedented mainly because the virus has evolved into multiple genetic lineages that differ antigenically (Webster *et al.,* 1992). The Qinghai strain, the predominant variant in Africa, has acquired several troubling properties, including respiratory rather than fecal transmission in poultry, increased thermal stability and PB2 gene mutation associated with pathogenicity in animals, including human beings.

Since the earliest known progenitor detected in China- A/goose/Guamdong/96, numerous lineages of HPAI (H5N1) viruses have emerged (OIE, 2009). Introduction and spread of distinct H5N1 genetic lineages were described in several Asian countries and Europe, such as Canada , Italy and France (Salsberg *et al,* 2007; Starick *et al,* 2007). Surprisingly, Nigeria is the only country in Africa where HPAI H5N1 belonging to distinct sub lineages have been detected (Ducatez *et al.,* 2007; Salsberg *et al.,* 2007).

Previous genetic characteristics of HPAI (H5N1) viruses isolated during 2006 and 2007 Nigerian outbreaks indicated the circulation of 3 distinct sub lineages designated I, II and IV (Joannis *et al.,* 2006; Owoade *et al.,* 2006). Sub lineages I and II appeared to be widespread in Nigeria during 2006 and 2007 outbreaks and their extended circulation enabled reassortment events between these sub lineages (Owoade *et al.,* 2006). The first reassortment virus was identified in June 2006, and early in 2007, an additional reassortment virus was identified in 7 of 22 Nigerian States where infection was found (Joannis *et al.,* 2006).

The Nigerian isolate A/duck/Nigeria/3724-2/2008 resulted in a genome almost identical to an isolate from a wild mute Swan- A/Cygnus olor/Czech Republic/10732/2007) (Ducatez *et al.,* 2007). This finding however did not shed light on how the virus was introduced into Nigeria because neither of the two means of spread, through wild birds or the poultry trade can be excluded.

Until the recent H5N1 outbreaks involving ducks, geese, swans, egret and black headed gull, naturally occurring outbreaks of lethal avian influenza has not been reported in aquatic birds worldwide since 1966 (Becker, 1966).

Occasional wild bird deaths have occurred at the time of poultry outbreaks, as was the case with H7N1 infection in a shaker falcon in Italy (Magnino *et al.,* 2000). It had been generally considered that avian influenza viruses characterized as highly pathogenic in chickens did not cause death or clinical disease in ducks or wild aquatic birds (Swayne and Suarez, 2000).

Experimental studies with a 1997 Hong Kong Origin H7N1 viruses (A/chicken/Hong Kong/220/97) in a wild range of avian species showed that this viruses could infect multiple avian species and its virulence varied significantly among avian species including species from the same order (Swayne, 2003). This suggests that there may be difference in inherent resistance to this H5N1 virus in some of the water fowl species (Swayne, 2003).

The immunoxidase staining using anti-H5 monoclonal antibodies on lung and brain cryostat sections from infected birds clearly demonstrated that brain and lungs lesions were associated with H5 virus replication (Swayne, 2003). Similarly isolation of H5N1 viruses from the lung and brain tissue showed that H5N1 virus replication was occurring in a broad range of tissues than was considered usual for avian influenza virus in aquatic birds, where infection is usually asymptomatic and enteric (Swayne and Suarez, 2000).

Recent avian influenza H5N1 virus isolate obtained from the meat of domestic ducks originating from mainland China caused lesions and mortality pattern of HPAI when inoculated into chickens but no clinical disease in ducks, although virus replication occurred in the lungs, brain and skeletal muscles of these ducks (Tumpey *et al.,*2002).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The study was carried out in Abuja, the Federal Capital Territory (FCT) of Nigeria. Abuja is located just north of the confluence of the Niger and Benue Rivers. It is bordered by Kaduna and Niger States to the North East, Nasarawa State to the East and Kogi State to the south. It lies between latitude 8.25^0 and 9.2^0 north of the equator and longitude 6.45^0 and 7.39^0 east of the Greenwich meridian. The FCT has a land mass of approximately 800 km^2 of which the actual city occupies 250 km^2 and is situated within the Guinea savannah. The FCT comprises six area councils namely Abuja municipal, Gwagwalada, Kuje, Bwari, Kwali and Abaji Area Councils.

3.2 SAMPLE SIZE

The sample size was determined by using the formula:

$$N = \frac{Z^2pq}{d^2}$$ Mahajan (1997)

Where N = Sample size; Z= 1.96; p = prevalence; q = 1-p; d = Allowable error

Therefore, using the prevalence of 18.2% (Durosinlorun, 2008)

$$N = \frac{1.962 \times 0.192 \times (1-0.192)}{0.052} = 238 \text{ X } 4 = 952$$

3.3 BLOOD SAMPLE COLLECTION

After obtaining due permission from the fowl sellers, blood samples were collected at the live bird markets in Abuja from chickens, guinea fowls, pigeon, turkeys and ducks within the months of June and August, 2010.

