SEROPREVALENCE OF AVIAN INFLUENZA, GUMBORO, NEWCASTLE DISEASE AND PRACTICES OF POULTRY TRADERS IN SOUTHERN PARTS OF KADUNA STATE

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NOVEMBER, 2011

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M.Sc/Vet.Med/10423/2007-2008

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF

MASTER OF SCIENCE IN VETERINARY MEDICINE
DEPARTMENT OF VETERINARY SURGERY AND MEDICINE
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

NOVEMBER, 2011

DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Surgery and Medicine, Ahmadu University, Zaria under the supervision of Dr. Lawal Sa'idu, Dr. M.A. Raji and Prof. P.A. Abdu. The work of other investigators referred to in this study is duly acknowledged. No part of this study has been previously submitted for a degree or diploma.

_____ _____

Kambai, Francis Mba'aya Date

CERTIFICATION

This thesis entitled "**SEROPREVALENCE OF AVIAN INFLUENZA, GUMBORO, NEWCASTLE DISEASE AND PRACTICES OF POULTRY TRADERS IN SOUTHERN PARTS OF KADUNA STATE"** by Kambai, Francis Mba'aya meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my parents Mr. Kambai Mallam of blessed memory and Mrs. Nachen 'Aya' Kambai who persevered so that all their children go to school, my dear wife Ninali who has always encouraged me and has so much believes in me and my children Zigwai, Koni, Faith and Dyep.

ACKNOWLEDGEMENTS

I wish to gratefully acknowledge my supervisors Drs. L. Sa'idu, M. A. Raji and Prof. P. A. Abdu in preparing and conducting this investigation. Their advice, suggestions and corrections had assisted in bringing this thesis to its present state. I have learnt from their wealth of professional and research experiences.

My special thanks goes to Dr. Assam Assam who has been of utmost assistance during the course of this study.

I am also grateful to Mr. David Leo, Mal. Lawal Musa and Mr. Adejoh Musa who assisted me during my laboratory work.

My special thanks goes to my dear wife without whose support and encouragements, this work would not have been possible. I appreciate my children Zigwai, Koni, Faith and Dyep for their patience and understanding as I have had to be away from them due to commitments to this project.

Finally my greatest thanks goes to God Almighty my creator who has given me all the potentials I need to go this earthly journey triumphantly.

ABSTRACT

The first HPAI outbreak in Africa and in Nigeria occurred in Kaduna State. The study investigated factors favouring the introduction and spread of highly pathogenic avian influenza and the prevalence of H5 subtypes, Newcastle and Gumboro diseases antibodies in live bird markets. One hundred and twenty three questionnaires were administered to live bird marketers and 230 blood samples were collected from poultry. Sera from these blood samples were analysed using agar gel immunodiffusion test for Gumboro disease and haemagglutination inhibition tests for avian influenza and Newcastle disease antibodies. The mean chicken antibody titre and prevalence of Newcastle disease antibodies were 4.46 ± 0.25 $\log_2$ and 61.9% respectively. The avian influenza H5 subtype sero-prevalence rates in chickens and guinea fowls were 34.9% and 60.0% respectively with a chicken mean antibody titre of 3.90 ± 0.42 $\log_2$. Gumboro disease sero- prevalence rate in chicken was 58.6%. Cages were used by 42.6% of marketers though 66.0% use wooden cages. Marketers trading in markets with history of the outbreak of the highly pathogenic avian influenza were more likely to separate their poultry based on breed and species compared to those from markets without history of outbreak of the disease. Live bird marketers engaged in risky biosecurity practices such as eating, selling and purchasing of sick poultry which will increase the likelihood of introduction and spread of highly pathogenic avian influenza in live bird markets. These practices together with failure to wash hands with water and soap increase the human exposure. The risk perception of live bird marketers with history of AI outbreak was higher than for those without history of AI outbreak. The period of high sales of live birds were Christmas, New Year and Eid-el Fitr. Chickens and guinea fowls in live bird markets sampled have been exposed to H5 subtype, Gumboro and Newcastle disease antigen. Practices such as selling, eating and purchasing of sick birds are factors likely to enhance the introduction

and spread of highly pathogenic avian influenza in live bird markets. Government interventions have improved the risk perception of highly pathogenic avian influenza in the intervened markets. Hence there is need for intervention in other markets.

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LIST OF ABBREVIATIONS

AGID	Agar Gel Immunodiffusion
AICP	Avian Influenza Control Project
AI	Avian Influenza
AIV	Avian Influenza Virus
DIVA	Differentiated Infected from Vaccinated Animals
EDTA	Ethylene Di amine Tetra Acetate
ELISA	Enzyme Link Immuno-Sorbent Assay
EPP	Emergency Preparedness Plan
FCT	Federal Capital Territory
FSA	Fowl Seller's Association
GD	Gumboro Disease
HA	Haemaglutinin
HAU	Haemaglutinin unit
HI	Haemaglutition Inhibition
HPAI	Highly Pathogenic Avian Influenza
IBD	Infectious Bursal Disease
IVPI	Intra venous Pathogenic Index
LBM	Live Bird Market
LGA	Local Government Area
LPAI	Lowly Pathogenic Avian Influenza
NA	Neuraminidase
ND	Newcastle Disease
NVRI	National Veterinary Research Institute
OIE	Office International Des Epizooties
p	Prevalence value
PBS	Phosphate Buffered Saline

PCV	Packed Cell Volume
RBC	Red Blood Cell
RNA	Ribonucleic Acid
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction
SE	Standard Error
Spp	Species
SPSS	Statistical Package for Social Science
WHO	World Health Organization
X^2	Chi Square value
^oC	Degrees centigrade

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Avian influenza (AI), commonly known as 'Bird Flu' is an infectious disease of birds caused by viruses that are members of the family Orthomyxoviridae and are placed in the genus influenza virus A (Webster *et al*., 1992). There are three influenza genera, A, B and C. Only influenza A viruses are known to cause natural infection in birds (Gunter, 2007; Webster *et al*., 1992). The influenza A virus is generally spherical and between 80-120 nm in diameter (Easterday *et al.,* 1997). Its genome is made up of eight different pieces of single stranded RNA, which encode 11 different viral proteins (Easterday *et al.*, 1997). Of these proteins, the best characterized are the haemegglutinin (H or HA) and the neuraminidase (N or NA) glycoproteins. These proteins are found on the outside of the virion and are important in infecting host cells as well as elicit host antigenic response (Causey and Edwards, 2008). Although all species of birds are thought to be susceptible to infection with AI viruses, many wild and domestic bird species carry the viruses without any clinical manifestation of the disease (Abdu *et al.,* 2006). Chickens, quails and turkeys are especially susceptible while ducks may show no signs of the disease even when infected but act as reservoirs of the virus (Martin *et al*., 2006).

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

Highly pathogenic avian influenza (H5N1) virus is an influenza A virus subtype that occurs mainly in birds and is highly contagious causing high mortality (CDC, 2007). It was first defined in 1878 in Italy and became known as "fowl plague" (Bankowski and

Samadieh, 1981; WHO, 2006). The causative organism for this disease was shown to be a virus as early as 1901 but it was not until 1955 that the relationship to the mammalian influenza A virus (first isolated in the 1930s) was demonstrated (Easterday *et al*., 1997). This finding led to the recognition that other isolates from birds, which were serologically distinguishable from "fowl plague" viruses and usually caused only mild disease were also influenza A viruses.

However, influenza A viruses were found to be capable of causing the mild form of the disease in chickens could be antigenically distinguishable from those of high virulence. This resulted in some confusion as to the identification of the specific serological subtypes. As a result, the term "highly pathogenic avian influenza (HPAI) has been adopted to apply to viruses capable of causing severe disease in poultry regardless of their subtype (Jordan and Pattison, 1998).

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

Although limited serological studies showed the presence of antibody to influenza virus type A in Nigeria (Adeniji *et al*, 1993;Owoade *et al*, 2002) there was no evidence of clinical disease from A/H5N1 in Nigeria until January 2006 when the disease was reported in Sambawa farm (Adene *et al*., 2006). The National Veterinary Research Institute (NVRI) laboratory, Vom confirmed the disease to be type A influenza virus infection on February 6, 2006, and the OIE/FAO reference laboratory at Padova, Italy, finally confirmed the disease as HPAI caused by a H5N1 virus (profile: PQGERRRKKRGFG) on February 7th, 2006 (Anon, 2006). One human death due to the disease has been reported in Nigeria in January, 2007 (WHO, 2008). Despite immediate

containment measures taken by the veterinary and public health authorities in conformity with standard operation procedures, the disease spread to other parts of the country (Anon, 2006). As at November, 2008, the disease has been reported in 27 States and the FCT (Lombin, 2008).

Following the confirmation of avian influenza (H5N1) in Sambawa farm, Igabi Local Government Area of Kaduna State, the State Government inaugurated the Kaduna State Bird Flu Response Committee on the 9th of February, 2006. The terms of reference of the committee were as follows:-

i) Preparation of rapid response plan to combat the spread of the disease in the state.
ii) Enlightenment of the general public on steps to be taken to contain the disease.
iii) Depopulation of all the remaining poultry in Sambawa farm and screening of the personnel on the farm.
iv) Census of all commercial poultry in the state.
v) Visits to such farms and enlightenment of owners on how to combat the spread of the disease.
vi) Identification of other poultry farms which have reported such outbreaks and examination of their personnel.
vii) Advice Government on measures to contain the spread of the disease.

Despite efforts by this committee, the disease spread to six Local Government Areas out of the 23 Local Government Areas of the State. During these outbreaks over 114,000 birds of various species died and over another 92,000 were culled and compensation of about ₦69,000,000.00 (Sixty-nine million) paid to the farmers in Kaduna State (AICP, 2009).

1.2 STATEMENT OF THE PROBLEM

Nigeria's poultry population is put at about 140 million. Out of this number, 25% are commercial, 15% semi-commercial and 60% backyard. (FGN, 2007). In Kaduna State, the Poultry population is put at about 5,200,000 (KDSG, 2007).