Two millilitres (2 ml) of blood was collected from the brachial vein of each bird using a sterile of needle and syringe. The blood was carefully transferred into a sterile sample bottle and labeled with an acronym number, LBM, poultry species and date of collection and kept in a slanting position in a shade for 24 h to allow for clothing and formation of sera. The sera was harvested into sterile serum bottles and kept at -20^0C until tested.

3.4 SEROLOGICAL TEST PROCEDURE

3.4.1 Preparing 1% Chicken Red Blood Cells (Rbcs) Suspension

The reagents required are isotonic PBS (0.01M), pH 7.0-7.2 and red blood cells (RBCs) taken from a minimum of three different chickens and pooled into an equal volume of Alsever's solution. The red blood cells were washed by centrifugation at 220 **g** for five minutes three times in Phosphate Buffered Saline (PBS) [pH7.4]. One millilitre of RBCs was added to ninety-nine millilitre of PBS to prepare 1% of RBCs.

3.4.2 Haemagglutination Test as conducted by OIE, 2009

i. 0.025 ml of PBS was dispensed into each well of a microtitre plate.

ii. 0.025 ml of antigen was dispensed into the first well. For accurate determination of the HA content, serial double dilutions, i.e. 1/2, 1/4, 1/8, 1/16, etc. was used.

iii. Two fold dilutions of 0.025 ml of the antigen suspension across the plate was made.

iv. 0.025 ml 0f 1% (v/v) chicken RBCs to each well was dispensed

v. The plate was gently tapped for proper mixing, and then allowed the RBCs to settle for about 15-20 minutes at room temperature.

vi. The HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration reading the highest dilution giving complete HA (no streaming); this represents 1HA unit (HAU) and was calculated from the initial range of dilutions.

3.4.3 Detection of Avian Influenza and Newcastle Disease Antibodies

3.4.3.1 *Haemagglutination inhibition test as conducted by* OIE, 2009

vii. 0.025 ml of PBS was dispensed into each well of a microtitre plate.

viii. 0.025 ml of serum was added into the first well of the plate

ix. Two fold dilutions of 0.025 ml volumes of the serum across the plate were made

x. 4 HAU of antigen in 0.025 ml was added to each well and left for 20 minutes at room temperature.

xi. 0.025 ml of 1% (v/v) chicken RBCs was added to each well and gently mixed and was left to settle for 15-20 minutes at room temperature.

xii. The HI titre was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.25 ml RBCs and 0.05 ml PBS only) were considered to show inhibition.

xiii. The validity of result was assessed against a negative control serum, which should not give a titre > 1/4 (> 2^2 or > log 2^2 when expressed as

the reciprocal), and a positive control serum for which the titre was within one dilution of the known titre.

The HI titre was regarded as being positive if there was inhibition at a serum dilution of 1/16 (2^4 or $\log 2^4$ when expressed as the reciprocal) or more against 4 HAU of antigen.

The true prevalence was determined by using the formula:

Prevalence = no. of Positive samples/Total No. of sample tested x 100/1

3.4.4 Detection of Gumboro Disease Antibodies

3.4.4.1 *Agar gel immunodiffusion test*

Agar gel preparation

The agar gel was prepared by mixing 8 g of sodium chloride 0.02 g of sodium azide and 1.25 g of agar in 100 ml of distilled water and heating the mixture slowly to boiling. Twenty five mls of the boiled mixture was then poured into 9 x 9.5 Perspex dishes to form gels (Harai *et al.,* 1972).

Using a template cutter, wells of 6mm diameter with 3mm distance from the other were cut in the agar gel. An offset linear pattern of 12 parallel rows was used with the middle row of every three having every hole cut. The middle row was for antigen while the two complete rows to either side were for antiserum dilutions. This made it possible for one antigen to serve four adjacent antiserum wells. The cut agar plugs were then removed from the wells with a hypodermic needle (Harai *et al.,* 1972).

Gumboro Disease (GD) Antigen used for the Test

The GD antigen was derived from infected bursae of chickens that died from natural GD. The infected bursae were removed, ground in a mortar and diluted 1:1 (w/v) with PBS (pH 7.6) and centrifuged at 1000 g for 5 minutes. The supernatant was then used as GD

antigen. A positive control and a known negative antiserum were included in each test run.