With few exceptions, notably in large commercial farms, surveillance for avian diseases is limited (FGN, 2007). Nutrition of the birds is poor and high mortality is common, increasing the likelihood that outbreaks of H5N1 will be missed (FGN, 2007). Experience in Asia has shown that late detection of outbreaks increases the likely hood that the virus will become enzootic (Kaleta and Rulke, 2008). Deaths of a large number of poultry, whether due to disease or culling for control purposes, would deprive already impoverished populations of an important source of income and protein, particularly for women and children (FGN, 2007). The HPAI epizootic in Asia, Europe, the Near East and Africa is not expected to diminish significantly in the short term (Kaleta and Rulke, 2008). It is likely that H5N1 virus infections among domestic and wild birds will continue to occur especially with Newcastle disease and immunosuppressive infectious bursa disease being endemic in Nigeria. So far, the spread of H5N1 virus from person-to-person has been limited and unsustained. However, this epizootic continues to pose an important public health threat (CDC, 2008). One human death due to the disease has been reported in Nigeria in January, 2007 and the source was traced back to a poultry market in Lagos (WHO, 2008). After nearly 10 months (Oct, 2007 to July 2008), Nigeria announced an outbreak of H5N1 in Kano and Katsina States (Lombin, 2008). There is therefore the need for continuous surveillance for the disease.

1.3 JUSTIFICATION

The first HPAI outbreak in Africa and Nigeria occurred in Kaduna State (Adene *et al*., 2006; Anon, 2006). This outbreak led to trade bans and restrictions on poultry and poultry by-products which negatively affected the poultry industry and subsequently the economy of Kaduna State and Nigeria (Ahmed, 2006; Abdu *et al.*, 2006). Since concurrent infections of Newcastle and Gumboro diseases influence the clinical outcome of avian influenza infection, there is the need to assess the immune status of poultry with respect to Newcastle and Gumboro diseases which are important endemic diseases in Nigeria. There is also the need to reactivate the poultry industry, create jobs and create sources of revenue generation for individuals, households, firms and governments (Ocholi *et al*., 2006). Highly pathogenic avian influenza has also been found to cross species barriers to infect cats, dogs, pigs and humans leading to disease and high rate of fatality (Kaleta and Rulke, 2008). HPAI is a devastating disease in poultry as it is associated with high death rate which disrupts poultry production and trade and can be transmitted to humans (Kaleta and Rulke, 2008).

1.4 AIM AND OBJECTIVES

1.4.1 Aim

This study was conducted to determine some aspects of epidemiology of HPAI, Newcastle disease and infectious bursal disease and the biosecurity practices in live bird markets in Kaduna State.

1.4.2 Objectives

The objectives of the study were to determine the:

1. Factors that favour the introduction and spread of HPAI in LBMs and trade in live birds in some Local Government Areas of Kaduna State.

2. Sero-prevalence of H5 Avian Influenza, Newcastle disease and infectious bursal disease antibodies in poultry in LBMs in some Local Government Areas of Kaduna State.

CHAPTER 2

LITERATURE REVIEW

2.1 AVIAN INFLUENZA

2.1.1 Historical Perspective of Avian Influenza

The severe form of avian influenza (AI) infection also known as "epizootic typhoid of fowl", 'fowl plague', 'Vogelpest', 'European fowl pest' was first described in Italy in 1887 by Perroncito (cited by Kaleta and Rulke, 2008). By 1901, even though the agent of 'fowl plague' was known to be a 'virus', its relationship with other mild viruses isolated from birds with influenza was not demonstrated until 1955 (Easterday *et al*., 1997). It is thought that AI was endemic during late nineteenth and early twentieth century in Europe and Egypt meaning that outbreaks were not swiftly controlled during these periods. However, there are suggestions that AI might have been misdiagnosed for fowl cholera and Newcastle disease (Alexander, 1987).

Worldwide between 1959 and 1999 there were 18 reported outbreaks of HPAI caused by avian influenza viruses, with nine due to H5 viruses and nine due to H7 viruses (Swange and Suarez ,2000). Since then, HPAI outbreaks caused by H5N1 avian influenza viruses have occurred in gallinaceous poultry in Hong Kong in 2001, 2002 and 2003, and by H7N7 viruses in Netherlands, Belgium and Germany in 2003 (Sims *et al.,* 2003; Ellis *et al.,* 2004). High mortality and severe pathology in birds in these outbreaks has generally been confined to domestic poultry, and HPAI viruses have rarely been isolated from wild birds even on farms experiencing HPAI outbreaks in poultry (Swayne and Suarez, 2000).

The AI infection has spread to over 62 countries across Asia, Europe, Africa and Middle East (OIE, 2008). The 1997 human and poultry outbreak of fatal H5N1 AI in Hong Kong

resulted in worldwide interest in highly pathogenic avian influenza viruses (HPAI) (Alexander and Brown, 2000). Avian influenza virus (AIV) of the H5N3 subtype was first isolated in South Africa from the common tern (*Sterna hirundo*) in 1961 and this was also the first reported case of AI in wild birds (Alexander *et al*., 2008). In 2005, AI infection in ostriches caused by H5N2 subtype was reported in Zimbabwe (Alexander *et al*., 2008). Since then there have been AI outbreaks in other African countries.

2.1.2 Distribution of Avian Influenza

Avian influenza has a worldwide distribution which includes Asia and Middle East, Europe, Africa, North and South America (Easterday *et al*., 1997; DEFRA, 2003). The first outbreak on the African continent was reported in 2006 in Kaduna State, Nigeria and thereafter spread to other African countries including Ivory Coast, Niger, Cameroon, Burkina Faso, Egypt, Sudan Benin Republic, Ghana, Togo and Djibouti (OIE, 2008). In Nigeria, as at October, 2008, HPAI has been reported in 25 states and FCT involving 97 local government areas (AICP, 2009). There are reports of AI antibodies and influenza A antigen in local poultry in Kaduna State (Durosinlorin, 2008; Assam, 2010).

2.1.3 Incidence of Avian Influenza

The incidence of AI is influenced by the geographic region, species and age of bird, the time of year and environmental or agricultural system (Swayne *et al*., 1998). However, AI is a seasonal disease in high risk areas due to the migratory activities of waterfowls (Abdu *et al*., 2005).

Avian influenza outbreaks usually occur during the cold months since the virus is easily destroyed by heat and ultra violet light. Most AI outbreaks in Nigeria occur during the harmattan period (Wakawa *et al.*, 2008). However, Wakawa *et al*. (2008) reported an outbreak in waterfowls in the hot month of April in Zaria. Some factors attributed to the

increased incidence of AI include increase poultry population, intensification of the poultry industry with a concurrent increase in free-range production and climate change which led to alterations in wild bird migratory paths (Capua and Alexander, 2004).

2.1.4 Aetiology of Avian Influenza

Avian influenza is caused by avian influenza viruses which are negative stranded medium-sized pleomorphic RNA viruses belonging to the *Orthomyxoviridae* family. The AIV have helical symmetry and glycoprotein projections from the envelope having haemagglutinating and neuraminidase activity (Easterday *et al.*, 1997). The AIV can be classified based on either their pathogenicity or the surface glycoprotein-haemagglutinin and neuraminase (Easterday *et al*., 1997).

Avian influenza has two surface glycoprotein-haemagglutinin and neuraminidase which gives the virus its antigenic subtype specificity (Swayne *et al.*, 1998; Abdu *et al.*, 2005). There are currently 16 haemagglutinin subtypes (H1-16) and nine neuraminidase subtypes (N1-9) with 144 possible avian influenza subtypes with no cross protection between haemagglutinin subtypes (Swayne *et al*., 1998).

Based on their pathogenicity, HPAI viruses have an intravenous pathogenicity index (IVPI) in 6-week old chickens greater than 1.2 or cause at least 75% mortality in 4 to 8-week-old chickens infected intravenously; or H5 and H7 viruses with multiple basic amino acids at the haemagglutinin cleavage site (OIE, 2009). However, low pathogenic avian influenza (LPAI) viruses are from any of the 16 haemagglutinin (H1-H16) and 9 neuraminidase (N1–N9) subtypes not fulfilling the aforementioned conditions (OIE, 2009).

Currently, AI viruses are classified based on the potential of the LPAI to become HPAI after circulating in poultry (OIE, 2009). The AIV having multiple basic amino acid

sequences at the cleavage site are considered notifiable and the viruses are denoted as highly pathogenic notifiable avian influenza (HPNAI) while all H5 and H7 subtypes that are not HPNAI are low pathogenic notifiable avian influenza (LPNAI) (OIE, 2009).

The diversity of the AI viral population is guaranteed by their segmented nature and the presence of different surface protein (Easterday *et al.*,1997). 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 the same time can occur (Beard, 1998). These antigenic differences have varied implications for vaccination though the importance and interpretation of antigenic drift in poultry causes changes at antigenic sites affecting specificity of neutralizing antibodies (Suarez, 2008).

2.1.5 Resistance of Avian Influenza Virus to Chemical and Physical Agents

Physical and chemical agents can be used to destroy AIV. Avian influenza viruses are susceptible to high temperature, extremes of pH, dryness, dry heat, steam and ultra violet (UV) lights (Abdu *et al*., 2005; Birnbaum and O'Brien, 2008). Organic materials shield the virus from UV light rendering it ineffective at inactivating AIV. The UV light damage the viral nucleic acid though most effective on clean surface. However, AIV in manure are inactivated by heating poultry house to 32^{o}C for three hours or for 30 minutes at 38^{o}C (Cardona, 2007). Steam at 100^{o}C when used to treat surfaces for a long time also inactivates AIV though is difficult maintaining a constant temperature (AusVetPlan, 2000). Proper compositing of poultry litter would generate enough heat to inactivate the AIV (Easterday *et al.*, 1997).

Several chemical agents have been used to inactivate AIV. Washing of hands with soap and water is a proper way of disinfecting the hands. Soap and detergents remove the organic materials on surfaces as well as inactivate AIV by destroying the lipid outer

membrane used for viral attachment (Birnbaum and O'Brien, 2008). Avian influenza viruses are susceptible to either inorganic or organic disinfectants such as iodine and iodophors, peroxygen compounds, alcohols, phenols, cresols, quaternary ammonium compounds, hypochlorites compounds, aldehydes, acid and bases. Peroxygen compounds are strong oxidizers and produce free radicals which inactivates AIV though peracetic acid remains active in the presence of organic matter unlike hydrogen peroxide (Birnbaum and O'Brien, 2008). However, inactivation of AIV in small hatchery equipments is possible by using 70% ethanol though its activity is limited in the presence of organic materials (Birnbaum and O'Brien, 2008).