About 15 ul of antign was put into the central well and test sera were added to adjacent wells. The Petri dishes were incubated at room temperature in a humid chamber for 24-48 hours before being read. Positive test samples showed precipitin lines of identity to the GD antigen.

3.4.5 Questionnaire

Fourty eight (48) Structured questionnaires were administered by myself to fowl sellers at LBMs to asses their knowledge on avian influenza, biosecurity practices, system of management, readiness to disclose outbreaks of bird flu, method of disposal of dead birds and offal, handling of sick poultry, sources and destination of birds bought at LBMs.

3.5 DATA ANALYSIS

Data obtained from test results and questionnaires were entered into Excel spreadsheet and analyzed using the Statistical Package for Social Science (SPSS) version 17 to determine frequency and mean and test for association by cross-tabulation. Where values of $p<0.05$ was considered significant by chi-square test.

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CHAPTER 4

RESULTS

4.1 AVIAN INFLUENZA, NEWCASTLE AND GUMBORO DISEASE ANTIBODY IN POULTRY IN LIVE BIRD MARKETS IN ABUJA

A total of eight hundred and ten poultry sera samples were collected from nine live bird markets in the FCT though only 329, 266 and 215 were tested for AI, ND and GD antibodies respectively due to unavoidable loss of some samples. However, the antibodies prevalence for AI, ND and GD in chickens were 63.3%, 63.8% and 69.4%, respectively. Nonetheless the mean antibodies titre for AI was 10.40±0.94 $\log_2$, while ND mean antibodies titre was 5.91±0.27 $\log_2$.

The prevalence of AI in chickens was 63.3% with ND prevalence in ducks being 43.8% (Table 4.1). All the poultry species sampled in the study had a mean antibodies titre above 10 $\log_2$ though all ducks and 87.9% of chickens tested had AI and ND antibodies titre $\geq 7\log_2$, respectively (Table 4.2).This could be attributed to natural exposure or routine vaccination as in the case of chickens.

The prevalence of AI, ND and GD among the different LBM revealed that Gariki Area I LBM had an AI prevalence of 87.9 % with Garki II LBM having an ND prevalence of 82.4 %

All poultry in Abaji, Bwari, Garki makeshift and New extension LBMs tested for AI had antibodies titre $\geq 7\log_2$, though only 50 % of poultry in Bwari LBM had ND antibodies titre $\geq 7\log_2$ (Table 4.3) and this shows that birds in Bwari will be more susceptible to ND outbreak.

Table 4.1: Prevalence of Avian Influenza, Newcastle and Gumboro disease antibodies in poultry species in Abuja, Nigeria

Poultry species	Avian influenza (%)	Newcastle disease (%)	Gumboro disease (%)
Chicken	179/283 (63.3)	148/232 (63.8)	129/186 (69.4)
Duck	21/25 (84.0)	7/16 (43.8)	7/13 (53.7)
Guinea fowl	5/10 (50)	8/10 (80)	6/8 (75)
Pigeon	2/3 (66.7)	Not Tested	3/3 (100)
Turkey	8/8 (100)	8/8 (100)	5/5 (100)

Table 4.2: Mean Avian Influenza and Newcastle disease antibody titre and proportion of poultry species with antibody titre ≥ 7 Log_2 in Abuja live bird markets.

Poultry species	Avian influenza		Newcastle disease	
	Mean titre (Log_2)	**% Poultry with AI titre ≥ 7 Log_2 (p=0.001)**	**Mean titre (Log_2)**	**% Poultry with ND titre ≥ 7 Log_2 (p=0.37)**
Chicken	10.4	96.6 (173/179)	6.4	87.9 (131/149)
Duck	10.2	100 (21/21)	9.2	71.4 (5/7)
Guinea fowl	10.5	60 (3/5)	9.8	77.5 (7/9)
Pigeon	10.6	100 (2/2)	NT	NT
Turkey	10.6	87.5 (7/8)	8.6	87.5 (7/8)

Table 4.3: Prevalence of Avian Influenza and Newcastle disease and Gumboro disease antibodies in live bird markets in Abuja

Live bird market	Avian influenza prevalence (p=0.00)	Gumboro disease Prevalence	Newcastle disease prevalence (p=0.006)
Abaji	78.4 (29/37)	62.5 (20/32)	74.3 (26/35)
Garki Area I	87.9 (29/33)	66.7 (12/18)	62.5 (15/24)
Garki II	77.3 (17/22)	77,8 (14/18)	82.4 (14/17)
Gariki II make shift	66.7 (6/9)	NT	NT
Garki New Extension	22.2 (2/9)	71.4 (5/7)	50 (5/10)
Bwari make shift	40 (2/5)	NT	NT
Bwari	41.4 (12/29)	75 (12/16)	40 (2/5)
Gwagwalada	79 (64/81)	74.6 (44/59)	64.3 (27/42)
Kuje	60.7 (17/28)	56 (14/25)	66.9 (81/121)

NT= not tested

Table 4.4: Distribution of poultry with Avian Influenza and Newcastle disease Antibody titre ≥ 7 Log_2 within live bird markets in Abuja.

Live bird market	% Poultry with avian influenza antibody titre ≥ 7 log_2 (p=0.00)	% Poultry with Newcastle disease antibody titre ≥ 7 log_2 (p=0.00)
ABAJI	100 (29/29)	84.6 (22/26)
Garki area I	96.6 (28/29)	80 (12/15)
Garki II	82.2 (15/17)	75 (12/16)
Garki New Extension	100 (2/2)	60 (2/5)
Garki II makeshift	100(6/6)	Not tested
Bwari make shift	100 (2/2)	Not tested
Bwari	100 (12/12)	50 (1/2)
Gwagwalada	95.3 (61/64)	100 (27/27)
Kuje	94.1 (16/17)	88.9 (72/81)

4.2 ASSESSMENT OF FOWL SELLERS BIOSECURITY PRACTICES WITHIN LIVE BIRD MARKETS IN ABUJA

The assessment of fowl sellers' biosecurity practices revealed that all fowl sellers keep sick poultry in cages while another 93.8% would separate poultry by species. Similarly, all fowl sellers separate poultry by type and have poultry in crowded cages. However, 8.3% of the sellers sold wild birds in LBM with 33.3% selling waterfowls. All fowl sellers stack poultry cages on another with 68.8% selling their poultry from the ground and 72.9% reported presence of rodents in their LBM (Figure 1).

Also, practices that may lead to disease spread were assessed by their action on sick and dead poultry, disposal of poultry manure and offal. Among fowl sellers who participated in the study, 64.6% use sick poultry for food with 33.3% selling sick poultry though 8.3% burn sick poultry with 4.2% either burying or throwing away sick poultry. However, 2.1% would give away sick poultry as gift.

About half of the fowl sellers interviewed would burn dead poultry while 20.8% and 29.2% would bury or throw away dead poultry. Less than a third (29.2%) of fowl sellers would throw away offal, with 25.0 % giving out poultry offal as gift while 20.8% eat offal. However, 14.6% fowl sellers sell the offal, though only 8.3% and 2.1% would bury and burn poultry offal respectively. In addition, 4.2% of fowl sellers would either sell poultry manure or give as gift (Figure 2).

The study revealed that 62.6% fowl sellers' had knowledge of one sign or the other though 37.4% do not know any clinical signs of avian influenza (Table 4.5).

On the knowledge of the routes of avian influenza transmission, 69.8% fowl sellers had knowledge of at least one routes while 31.2% had no knowledge of any means (Table 4.6).

The study also revealed that 90.0% of fowl sellers would report suspected Avian Iinfluenza outbreak to Veterinarians with 4.0% willing to report to local government authority. Nonetheless, 4.0% fowl sellers would report to extension health workers and 2.0% would not report to suspected Avian Influenza outbreak (figure 3).