2.1.6 Susceptible Hosts to Avian Influenza

In a variety of animal species including humans, pigs, horses, sea mammals, mustelids and birds, avian influenza infections have been reported to occur naturally (Alexander and Brown, 2000). Though chickens and turkeys are usually severely affected by AIV, birds of all species and ages are susceptible (Hanson, 2005). Domestic and wild aquatic birds have been identified as the natural reservoirs of AIV as they harbor and excrete more than one subtype of AIV without showing clinical disease or rarely produce precipitin antibodies (Abdu *et al.*, 2005).

Current evidence suggest that HPAI only arise when H5 or H7 LPAI viruses mutate after being introduced to poultry from wild birds (Gracia *et al*., 1996; Perdue *et al*., 1998). Nicolas *et al*. (2008) reported H5N2 subtype of AIV with highly pathogenic genotype in healthy water fowls species in Nigeria.

2.1.7 Transmission of Avian Influenza

Avian influenza virus is usually present in oral, nasal and ocular secretions and faeces of infected birds (Easterday *et al.*, 1997). Viral transmission can be by direct transmission of

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

Ingestion of contaminated food, water or infected carcass is usually the mode of transmitting AIV indirectly (Easterday *et al.*, 1997). The most common route of spread of AIV among birds is the faeco-oral route (Abdu *et al.*, 2005; Umoh, 2005). Non-composted contaminated litter or manure have been implicated in avian influenza outbreaks (Brugh and Johnson, 1987). About one gram of contaminated faeces from infected chickens can contain enough of HPAI viruses to infect 1,000,000 birds (Chalmers, 2005).

Other methods of AIV spread are live bird markets (LBM), movement of infected birds, contaminated equipments, clothing, shoes, vehicles and eggs (Mubiru, 1998; Assam, 2010).

2.1.8 Incubation Period of Avian Influenza

The HPAI has an incubation period of 3-7 days in individual birds and 14 days between flocks, depending on the subtype, the viral dose, the species and age of the birds and route of infection (Easterday *et al.*, 1997; Abdu *et al.*, 2005).

2.1.9 Clinical Signs of Avian Influenza

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

The LPAI in chickens and turkeys, causes an infection with no apparent clinical signs to a drop in egg production with mild respiratory signs and low mortality. Secondary bacterial infection may cause a severe disease with high mortality (Swayne, 2003).

Highly pathogenic avian influenza infection is characterised by sudden onset of high morbidity which may reach up to 100% and mortality rate ranging from 50 to 100 %; cessation or drop in egg production and 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 also reported (Easterday *et al.*, 1997; Adene *et al*., 2006; Wakawa *et al*., 2008). Nervous signs such as excitation, convulsion, circling and ataxia are common in poultry that survive the acute form of AI (Swayne *et al*., 2003).

2.1.10 Pathology of Avian Influenza

2.1.10.1 *Gross lesions*

Avian influenza lesions depends on the severity of the disease and species of poultry affected (Abdu *et al*., 2005). In LPAI, lesions include catarrhal, fibrinous, serofibrinous, mucopurulent or caseous inflammation of the sinuses (Adene *et al*., 2006). Air sacs are thickened with fibrinous or caseous exudates. In the peritoneum there is catarrhal or fibrinous peritonitis and egg yolk peritonitis. In the intestine and ceca, fibrinous enteritis are seen. In the oviduct are presence of exudates and oviduct involution (Abdu *et al*., 2005).

In HPAI infections involving gallinaceous birds, there are oedematous, haemorrhagic and necrotic lesions in visceral organs, cardiovascular and nervous systems (Swayne and Jackwood, 2008). There is usually no gross lesions in per acute cases of HPAI though the acute cases in chickens results in 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 (Easterday *et al*., 1997). In chickens, there is congested and swollen comb and wattles; oedema of surrounding eyes with nasal

and oral discharges. Legs are congested and haemorrhagic with severe congestion and oedema of the lungs; enlargement and congestion of the kidneys with urates. Necrotic foci are present on the wattles, skin, liver, spleen, kidneys and lungs (Swayne and Jackwood, 2008).

2.1.10.2 *Histopathology*

Microscopic lesions depend on the infecting AI virus, the species and type of bird. In LPAI caseous exudates are seen in the nares and paranasal sinuses. There is loss of cilia, swelling and vacuolation and necrosis of epithelial cells in the sinuses associated with oedema, heterophil and mononuclear cell infiltration (Easterday *et al.,* 1997). In the trachea, there is necrosis of the epithelium, heterophil infiltration, hyperplasia and mononuclear cell infiltration (Easterday *et al.,* 1997). In the lungs, the bronchi, atria and air capillaries are filled with sero-fibrinous exudates mixed with macrophages, heterophils and epithelial cells. Some lobules are completely necrotic. There is congestion of the spleen with fibrin deposition and lymphocyte depletion. The blood vessels of the kidneys are severely congested (Easterday *et al*., 1997).

In HPAI, there is necrosis and inflammatory changes in multiple organs such as the skin, brain, heart, pancreas, lungs, adrenal glands and lymphoid organs (Swayne and Jackwood, 2008). The per acute phase is characterised by little or no microscopic lesions though the acute cases involve multiple organs with predominantly necrotic lesions and apoptotic cell death associated with inflammation, haemorrhages and oedema (Swayne and Jackwood, 2008). The lesions seen on the wattles, are oedema, hyperaemia, haemorrhages and foci of perivascular cuffing. There is severe myocarditis and myocardial necrosis, oedema, hyperaemia and haemorrhages in the heart (Swayne and Jackwood, 2008).

2.1.11 Differential Diagnosis of Avian Influenza

Avian influenza should be differentiated from other diseases having similar clinical signs and postmortem lesions such as acute fowl cholera, Newcastle disease, infectious bronchitis, infectious coryza, chlamydiosis, adenoviruses causing respiratory disease or drop in egg production (e.g. EDS76) and mycoplasmosis (Easterday *et al.*, 1997; Swayne and Jackwood, 2008).

2.1.12 Diagnosis of Avian Influenza

A tentative of AI can be arrived at based on history, clinical signs and post-mortem lesions while final diagnosis is through detection of AIV antigen or antibody in infected poultry by any of the diagnostic methods recommended by the OIE (OIE, 2009). The gold standard method for AIV diagnosis is viral isolation in embryonated fowl eggs. Other methods include agar gel immunodiffusion (AGID) test, haemagglutination inhibition (HI) test, enzyme linked immune sorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) or real-time reverse transcription polymerase chain reaction (rRT-PCR) test. Reverse transcriptase polymerase chain reaction 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).

2.1.13 Highly Pathogenic Avian Influenza Control

The strategy for control of AIV undertaken by a country depends on the status of the infection in that country. A control strategy has three possible outcomes which include prevention, management and eradication (Swayne, 2008). In a country free of infection, the goal is usually to prevent introduction of AI. However, when AI is present in the country, the goal of a control programme will be to manage the infection to avoid spread, reduce economic losses and in the long term to eradicate the infection (Swayne, 2008). The aforementioned goals can be achieved through development of a strategy involving

education, biosecurity, diagnosis, surveillance, removal of source of infected and reducing host susceptibility to the virus (Swayne, 2008).

In Nigeria, the strategy of control of AI is swift detection and control of HPAI outbreaks and close monitoring of the situation (AICP, 2007). The strategy involves establishing a viable epidemio-surveillance and laboratory network for proactive surveillance, ensuring swift disease reporting within 12 hours; stamping-out, disinfecting farms and restriction of movement of equipment and personnel (AICP, 2000).

Knowledge on AI transmission and the role of individuals in the prevention, management and eradication is a critical aspect in any AI control program (Swayne, 2008). It involves training of stakeholders in the poultry industry on AI control highlighting how the AIV can be introduced, spread and prevented. The need to educate these stakeholders on the control of risky practices that would reduce the spread of the viruses is essential. In Nigeria, the community dialogue system would complement other education strategies to educate and identify risky practices, attitudes and believes among Nigerians (AICP, 2007).

The ability to detect the virus or evidence of infection within a population or environment can be achieved by diagnosis. Accurate and rapid diagnosis is essential for prompt control of AI (Easterday *et al*., 1997). Passive surveillance is essential to identify LPAI due to the mild nature of LPAI viruses. However, HPAI characterized by high mortality requires active surveillance to identify where AIV infection is located within the country (Swayne, 2008).

Surveillance is important to evaluate the outcome of a control strategy and for decision making. In developing countries like Nigeria, poor veterinary infrastructure presents serious problems with surveillance. Some of the problems include the development of a

mechanism to trace and monitor poultry and poultry product movements; fortifying veterinary quarantine infrastructure and control posts while promoting the training of Nigerians on HPAI recognition and control (AICP, 2008).

Stopping an outbreak and eradicating the disease from a farm involves either removal of the source of infection source or the susceptible hosts. These measures also prevent continued environmental contamination and dissemination of the AIV (Swayne, 2008). Elimination method should be safe and economically viable though this usually depends on the viral strain, local conditions, farm biosecurity level and financial and human resources available (Swayne, 2008).

In HPAI, viral source elimination involves humane depopulation and disposal of carcasses, eggs and manure through composing, incineration, rendering or landfill burial while in some developed economies, LPAI is eliminated by controlled marketing of birds 2-3 weeks after they recover from infection and washing of eggs prior to marketing (Swayne, 2008). Though stamping-out is recommended for notifiable LPAI infection by OIE, this would only be achieved if governments pay compensation and on time (Swayne, 2008; OIE, 2009).

In Nigeria, a modified stamping-out policy is practiced where poultry on infected farms are depopulated and the farms decontaminated. This is later accompanied by compensation aimed at encouraging farmers to report disease outbreak (AICP, 2007).

The effects of AIV could be decreased by boosting host resistance to AIV. This measure could also prevent AI infection when birds are exposed to virus and is achievable either through using genetically AI resistant breeds or producing passive or active immunity to susceptible birds (Swayne, 2008).

The Thailand native chicken breeds have been reported as being more frequently present in survivors after HPAI (H5N1) village outbreak (Boonyanuwat *et al*., 2006). However, some AI viral genes expressions in avian and mammalian cells have been silenced using small interfering RNA (siRNA) thereby conferring resistance to AI viral replication (Li *et al.,* 2005).