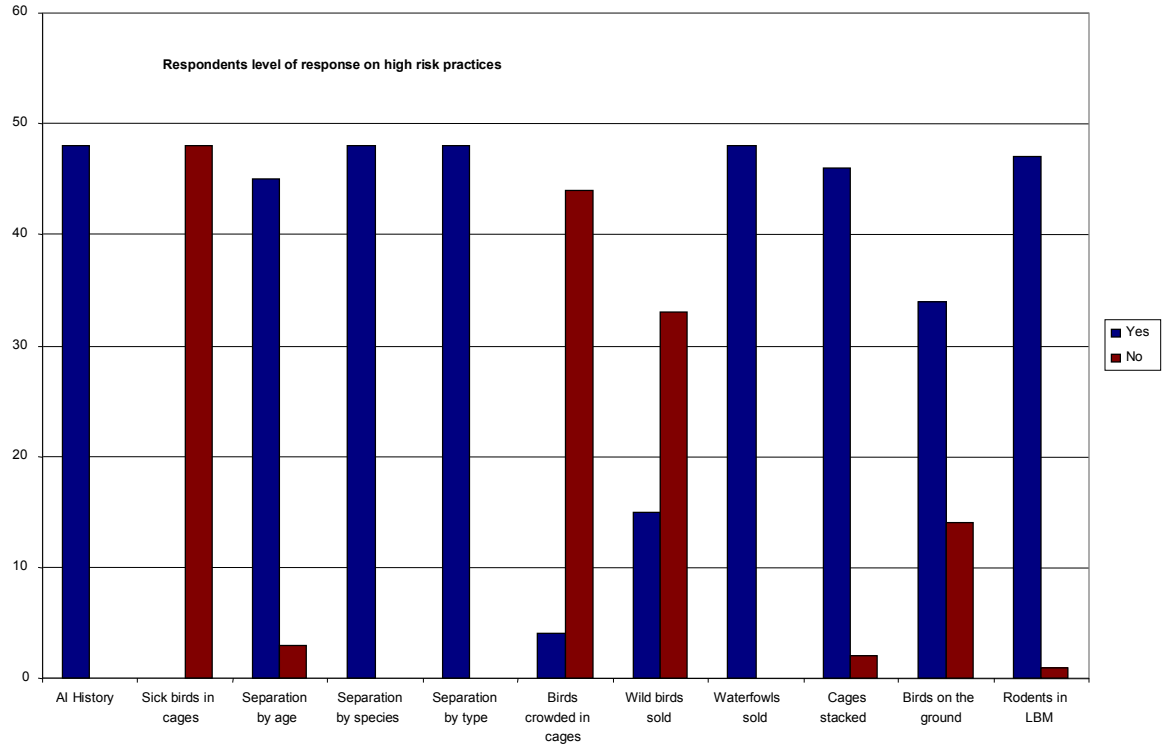


Figure 1: Fowl sellers' awareness and trading practices in Abuja live bird markets.

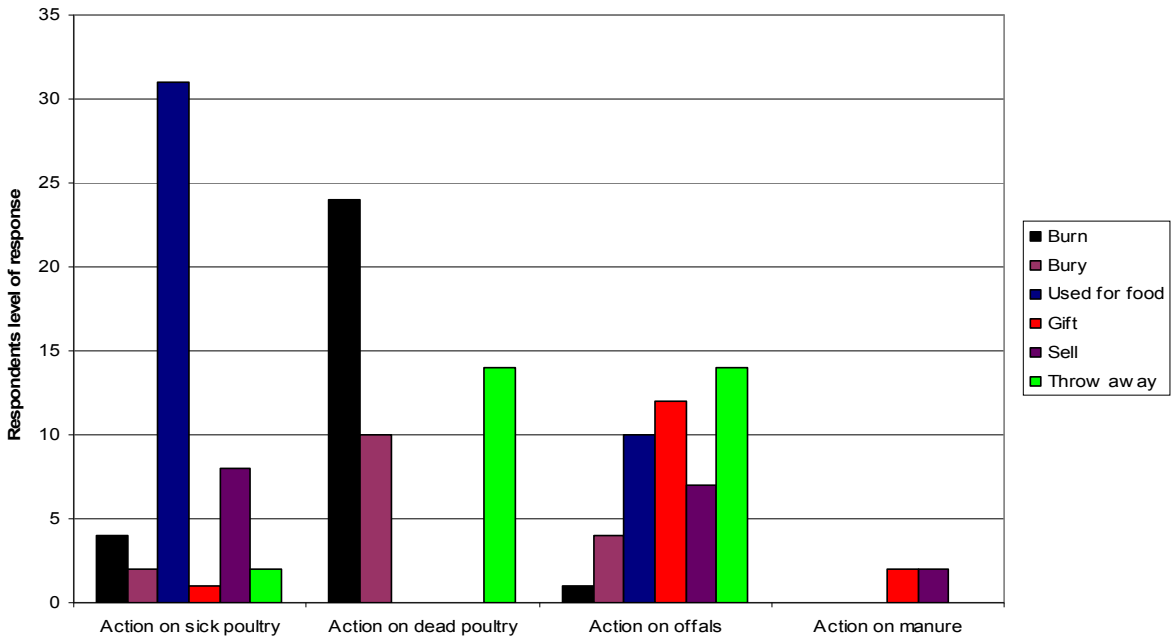


Figure 2: Fowl sellers actions on sick and dead poultry, offal and manure.

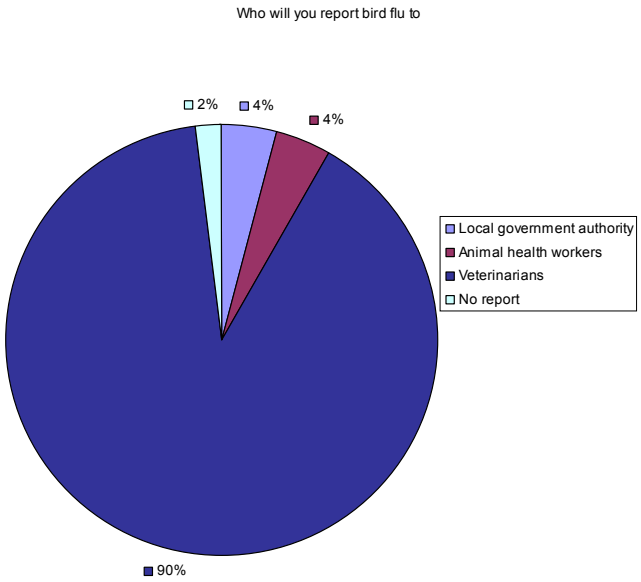


Figure 3: persons fowl sellers prefer to report suspected avian influenza outbreak.

Table 4.5: Fowl sellers' knowledge of the clinical signs of avian influenza

Signs of avian influenza	No. of sellers (%)
High morality	3 (6.3)
Sudden death	11 (22.9)
Sneezing and swollen head	5 (10.4)
Swollen head and coughing	1 (2.1)
Coughing	3 (6.3)
Bluish comb	6 (12.5)
Diarrhoea and weakness	1 (2.1)
No sign	18 (37.5)
Total	**48 (100.0)**

Table 4.6: Fowl sellers' knowledge of ways humans contact avian influenza

Way of infection	No. of sellers (%)
Contact with sick poultry	19 (39.6)
Eating uncooked meat and egg	7 (14.6)
Eating infected chicken	4 (8.3)
From any chicken	3 (6.3)
None	15 (31.3)
Total	**48 (100.0)**

CHAPTER 5

DISCUSSION

All the species had antibodies to AI, ND and IBD, indicating previous exposure to these viruses. However, among the poultry species, ducks and turkeys had the highest AI prevalence while guinea fowls and turkeys had high ND prevalence. Similarly, GD prevalence was highest in pigeons and turkeys.

The fact that there is no legal provision for vaccinating against AI in Nigeria the antibody detected may have been produced as a result of natural infection. The antibodies to ND, GD and AI found in duck guinea fowls and pigeons are natural exposure because they are often not routinely vaccinated.

The study revealed that poultry from these live bird markets could be sources of avian influenza viruses to other poultry, especially local poultry as local poultry farmers source their breeding stock from LBMs. Ducks have been implicated as a frequent cause of epidemics in poultry populations and the study reveals high avian influenza titre indicative of recent exposure with high chances of actively be shedding the virus to poultry in the cages and other in contact birds without necessarily showing clinical disease of avian influenza viruses (Boven *et al.,* 2008).

Excretion of avian influenza viruses by these sero-positive poultry in the LBMs would lead to contamination of the cages which are made of wood and difficult to clean and disinfect. The inability to properly clean and disinfect these wooden cages would ensure spread of the virus to new poultry introduced into these cages since the virus would be protected in organic materials in the cage. It is generally accepted that ducks and other

waterfowls are the natural reservoirs of AI viruses which can be transmitted to domestic bird population and to commercial poultry (WHO, 2005).

Turkey had 100 % antibody prevalence to ND, while guinea fowl has 80 % antibody prevalence to ND. Routine vaccination is rarely practiced in these two species and so natural exposure is more likely responsible for this high prevalence. Turkey and pigeon may develop generalized disease but clinical signs are rare in ducks and geese (Nwanta *et al*., 2006).

The exposure of chicken to the virus may be due to routine vaccination. Nwanta *et al*. (2006) stated that effective control of ND in rural poultry would require application of effective vaccines. The high prevalence of poultry with ND antibodies titre > 7 $\log_2$ implies that the poultry have either become naturally exposed and recovered or have received booster vaccination and in this case only for ND and particularly by chicken and other poultry were naturally exposed since vaccination is not routinely done.

Poultry in all the studied LBM had antibodies to AI, ND and GD with Garki New Extension, Bwari makeshift and Bwari main market LBMs having the lowest prevalence for AI. For GD the prevalence was lowest in Kuje, while lowest ND prevalence was reported in Bwari main market and Garki New Extension. The implication of this finding is that birds that do not have antibody are susceptible to AI in Garki New Extension, Bwari makeshift and Bwari main market while sero-negative poultry in Kuje LBM either had no previous exposure to GD or their GD antibodies have waned. The study revealed that poultry in Garki New Extension, Bwari makeshift and Bwari main market LBMs are more at risk of AI and ND infection because of the increased chances of excreting the viruses. Apart from the chicken population routine vaccination against ND is not often

done in the other species but they may act as sources of infection to chickens. Ducks remain apparently healthy but actively shed AI and ND viruses to the chicken population (Swayne and Jackwood, 2008). Once in chicken flock, the previously stable mild LPAI strain of AI virus begins to evolve rapidly and can mutate over an unpredictable period of time into a highly lethal HPAI strain (WHO, 2005).