Resistance to AIV is usually produced through active and passive immunity, principally against the viral HA and to a lesser extent against viral NA (Easterday *et al*., 1997). Immunity is usually through vaccination although egg yolk might be a source of acquisition of maternal antibodies by chicks (Swayne, 2008). The control of AI through vaccination has been practiced in Mexico, Italy and Pakistan (Capua and Marangon, 2003). Incorporation of vaccination with other features of AI control such as controlled marketing and surveillance is cost-effective and utilizes the industry infrastructure, regulatory power and does not require either ethical destruction of healthy birds nor costly disposal (Halvorson, 2005). Birds vaccinated against AI are protected from clinical disease but continue to shed the virus in small quantities into the environment thereby making it impossible to differentiate infected from vaccinated birds when using inactivated homologous vaccines (Swayne, 2008). When inactivated heterologous vaccines are used, vaccinated and naturally infected birds could be differentiated by the "DIVA" strategy (Swayne, 2008). Vaccination advocates believes the ability to differentiate vaccinated and infected from vaccinated and non-infected and ethical question of depopulation of healthy birds calls for a re-think of using stamping-out as first option in AI outbreak (Capua and Marangon, 2003). Currently, in Nigeria vaccination is not an official policy (AICP, 2007).

The management practices and procedures undertaken to prevent virus introduction or if present from leaving a farm or live bird market (LBM) is known as biosecurity. It involves prevention of viral contamination, people, equipments and poultry or poultry products movement control or reduction of the amount of virus (Easterday *et al*., 1997). There are two categories of biosecurity namely inclusion biosecurity involving quarantine and other measures designed to keep the virus on infected farm or area and exclusion biosecurity aimed at keeping the virus out of AI- free farms or area (Swayne, 2008).

Nigeria, consisting mostly of sector 2 and 3 poultry farms with either little or no biosecurity and regular introduction of new birds from unknown source exacerbate the biosecurity problems (AICP, 2008). The rearing of different species within a farm increases the ease of interspecies transmission (Shane, 2002). The Federal Government of Nigeria has step up biosecurity measures in live bird markets (LBM) with routine disinfection of LBM and building of eight model LBMs with improved biosecurity features (AICP, 2009).

2.1.14 Highly Pathogenic Avian Influenza in Nigeria

In 2006 the first outbreak of HPAI in Nigeria was reported at Sambawa farms. Prior to the outbreak, federal government of Nigeria had made efforts at strengthening of institutional arrangements and conducting a study on the assessment of risk of introduction and spread of HPAI (Ahmed, 2000). Other measures undertaken include targeted surveillance at wetlands and other high risk areas and development of an Emergency Preparedness Plan (EPP).

After the outbreak, the avian influenza crisis management centre was established from where outbreak activities were coordinated. The centre consisted of the information, human and animal components of the avian influenza control program as instituted by the

EPP thereby ensuring effective collaboration of relevant ministries, development partners and donor agencies (FGN, 2007).

In the animal health component of the Avian Influenza Control Project, a holistic and coordinated veterinary services delivery system including ban on importation of poultry and poultry products has been adopted (FGN, 2007). Movement control with modified test and slaughter policy without vaccination was adopted as a means of eradication of HPAI (Obi and Ahmed, 2008).

There has been improved data collection and analysis with a multi-sectorial approach to control of TADs. The compensation has been improved and most affected farmers had been paid (Anon, 2006).

At the field level, the Federal Government of Nigeria has strengthened the disease surveillance and diagnostic capacity, veterinary quarantine services and improved the biosecurity in live bird markets and production together with registration of poultry farms (Ahmed, 2000).

The total number of HPAI suspicious cases and States and FCT reported as at July, 2008 was 1,590 and 32 respectively. Among these suspicious cases, 300 cases were positive in 25 States and FCT involving 97 local government areas. During the outbreak, a total of 1,264,191 birds were depopulated with six hundred and thirty-one million Naira paid as compensation to three thousand and thirty seven farmers (AICP, 2009).

Challenges identified in the control of HPAI in Nigeria includes lack of effective trace back and forward of cases, inadequate veterinary infrastructure and surveillance networks and difficulty in movement control of poultry and poultry products (Obi and Ahmed, 2008). The role of wild birds and poultry market chains in HPAI control are yet to be

ascertained as well as the development of suitable biosecurity and control strategies for rural and backyard commercial poultry. The communities need to be mobilised for prompt reporting of HPAI-like infections as well as the need to improve on the feedback mechanism (Obi and Ahmed, 2008).

2.1.15 Highly Pathogenic Avian Influenza Control in Live Bird Markets

In Nigeria, about 90 % of poultry marketing is by sales of live birds with less than 2 % comprising processed or frozen chicken (Muhammed, 2008). Over 40 % of live bird markets (LBMs) are located within residential areas (Muhammed, 2008). The live bird marketers are not organised with about 78 % operating as individuals with limited infrastructure though some are Fowl Sellers Association (FSA) or co-operative members (Muhammed, 2008).

The marketers usually keep their birds in cages with 45% using wooden cages, 29% of birds kept in metal cages and 26% kept in baskets (Muhammed, 2008). Though most local poultry originate from Northern Nigeria, they are marketed in the South (Muhammed, 2008). The long distance movement of birds to LBMs increases the potential of these markets as centres for HPAI (H5N1) virus dissemination. The different species are usually kept together both during transportation and in markets (Muhammed, 2008).

Most marketers do not understand the need for biosecurity in LBMs with only 10 % using protective clothing during slaughtering. Muhammed (2008) reported that the most common biosecurity measures undertaken in LBMs was regular sweeping of floor with only 24% of LBMs disinfecting their markets with 62% of these markets being disinfected on weekly basis. Most marketers do not bury or incinerate dead birds or waste from slaughtering which are usually disposed into public waste bins (Muhammed, 2008)..

Among the LBMs, 78% had a form of slaughter facility with about 66% practicing biosecurity measures with 57% of these LBMs receiving veterinary attention (Muhammed, 2008).

It is estimated that over 2 million live birds are sold daily in LBMs across Nigeria which highlights the position of LBMs in the control of HPAI in Nigeria (Muhammed, 2008). Improved biosecurity will minimize or stop the spread of HPAI among poultry in LBM thereby reducing human exposure during slaughtering and processing (Muhammed, 2010). This led to the Federal Government of Nigeria to embark on measures such as introduction of plastic cages for transportation of birds and metallic cages for holding in markets, design and construction of standard LBMs which provides markets with minimal standard facilities to enable them operate (Muhammed, 2008).

These biosecured LBMs would be located away from residential areas and encourage species separation within the LBMs. These new LBMs are mostly established through partnership between Federal Government of Nigeria (FGN) and FSA. The partnership would ensure sustainability and ownership. However, the FGN, State or Local governments can construct the LBMs and give to the FSA for use either for free or on lease (Muhammed, 2010).

2.1.16 The Biosecure Live Bird Market

The Federal Government of Nigeria decided to restructure the LBM as intervention to assist in the control of HPAI so as to break infection chain from farm to consumer by breaking the transmission cycle of the HPAI virus; ensure that poultry and other birds are

slaughtered and processed hygienically for human consumption; maintain biosecurity and prevent the introduction or spread of HPAI in the LBM (Muhammed, 2010).

In the restructured LBMs, the rule of "one way traffic only" must be maintained to ensure good biosecurity. Also only metal, plastic or glass would be allowed in the LBM because of ease of cleaning and disinfection. Hence no wood, cane or mat cages are allowed because of difficulty in cleaning and disinfection (Muhammed, 2010).

The restructured prototype LBMs consist of three phases which comprises of phase one – delivery of poultry and storage of transport plastic cages which consists of cement floor with drainage and chicken crates wash up and storage area. The second phase is the selling section consisting of chicken and duck steel cages. The third phase is the slaughtering and processing section comprising of the chicken slaughtering and processing area and/or duck/geese slaughtering and processing area.

Firewood is used for heating water for scalding instead of electric water heaters because of the availability and affordability of firewood, charcoal or coal and ease of maintenance (Muhammed, 2010).

For FGN to intervene in an LBM there must be evidence of registration of FSA as a cooperative entity with possession of a viable bank account having 15% contribution of the total cost of the project (LBM). There should also be evidence of allocation or ownership of land in which the project is to be sited with bill of quantity and drawings of the LBM. The LBM plans should have received approval from town planning, environment and other relevant agencies (Muhammed, 2010).

The LBM should be constructed against the direction of sun and wind, preferably East-West position with adequate ventilation, preferably with ridge roofing. Oil paints are used for the interior of the building to ease daily washing and cleaning with strong floor that

can withstand daily washing. Also the tap heads with nozzles are used for water pressure (spraying) and effective cleaning (Muhammed, 2010).

The slaughter and processing unit is a restricted area with 2 sections: the dirty operation section comprising a slaughter area, chimney, and scalding and depluming areas and the clean operation section where the poultry are opened, cut, cleaned and packaged for the customer. This section of the LBM should be properly tiled for easy cleaning with strong and well drained floor because of use of hot water and regular cleaning. The wall should be painted with oil paints, well ventilated and netted to protect it from flies and other insects (Muhammed, 2010).

The cages in the selling area should be made of metal or galvanized steel with a height of 7 ft (2.13 M); distance between floors of about 1.5 ft (0.45 M), length of the leg from the floor is 1ft (0.30 M) and size of tray/floor is 4.5 ft by 4.0 ft (1.22 M by 1.37 M).

Each cage floor has a tray under the wire mesh for collection and evacuation of faeces made of strong aluminum or plastic because of rusting and washing with a capacity of 20 matured heavy breed of layer chicken (20 X 4 = 80 birds).

The transport crates must be cleaned and disinfected after each delivery before going for another delivery. Over stocking of transport crates should be avoided by following the manufacturer's recommendation of 12 matured heavy breed per crate or 15 matured light breed per crate (Muhammed, 2010).

Some basic rules in a biosecure LBM include the use of only metal/steel and plastics cages, ensuring regular water supply necessary for cleaning and disinfection and use of disinfectant for washing the market, empty poultry cages and transport crates. Also, live bird marketers should ensure that washing water drains through openings in the floor and these openings should not be blocked. The live bird markets designed to have one - way

flow of traffic to prevent spread of disease. The one way flow applies to all customers, sellers and all poultry (Muhammed, 2010).

Poultry transporters should work only in the delivery section and never be allowed into the other sections unless they are also sellers. However, customers should enter the LBM through the entrance door, walk through the sellers section and then leave through the exit door. Walking inside the selling section to select birds is allowed. However, every FSA member must understand and accept the LBM rules before he or she can be allowed to sale or do business in the LBM. However, sanctions will be applied to all defaulters of the LBM rules (Muhammed, 2010).

For effective daily operation of the LBM, the FSA form four specialized teams for activities in the LBM: namely the biosecurity team, poultry delivery team, poultry sellers team and the poultry slaughtering and processing team.

The biosecurity team is composed of the FSA leader, 2 elected senior members, the State and Local Government Avian Influenza Control Project (AICP) desk officers. The responsibilities of the biosecurity team amongst others include enforcement of the golden rules in the LBM and sanctioning of violators. The team inspects the LBM daily and ensures that members and customers follow the rules and at the end of each market day, they make sure that the LBM is washed and disinfected properly (Muhammed, 2010).

2.1.17 Achievements of Federal Government Intervention in Live Bird Markets in Kaduna State

Among the achievements of the AICP in Kaduna state are the setting up of the State Desk Office, sensitization workshops on avian influenza for Heads of Agriculture, Heads of Veterinary Units, Heads of Information, Zonal/Area Veterinary officers of all the 23 Local Governments of the state and the appointment of Local government Desk Officers

(Animal Health) in all the 23 local government areas to coordinate the activities of the project at the local government level.

The backyard poultry farmers were also encouraged to disinfect their poultry premises regularly with disinfectants supplied by the AICP though subsequent disinfections will be their responsibility to protect their investments. The AICP have been organizing disinfection of village poultry markets on market days. Moreover, all major LBMs in the state have been registered and are fumigated on regular basis by the Local Government Desk Officers with disinfectants supplied by the AICP.

A model LBM has been constructed and commissioned at Sokoto road LBM, Kaduna while the LBM at Sheik Abubakar Gumi market has also been renovated and is fully operational. The project also ensured that operators of LBM disinfect the market on daily basis and carry out sanitation exercise on a weekly basis. The registration of all commercial poultry farms in the state and these are encouraged to operate standard biosecurity measures.

2.2 NEWCASTLE DISEASE

2.2.1 Historical Perspective

Newcastle disease outbreak was first confirmed and documented in Nigeria between 1952 and 1953 in Ibadan (Hill *et al*., 1953) and is currently endemic in the country (Abdu *et al*., 1985; Nwosu and Okeke, 1989). Newcastle disease is currently identified as the single most dreaded disease in the Nigeria poultry industry (Abdu *et al*., 1985).

2.2.2 Distribution

Newcastle disease is widespread in Africa, Asia and the Americas though the disease is endemic in Nigeria (Abdu *et al*., 1985).

2.2.3 Aetiology

Newcastle disease virus belongs to the Avulavirus genus of the Paramyxoviridae family in the Order Monoegavirales (Mayo *et al.*, 2002). Newcastle disease virus also known as avian paramyxovirus 1 is one of the nine identified serotypes of Paramyxoviruses known to infect birds. Strains of NDV have been distinguished on the basis of their clinical manifestion in chickens. Five pathotypes that have been defined are the viscerotropic velogenic virus characterised by high mortality with haemorrhagic lesions in the intestine; neurotropic velogenic virus characterised by high mortality following respiratory and nervous signs though gut lesions are usually absent. However, mesogenic strain is characterised by modrate to low mortality with respiratory signs and occasionally nervous signs. Nevertheless, there is no mortality associated with lentogenic and asymptomatic enteric strains of NDV but characterised by only mild respiratory signs and subclinical infection of the intestines respectively.

2.2.4 Susceptible Hosts

Newcastle disease virus infects both domestic and feral birds with reports of more than 250 species of free-living and caged birds have being infected with the virus though chickens are highly susceptible (Kelata and Baldauf, 1988). However, the disease manifestation will depend on the viral strain and the host species. Ducks have been reported to spread NDV after infection without necessarily exhibiting any clinical signs (Kingston and Dharsana, 1979). Turkey are reported to be less susceptible to ND infection though there are reports of some strains of NDV being adapted to pigeon but causes

disease in both pigeons and chickens (Alexander, 1997). However, there are reports of clinical manifestation of ND in geese caused by geese adapted strains of NDV (Luchuan and Hong, 1988).

Newcastle disease virus have been isolated from mammals though the viral replication in these hosts such as laboratory workers or vaccination crews might not be of epidemiological importance to avian host though there are reports of serious illness with most human infection by virulent strains of NDV resulting in severe conjunctivitis. (Alders and Spradbrow, 2001). Newcastle disease viruses have also been isolated from pigs (Alexander, 1997).

2.2.5 Transmission

Newcastle disease virus is transmitted by either inhalation of excreted droplets or ingestion of infective or contaminated materials such as faeces, feed and water (Alexander, 1995). Predator or scavenging wild birds are exposed to NDV upon ingestion of untreated contaminated swill or poultry meat. Transmission through aerosol is likely in densely populated poultry houses having infected poultry though airborne transmission of NDV has been reported to a distance of about 40 kilometre (Alders and Spradbrow, 2001). Other factors implicated in NDV spread include contaminated shoes and clothes, egg crates, cartoons, eggs and vaccines (Majiyagbe and Nawathe, 1981; Alders and Spradbrow, 2001). There are no reports of transovarian transmission of NDV though embryo death may occur during first five days of incubation in eggs laid by ND infected hens (Bivins *et al*., 1950 cited by Alexander, 1995).

2.2.6 Incubation Period

The incubation period of ND after natural infection is 2 to 5 days though can be as long as 5-6 days. There are reports that incubation period and disease severity gradually decrease from hatching to maturity (Hanson, 1972).

The incubation period of experimental infection of Newcastle disease range from 1 – 25 days with an average of 4 – 5 days though the incubation period is shorter if route of infection is aerosol or environmental temperature is high (Doyle, 1927; Sinha, 1958).

2.2.7 Clinical Signs

The clinical manifestation of ND depends on the infectivity and dose of the virus, the host species, age and immune status, concurrent infections, environmental conditions and route of exposure (Alexander, 1997). Clinical signs include respiratory and nervous signs such as coughing and gasping, drooping wings, dragging legs, torticollis, cycling, depression, inappetence and complete paralysis. There is either a drop or total cessation of egg production. However, eggs are often misshapen, rough or thin shelled with watery albumen. Birds usually develop a greenish watery diarrhoea.

2.2.8 Pathology

2.2.8.1 *Gross lesions*

The tissue or organs where gross lesions are usually depends the tropism of the strain causing the infection. The virulent NDV causes haemorrhagic lesions of the intestinal and respiratory tracts, although there are no pathognomic gross lesions.

2.2.8.2 *Histopathology*

Histopathology changes of NDV depends on the viral strain and route of infection. In the nervous system, there are foci of glia cells, perivascular infiltration of lymphocytes and proliferation of endothelia cells in the cerebellum, medulla and spinal cord (Alexander, 1997). However, there is hydropic degeneration, hyalinization of capillaries and areterioles. Simillarly, there is thrombosis in small vessels and necrosis of endothelia cells of the vessels. There are also changes in the intestinal, respiratory and reproductive system.

2.2.9 Diagnosis of Newcastle Disease

Diagnosis of ND is either through direct or indirect methods. The direct methods include haemagglutination inhibition (HI) test, enzyme linked immunosorbent assay (ELISA), virus neutralisation (VN) test and complement fixation test (CFT) (OIE, 2009). However, direct test includes virus isolation, electron microscopy and virus characterisation (OIE, 2009).

2.2.10 Control of Newcastle Disease

The objective of control is either to prevent susceptible poultry from becoming infected or to reduce the number of susceptible birds from becoming susceptible by vaccination. In Nigeria where ND is endemic, the control strategy is by vaccination. However, biosecurity is essential in reducing exposure of poultry to the virus.

2.3 GUMBORO DISEASE

2.3.1 Introduction

Gumboro disease or infectious bursal disease is an acute or occasionally subclinical , highly contagious disease of mostly young chickens in which the tissues of the immune organs especially the bursa of Fabricius are involved causing necrosis and atrophy targeted resulting in immunosuppression (Cosgrove, 1962). In the acute form mortality can be 25% or even higher.

The name ‘Gumboro’ comes from the area on the Delmarva Peninsula in the USA where the disease was a significant problem. However, synonyms include avian-nephrosis (Cosgrove, 1962), infectious bursitis (Ibragimov, 1976) and avian infectious bursitis (Rodon, 1982). Gumboro disease was first reported in Nigeria, in 1973 and confirmed in 1975 (Ojo *et al*., 1973; Onunkwo, 1975). Currently, Gumboro disease (GD) is endemic in Nigeria.

2.3.2 Aetiology of Gumboro Disease

Gumboro disease virus is a birnavirus of the Avibirnavirus genus (Brown, 1986). These viruses are called birnaviruses because their genomes consist of two (bi-) segments of double stranded RNA (MacDonald, 1980).

The Gumboro disease virus is very stable and resistant to a variety of chemicals. The virus is unaffected by an hour’s exposure to 0.5% phenol, six hours exposure to 0.5% formalin reduces, but does not eliminate, infectivity. However, it is inactivated at pH 12, but is unaffected by pH 2 (Lukert and Saif, 1997). Gumboro disease virus will survive temperatures of 60°C for 30 minutes but not at 70°C. The ability of Gumboro disease virus (GDV) to survive adverse conditions is responsible for its survival and persistence in poultry farms and other environment even after thorough cleaning and disinfection.

Two strains of Gumboro disease virus, serotypes 1 and 2, are known. Serotype 1 is the strain that causes the well known disease in chickens, while serotype 2 was first isolated from turkeys where it is apathogenic though it has also been reported in chickens (McFerran *et al*., 1980). Very virulent strains of the Gumboro disease virus have evolved from the serotype 1.

2.3.4 Pathogenicity of Gumboro Disease

Chickens are the only species known to develop the clinical disease and lesions when exposed to by the Gumboro disease virus (Lukert and Saif, 1997). However, the classical Gumboro disease is characterised by mortalities of 10-50%, while very virulent GD, mortalities are between of 50-100%.