More than 80% of all the poultry in the LBMs have antibody > $7\log_2$ for AI and 60% and above for ND. This percentage implies a recent exposure to ND or AI virus. However, for ND it may be booster vaccination in chickens with the exception of those chickens sampled from Garki New Extension and Bwari main market that do not have antibody >$7\log_2$. Birds in Garki New Extension and Bwari are susceptible to ND.

The fowl sellers interviewed indulged in both beneficial and risky practices as regards biosecurity. The beneficial practices include separating sick and healthy poultry, separation of birds by species age and type and not overcrowding birds. However, the risky biosecurity practices include selling of waterfowls and wild birds together with chickens; stacking of cages as well as keeping birds on the ground for sales with respect to control of AI and ND within studied LBMs.

The sale of wild birds in some of the LBMs is risky because wild birds are known to harbour AI viruses without showing clinical disease (WHO, 2005; Nicolas *et al*., 2008). Also, the AI viruses have stable adaptation in waterfowls, especially ducks that are commonly sold with other poultry species in LBMs.

The consumption of sick poultry would increase human exposure to avian influenza during processing of the poultry as their hands will be contaminated as infection through the oral mucus membrane or by aerosol becomes a possibility (Mabiru,1998). Moreover

the practice of throwing away contaminated litter materials on farms and offal in the refuse dump will lead to environmental contamination, maintenance and spread of AI as well as other contagious poultry diseases (Brugh and Johnson, 1987).

The willingness of fowl sellers to report AI outbreak to veterinarians is contrary to previous studies in Kaduna and Kogi States where in both studies less than 40% of the respondents were willing to report to veterinarians (Ameji, 2010; Assam *et al*., 2012). The aforementioned finding may not be unconnected with the awareness generated by the reported outbreaks between 2006 and 2007 in Bwari, Kuje and Abuja Municipal Area Councils of Abuja city. The community sensitization programmes embarked upon by the National Orientation Agency and Avian Influenza Control Project (AICP) may have had a positive effect on stakeholder awareness within the Federal Capital Territory as the fowl sellers had access to their educational materials unlike the local poultry farmers in the previous studies (Assam *et al*., 2012).

CHAPTER 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 SUMMARY

The study assessed Avian Influenza, Newcastle and Gumboro diseases antibodies status of poultry in Abuja LBMs by haemagglutination inhibition (HI) and agar gel immunodiffusion (AGID) tests respectively. Biosecurity practices were also assessed through administration of standard questionnaires. The data obtained were analysed using statistical package.

Prevalence and mean titre for avian influenza was 65.3% and 10.40±0.94 log_2, respectively. Newcastle disease mean antibody titre was 5.91±0.27 log_2 with prevalence of 64.3%. Gumboro disease prevalence was 69.8%. Among species tested, duck had the highest avian influenza prevalence of 84 % and chicken prevalence was 63.3%. Pigeons and turkeys have been exposed to Gumboro disease virus with chickens having Newcastle disease prevalence of 63.8 %. All the species had avian influenza antibodies titre $\geq 7log_2$ except guinea fowl. Garki Area I LBM had the highest avian influenza prevalence of 87.9 % with Garki II new extension market having the lowest prevalence of 22.2 %. Fowl sellers engaged in risky practices such as stacking of cages. Sixty four point six percent (64.6%) of fowl sellers use sick poultry for food, 33.3% sell sick poultry, 29.2% throw away offal and only 20.8% bury dead poultry. Furthermore, 37.4% of fowl sellers knew none of avian influenza signs though 69.8% were knowledgeable on its mode of transmission. Nonetheless, 90.0% of fowl sellers would report avian influenza outbreak to Veterinarians.

Poultry in Abuja LBMs have been exposed to avian influenza virus and are not protected from Gumboro disease and Newcastle disease outbreaks which might be confused with highly pathogenic avian influenza. The exposure of poultry to avian influenza antigen is most likely recent with the possibility of the bird species shedding the low pathogenic field strain at high levels . The risk of ducks spreading infection to household local chickens is high as local poultry farmers often get breeding stocks from the LBMs.

Fowl sellers engage in risky practices likely to expose them and poultry to avian influenza virus.

There is need to undertake Avian Influenza virus surveillance in LBMs in Abuja and better articulate avian influenza risks to fowl sellers.

6.2 CONCLUSIONS

Poultry in Abuja LBM have been exposed to AIV and are also not protected from ND outbreaks which might be misdiagnosed for Avian Influenza.

The exposure to avian influenza antigen is possibly recent with the likelihood of excretion of field low pathogenic strain by ducks in particular.