2.3.5 Distribution of Gumboro Disease

Infections with serotype 1 Gumboro disease virus are found around the world and are endemic in Nigeria (Abdu, 2005).

2.3.6 Transmission of Gumboro Disease

Gumboro disease virus persists in poultry house environments and houses from which infected birds are removed can remain infective for several months or longer (Benton *et al*., 1967). Feed, water and litter from such houses also remains infective for significant time periods (Benton *et al*, 1967). There is no evidence to suggest vertical transmission of Gumboro disease virus and latent carriers do not appear to exist in recovered flocks (Lukert and Saif, 1997).

Alphitobius diaperinus (lesser meal worm) has been reported to maintain GDV and infected chickens with Gumboro disease after a period of two months when fed to them (Snedeker *et al*., 1967). There are reports that mosquitoes, rats and dogs can carry Gumboro disease virus (Howie and Thorsen, 1981; Okoye and Uche, 1986).

2.3.7 Incubation Period and Clinical Signs of Gumboro Disease

The incubation period is short and clinical signs can be seen within two to three days after infection in birds at three weeks of age. Early signs are vent pecking in some birds followed by soiled vent feathers, diarrhoea, anorexia, depression, ruffled feathers, prostration and deaths.

In fully susceptible flocks the disease appears quickly with a high morbidity (up to 100%) and mortality can be 20-30% or higher (up to 90%) in outbreaks of very virulent GD (Abdu, 2005).

Mortality usually starts three days after infection and typically lasts for five days (Abdu, 2005).

2.3.8 Lesions of Gumboro Disease

The post-mortem lesions of GD include dehydrated and darkened breast muscles and haemorrhages are often seen in the wing and thigh muscles. Renal changes are often seen as is excess mucus in the intestines. The bursa of Fabricius is the primary target of GDV and three days after infection the bursa of Fabricius is enlarged (Abdu, 2007). Thereafter it atrophies and by day eight it is approximately a third or less of its original weight. However, the bursa of fabricius often shows areas of necrosis and haemorrhage and occasionally faeces is blood streaked and splenic enlargement (Abdu, 2007).

The histopathological lesions occur in most of the lymphoid organs especially in the bursa of Fabricius where lymphocyte depletion occurs (Abdu, 2007).

2.3.9 Immunity to Gumboro Disease

Following exposure to Gumboro disease virus active immunity is stimulated and the antibody response can be measured by VN, AGP or ELISA blood tests (Ismail and Saif, 1990). Antibody transmitted via the egg yolk protects chicks against early infections with Gumboro disease virus. Maternal or passive immunity can interfere with the stimulation of immunity from vaccines administered to the offspring (Abdu, 2007). Oil emulsion vaccines given to broiler breeders typically protect the progeny chicks for four to five weeks and live vaccines given to broiler breeders typically protect the offspring for one to three weeks (Abdu, 2007).

2.3.10 Diagnosis of Gumboro Disease

Acute outbreaks of Gumboro disease are diagnosed tentatively by clinical signs and post-mortem lesions. Typically mortality has a rapid onset with a rapid recovery with the whole episode typically being completed in under a week and is often referred to as spiking mortality (Abdu, 2007).

Confirmation is made by isolating and confirming the identity of GDV from the bursa of Fabricius and the spleen which contain high concentrations of the GDV. Nowadays, direct immunofluorescent staining of these organs is a useful additional test to confirm diagnosis (Abdu, 2005).

Nowadays, the ELISA is used for assessing GD antibody levels as it is a quantitative test and essential in assessing the likely depletion of maternal antibodies in progeny chicks which is important in to predict an optimal time for vaccination (Lukert and Saif, 1997).

2.3.11 Differential Diagnosis of Gumboro Disease

Gumboro disease signs are similar to those seen in acute coccidiosis and with of high mortality similar to virulent Newcastle disease or virulent avian influenza. The nephrosis is often seen in GD and other causes of renal pathology should be considered including water deprivation and nephrotoxic strains of infectious bronchitis. Bursal atrophy can be confused with Marek's disease (Lukert and Saif, 1997).

2.3.12 Prevention of Gumboro Disease

Vaccination is the way to control Gumboro disease infection and many vaccines, which vary in terms of virulence and antigenic diversity, are available in the market.

Live vaccines are classically known as mild, intermediate and 'hot' vaccines. Their use basically depends on the type of virus causing the disease and the level of maternal antibody that needs to be overcome. Vaccines containing an intermediate GDV and a

measured amount of antibody have been used with success in day olds and in ovo. New vaccine technology based on live recombinant virus vectors has also been successfully used.

Killed oil adjuvanted vaccines are used to provide a prolonged immunity in table egg layers and breeders and these are often administered in a multivalent vaccine that also contains Newcastle disease and infectious bronchitis vaccines and, in the case of table egg layers, EDS 76 vaccine.

Gumboro disease vaccination is a dynamic and movable feast dependent on many factors. Veterinary guidance on the design of your vaccination programme is to be recommended (Abdu, 2007).

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY AREA

The study areas were eight Local Government Areas of Kaduna State namely Jaba, Jema'a, Kachia, Kaduna North, Kaduna South, Kaura, Sanga and Zango Kataf.

Kaduna State is located in the North Western region of Nigeria. It is bounded by latitude 10^0 and 11^0 North and longitude 7^0 and 8^0 East. The state shares borders with Zamfara and Niger states to the west, Katsina and Kano states to the north, Bauchi and Plateau states to the east and Nassarawa and the Federal Capital Territory to the south. The state has an area of about 48,473.2 square kilometres with a human population of 6.3 million engaged in crop farming, livestock rearing, trading and fishing (KDSG, 2007). The cultivable land area is 4.5 million hectares, and only 2.02 million hectares is under actual cultivation. The estimated poultry population is 5,200,000 (KDSG, 2007).

Kaduna State is within the Northern Guinea Savannah with a land structure consists of undulating plateau. It has some major rivers that include River Kagom, River Galma and River Kaduna which stretches from the fringes of the Jos Plateau near Manchok through Kauru and Kaduna to join the River Niger (KDSG, 2007).

Kaduna State has two marked seasons made up of the dry, windy season extending from November to March and the wet (rainy) season which extends from April through October. There is a great variation in the wet season as one moves from the Northern part of the State to the Southern part of the State with an average of 5 months of rainfall of 1,016 mm. The southern part has heavier rainfall than the North (KDSG, 2007).

3.2 SAMPLE SIZE

The sample size of 228 was determined using the prevalence of avian influenza of 18.2 $^{0}/_{0}$ earlier determined by Obi and Ahmed (2008) in a previous study in LBMs in states that reported AI in Nigeria and the formula

$$N = \frac{Z^2 PQ}{D^2} \quad \text{(Mahajan, 1997)}$$

Where:-

N= Sample size

Z= the approximate value from the normal for the desired confidence (1.96)

P = Prevalence

Q = 1-Prevalence

D= Allowable error.

$$N = \frac{1.96^2 \times 0.192 \times (1-0.192)}{0.05^2} = 228$$

3.3 SAMPLE COLLECTION

The species sampled were chickens, guinea fowls, ducks, pigeons and turkeys at the randomly selected live bird markets in the different local governments of the study area. The LBMs are Central Market in Kaduna North LGA, Kwoi Market in Jaba LGA, Kafanchan Markets in Jema'a LGA, Kachia Crossing market in Kachia LGA, Sokoto Road LBM in Kaduna North LGA, Manchok Market in Kaura LGA, Railway Station Market in Kaduna South LGA, Gwantu market in Sanga LGA and Zonkwa and Katsit markets in Zango Kataf LGA.

In each of these markets, a total of 25 samples were collected proportionately depending on flock size of each poultry species.

A sample collection form was used to record vital information like serial number, date of sample collection, state, LGA , town/village, owner of poultry, breed, species and type of sample collected.

Blood samples were collected from the live birds. About two millilitres of blood was aseptically collected from the brachial vein from each bird, using 23 G needle and 5 ml syringe each, for each bird, after restrain by an aide. The blood was then carefully transferred into sterile test tubes, labelled with an acronym number, place and date of collection, kept at room temperature and allowed to clot and then packed in ice and transported to the laboratory were the sera was collected from the blood samples by centrifugation at 431gravitations per minute for 5 minutes. The sera was transferred into sterile boujour bottles and refrigerated until used for analysis by the HI test and agar gel immunodiffusion test.

3.4 DETERMINATION OF AVIAN INFLUENZA AND NEWCASTLE DISEASE ANTIBODIES

3.4.1 Avian Influenza Antigen and Positive Serum

The test antigen used was an inactivated H5 subtype–antigen while the positive serum was an H5N2 serum both prepared by Istituto Zooprofilattico OIE/FAO Laboratory for AI and NDV delle Venezie. Newcastle disease antigen was Newcastle vaccine (La Sota).

3.4.2 Preparation of 1% Red Blood Cells (RBCS) Suspension

Blood was collected from five day old chicks and pooled in an equal volume of Alserver's anticoagulant solution. Cells were washed three times in Phosphate Buffer Saline (PBS) by centrifuging at 431gravitations per minute for 5 minutes (Collee *et al*., 1982). Ninety-nine millitres of PBS was added to 1 ml of the RBCs to make a 1% suspension (Allan *et al*., 1978).

3.4.3 Determination of Avian Influenza type A H5 and Newcastle Disease Antigen Titre

Haemagglutination (HA) test was carried out according to the method described by OIE (2009). Twenty five micro litre of PBS was dispensed into each well of micro titre plates. 25 ul of antigen suspension was placed into first well. Two-fold dilutions of 25 ul of the antigen suspension were made across the plate. A further 25 ul of PBS was dispensed into each well. A 25 ul of 1% chicken red blood cells was dispensed into each well. These were mixed by gently tapping the plate and then allowing the RBC to settle for about 40 minutes at room temperature. The HA was determined by tilting the plate and observing for the presence or absence of tear- shaped streaming of the RBC. The titration was read to the highest dilution giving complete HA (no streaming); this represented 1HA unit (HAU) and was calculated accurately from initial range of dilutions from which 4HAU was calculated and used for HI test.

3.4.4 Haemagglutination Inhibition Test

The antibody titre was determined by haemagglutination inhibition (HI) test (OIE, 2009). Twenty five micro litres of PBS was dispensed into each well of a plastic U- bottom microtitre plate. Twenty five micro litre of serum was dispensed into each well of the plate. Two-fold dilutions of the 25 ul volumes of the serum were made across the plate. Four HAU of the antigen suspension in 25 ul was added to each well and left for a minimum of 30 minutes at room temperature. A 25 ul of chicken RBC was added to each well and after gentle mixing was allowed to settle for about 40 minutes at room temperature. The HI titre is the highest dilution of the serum causing complete inhibition of RBC agglutination by the 4 HAU antigens. Wells considered positive to HI were wells in which RBC stream at the same rate as positive control wells and the results were expressed in $\log_2$.

3.5 DETECTION OF INFECTIOUS BURSA DISEASE ANTIBODIES

Gumboro antigen was derived from affected bursae of chicken that died from natural GD. The bursae was removed, diluted 1:1 (w/v) with PBS (pH 7.6); ground with pestle and mortar and centrifuged. The supernatant was extracted and used as antigen. A positive control and a known negative antiserum were incorporated in each test run. The AGID test for Gumboro disease was performed as recommended by Harai *et al*. (1972).

3.6 THE ROLE OF LIVE BIRD MARKETS IN THE SPREAD OF AVIAN INFLUENZA

Selected live bird markets were identified by local government area, town/village and GPS co-ordinates and structured questionnaire administered (appendix).

Information regarding the management, biosecurity practices employed in each market, volume of trade, sources and destination of birds were sought. Additional information as regards the knowledge of the marketers on poultry diseases and their readiness to disclose outbreaks of poultry diseases especially AI were gathered. Other questions were aimed at determining the prices of birds at various levels of trade and the trade or otherwise in sick birds.

3.7 STATISTICAL ANALYSIS

All the data obtained were entered into the Excel data base and analysed using the statistical package for social science (SPSS) version 17. Data on antibody titre and from questionnaire on LBM were analyzed by descriptive statistics.

CHAPTER 4

RESULTS

4.1 LIVE BIRD MARKETS AND BIOSECURITY PRACTICES OF LIVE BIRD MARKETERS IN KADUNA STATE

Fifty six per cent of (LBM) studied were daily markets with 43.6% being weekly markets. The mean distance of LBMs from residential is 474.26 ± 27.60 m though the minimum distance was 0 m. However, 21.8% and 66.2% of LBMs were within 200 m and 500 m from residential areas though the maximum distance was 1 km.

Though 64.4% (66) of fowl sellers traded in LBMs without outbreak history, 56.4% (57) operated in daily LBMs with 43.6% (44) trading in weekly markets. Amongst fowl sellers who indicated their market days, 52.3% (23), 25.0% (11) and 2.7% (10) were Friday, Saturday and Thursday respectively. Seventy five (74.3%) LBMs were fenced with Kaduna being the nearest town to 19.8% (20) of the fowl sellers (Figure 1).

About 42.6% (43) of marketers kept poultry in cages with 34.0% (16) using metal cages while 66.0% (31) keep poultry in wooden cages though only 9.9% (10), 48.5% (49), 32.7% (33), 48.5% (49) separate poultry by age, breed, species and type respectively. However, 73.5% of fowl sellers in LBMs with history of HPAI separate poultry by breed (Figure 2). Daily LBMs (63.2%; x^2 = 43.18; p = 0.00) had outbreak history though LBMs without outbreak history (93.1%; 49.08, p=0.00) practiced keeping poultry in cages though all LBMs without outbreak don't keep poultry in metal cages (0%; x^2 = 7.42, p = 0.006). However, all LBMs with outbreak history separate poultry by age (100%; x^2 = 20.04, p = 0.00), breed (73.5 %; x^2 = 59.36, p = 0.00), species (60.6 %; x^2 = 13.31, p = 0.00), Type (73.5 %; x^2 = 59.36, p = 0.00). Similarly, all LBMs with outbreak history were fenced unlike those without outbreak history (13.3 %; x^2 = 63.21, p = 0.00).

Sixty five (64.4%) of fowl sellers reported that other animals are sold in their LBM with 11.8% (10), 74.1% (63), 76.5% (65) and 42.4% (36) selling cattle, sheep, goats and pigs respectively.

Most (59.4 %; x^2 = 53.14, p = 0.011) of fowl sellers prepare sick poultry for food (Figure 3) though 38.6% (39) and 54.5% (55) fowl sellers throw away dead poultry (Figure 4) and feathers respectively. However, 27 (66.7%) live bird marketers acknowledged purchasing sick birds usually because they are cheap (Figure 5) while amongst those who don't buy sick birds, only 9.8% (7) did so because it was not safe (Figure 6).

Poultry offal are reported to be eaten by 78.2% (79) of fowl sellers (Figure 7) while 95.0% (95) use poultry manure in farms and 5% (5) throw it away. All (100%) fowl sellers in Kafanchan throw away all dead poultry while most in Kachia (80%) would bury and 53.8% of fowl sellers in Kwoi would eat dead poultry (x^2 = 59.38, p = 0.002). However, in Kwoi, all fowl sellers eat poultry offal though fowl sellers in Sokoto road (12.5%) and Kwoi LBM (40.0 %; x^2 = 40.63, p = 0.018).

All fowl sellers in Sokoto road LBM burn poultry feather though most in Kachia LBM (70%) use feather in farms while all fowl sellers in Kaura and Kafanchan LBM would throw away feathers (x^2 = 168.46, p = 0.00). Weekly markets are likely to throw away (77.3%) feathers compared to daily marketers who either bury (10.5%), burn (40.4%) or use in farms (12.3 %; x^2 = 17.96; p=0.00).

Poultry faeces is used in farms by fowl sellers in all studied LBMs except Kafanchan LBM (45.5 %; x^2 = 43.04, p = 0.00).

None of the fowl sellers wear overalls, boots, face mask, hand gloves or goggles. All fowl sellers in all the LBMs wash their hands after handling poultry except marketers in

Crossing (20%), Kafachan (27.3%) and Zonkwa (45.5 %; x^2 = 62.8; p =0.00). Though only 46.5% (47) of fowl sellers trade in LBM having processing area, 23.4% (11) reported that well was the source of water used in the processing and 53.5 % (54) using tap water even though only 46.5% (47) acknowledged that the LBM is regularly cleaned and disinfected.

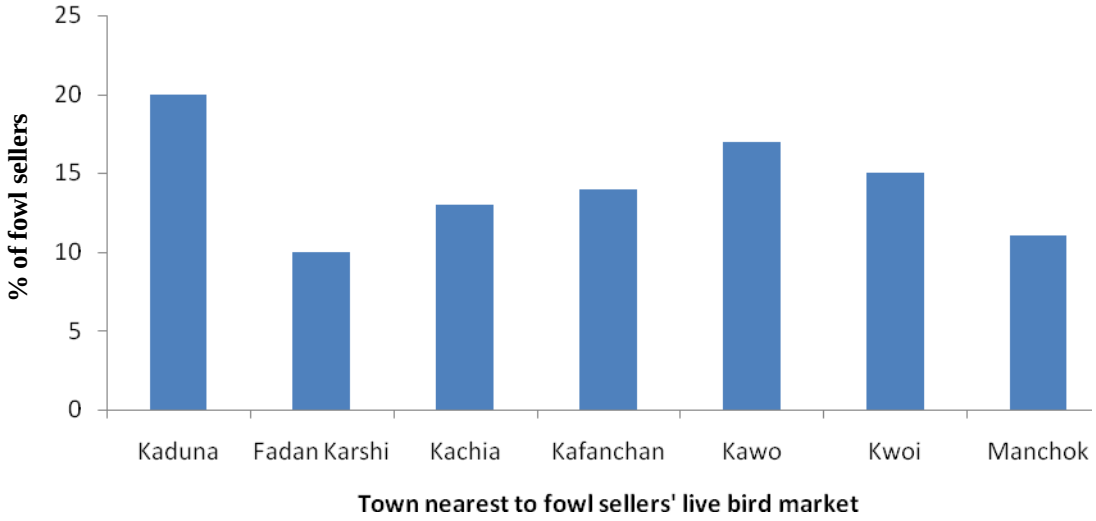


Figure 4.1: Distribution of town nearest to fowl sellers' live bird markets

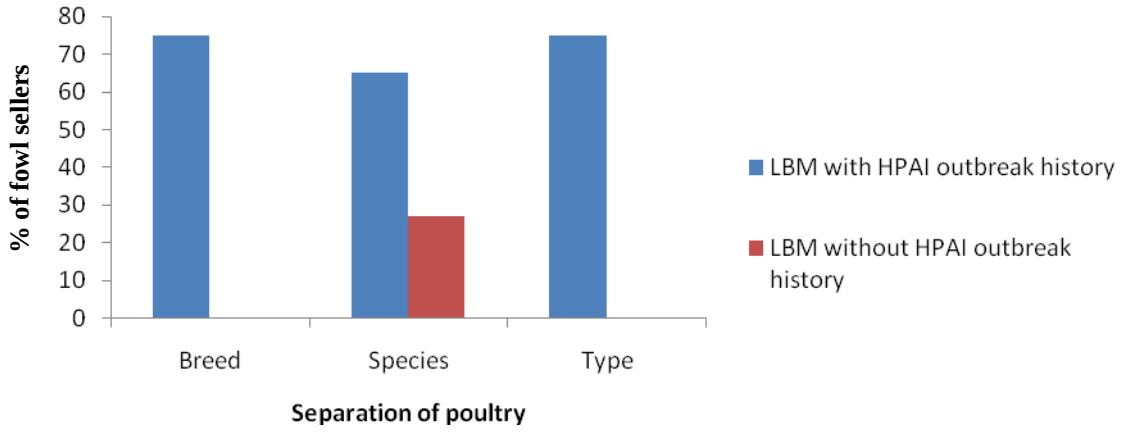


Figure 4.2: Fowl sellers' practices on poultry separation by breed, species or type