There is high risk of poultry purchased from these LBMs spreading infection to household local chickens when local poultry farmers get their breeding stocks from live bird markets.

Fowl sellers engage in high risk practices likely to expose them and their poultry to avian influenza virus as biosecurity measures are grossly inadequate within the LBMs.

6.3 RECOMMENDATIONS

1. There is need to undertake avian influenza virus detection in LBMs in Abuja.

2. It is recommended that vaccinations against ND and GD should be encouraged to prevent outbreaks of these diseases in commercial flocks.
3. Fowl sellers and the public should be enlightened on the importance of biosecurity towards AI and other contagious poultry diseases.
4. Efforts should be made by fowl sellers to put ducks and other water fowls in different sales units to minimize interspecies cross contaminations.
5. The Federal Capital Development Authority should enhance available infrastructure to make optimum biosecurity programs workable within all the LBMs in Abuja.

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Appendix 1
A structured questionnaire on biosecurity in live bird markets in Abuja, Nigeria

Please answer yes or no where applicable

SECTION A: MARKET SURVEY

1. Serial Number: ---------- Date: ----------

2. State: ----------

3. Local Government Area: ----------

4. GPS coordinators: Latitude ---------- Longitude ----------

5. Has there been any report of bird flu in the local government? Yes [ ] No [ ]

6. Town/Village: ----------

7. Nature of live bird market: ----------

8. Market type: (a) Daily [ ] (b) Weekly [ ] (c) Informal [ ] (d) Others (specify)---

9. Market days: M [ ] T [ ] W [ ] Thr [ ] S [ ] S [ ]

10. Where is the LBM located? (a) Within main market [ ] (b) Outside main market [ ]

SECTION B: BIOSECURITY IN THE MARKET

11. Is the market fenced off? Yes [ ] No [ ]

12. If yes, are entry and exit doors separated: Yes [ ] No [ ]

13. Which is the nearest town/village? ----------

14. Are birds kept in cages? Yes [ ] No [ ]

15. If yes, describe type of cage and its materials used ----------

16. Are some birds on ground? Yes [ ] No [ ]

17. Are cages stacked on top of each other? Yes [ ] No [ ]

18. Are birds in cages overcrowded? Yes [ ] No [ ]

19. Are birds in cages sick? Yes [ ] No [ ]

20. Are the birds kept separately by?

    1. Age Yes [ ] No [ ]

    2. Breed Yes [ ] No [ ]

    3. Species Yes [ ] No [ ]

    4. Type Yes [ ] No [ ]

21. Are rodents in the market? Yes [ ] No [ ]

22. If yes, how are they controlled? ----------

23. Are flies many in the market? Yes [ ] No [ ]

24. If yes, how are they controlled? ----------

25. Are ducks/geese water fowls sold in the market? Yes [ ] No [ ]

26. Are other animals sold in the market? Yes [ ] No [ ]

27. If yes, list the other animals sold: 1. Cattle [ ] (2) Sheep [ ] (3) Goats [ ]

    (4) Pigs [ ] (5) Donkeys [ ] (6) Horses [ ] (7) Rabbits [ ] (8) Camels [ ]

    (9) Guinea pigs [ ] Others ----------

28. Are stray dogs and cats in the market? Yes [ ] No [ ]

29. Are wild birds in the market? Yes [ ] No [ ]

30. What do you do with?

	Prepare for food	Throw away	Gift	Bury	Burn	Sell
1. Sick bird	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Others--

2. Dead birds	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Others--

3	Offals	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Others--

4	Feathers	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Others--

5	Manure	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

Others—

SECTION C: KNOWLEDGE OF FOWL SELLERS ON POULTRY DISEASES

31. Do you know any poultry disease(s)? Yes [ ] No [ ]

32. If yes, which one(s)? ----------

33. Do you know of bird flu? Yes [ ] No [ ]

34. Do you know of the sign of bird flu? Yes [ ] No [ ]

35. If yes, list some signs of bird flu: ----------

36. Do you know bird flu affects human beings? Yes [ ] No [ ]

37. Signs of bird flu in humans: ----------

38. Do you believe bird flu affects human beings? Yes [ ] No [ ]

39. If yes how do human beings get the disease? ----------

40. Do you know of any disease(s) similar to bird flu? Yes [ ] No [ ]

41. If yes, which one(s)? ----------

42. If you or your family has signs of bird flu, what will you do? ----------

43. In case of bird flu outbreak, will you report? Yes [ ] No [ ]

44. If no, why? ----------

45. If yes, to whom? ----------

46. Name of person interviewed: ----------

47. Name of interviewer: ----------