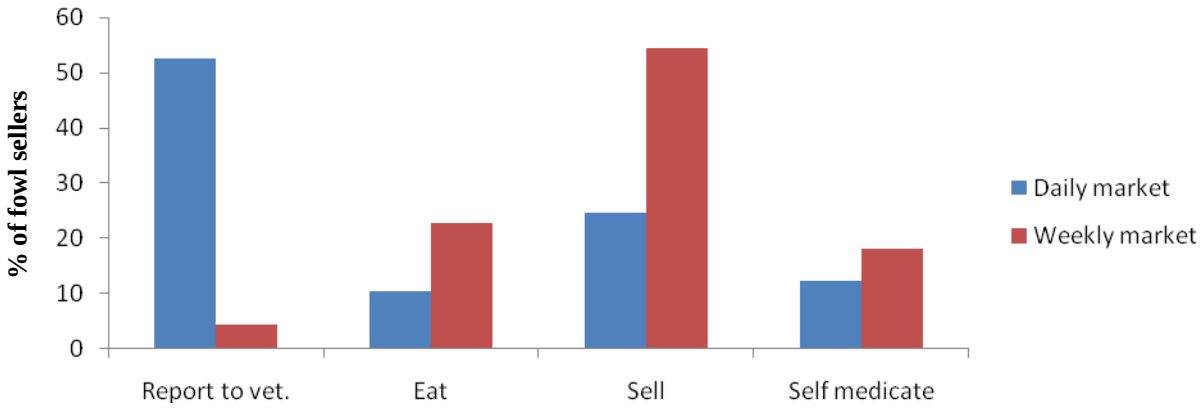


Figure 4.3: Behaviour of fowl sellers' in different types of live bird markets on sick poultry

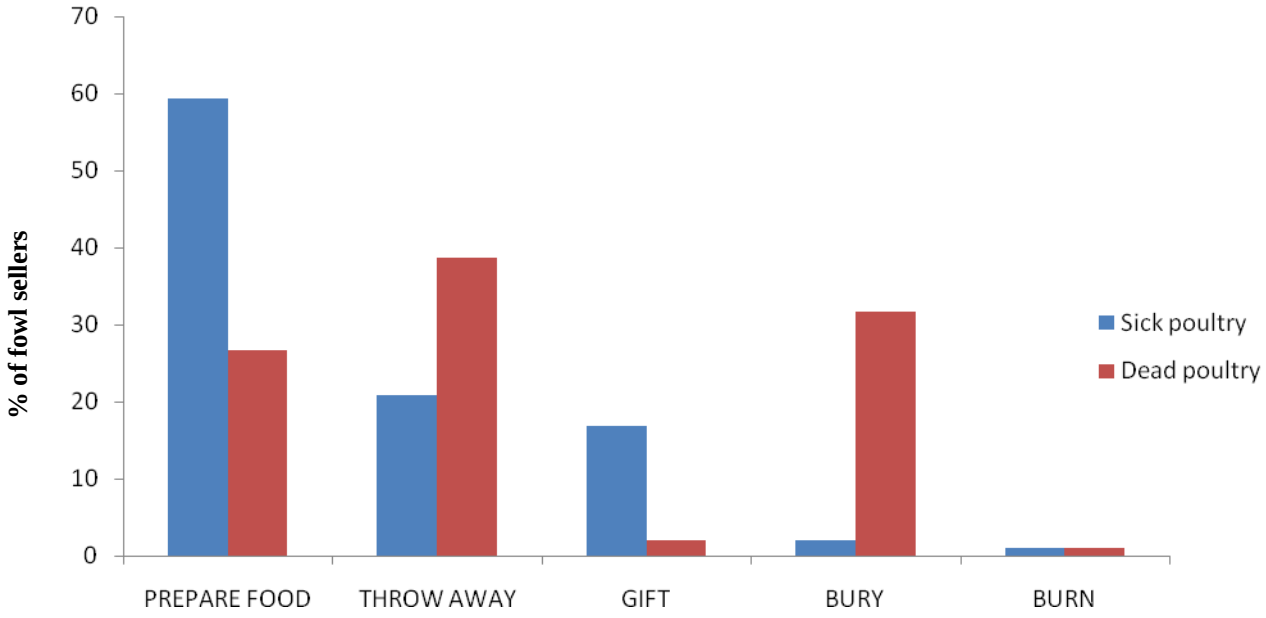


Figure 4.4: Action of fowl sellers on sick and dead poultry

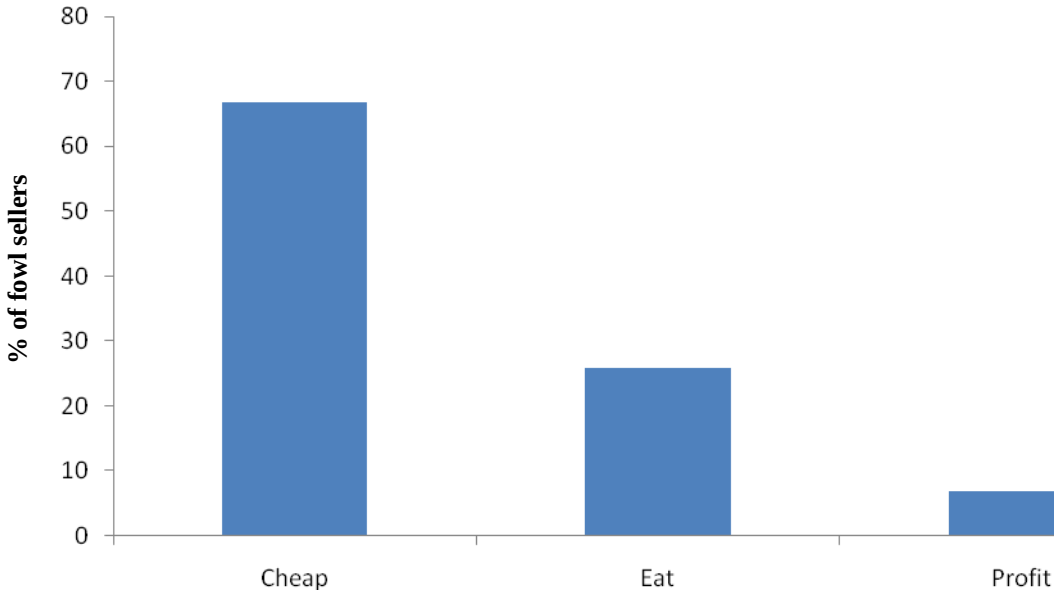


Figure 4.5: Reasons for buying sick birds by fowl sellers



Figure 4.6: Reasons fowl sellers do not buy sick birds

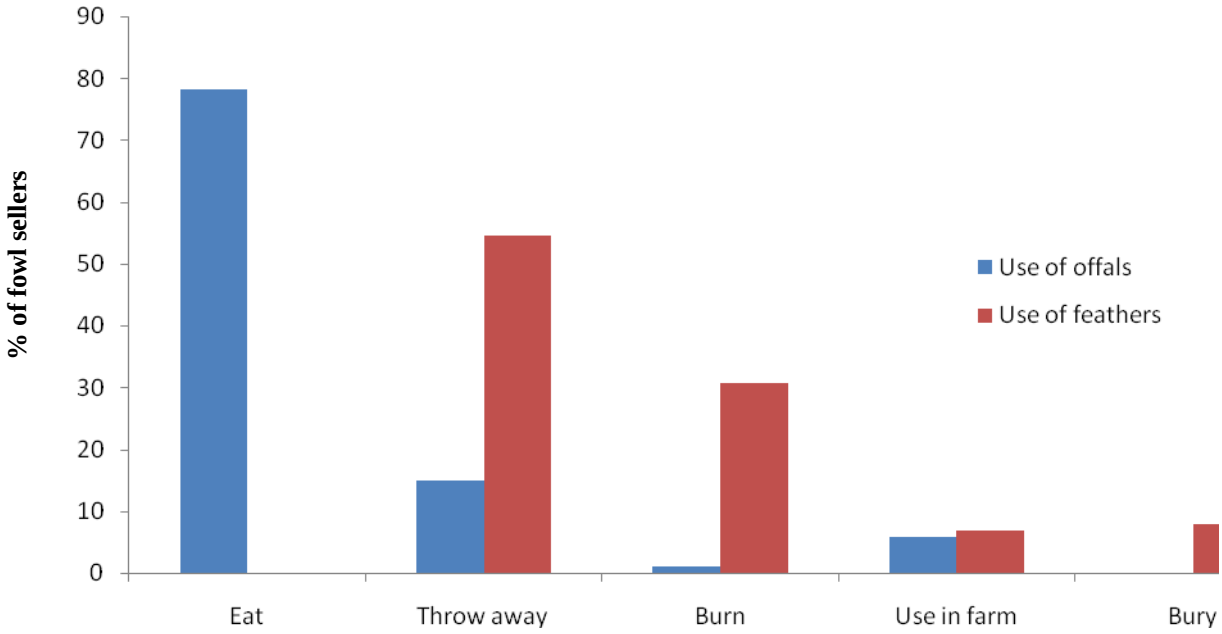


Figure 4.7: Use of poultry offal and feathers among live bird marketers

The processing area is usually cleaned by either live bird marketers (57.4%) or processors (42.6%). About 55.3% (26) of fowl sellers reported that they drain water from the processing area into drainage channels while 44.7% (21) drains into a river.

Live bird markets are either managed by local government staff (54.5%) or live bird marketers (44.6) or others (1%) though only 46.5% (47) acknowledged government intervention, 48.9% (23) listed building while 51.1% (24) reported fumigation as

government contribution. Fowl sellers reported government intervention in Sokoto road, Station, Kafanchan and central market LBMs (46.5 %; x^2 = 101.00, p = 0.00) though intervention reported in Sokoto road LBM involved building while Station LBM was regular fumigation but Central market LBM involved both building and fumigation (33.3 %; x^2= 263.86, p = 0.00). Government intervention is likely in daily market (100 %) than in weekly markets (x^2 = 101.0; p = 0.00).

None of the fowl sellers wear overalls, boots, face mask, hand gloves or goggles. However, 21.6 % (22) do not wash their hands after handling poultry.

4.2 POULTRY TRADE IN LIVE BIRD MARKETS AND LIVE BIRD MARKETERS' KNOWLEDGE ON POULTRY DISEASES

Eighty (79.2%) of fowl sellers reported that their LBMs have fowl sellers association (FSA). Most fowl sellers (89.1%) reported adults as those who bring poultry to LBMs, 10.9 % (11) reported adult and young people. Live bird marketers in Kafachan are likely to buy poultry from children though most fowl sellers buy from adults and young people (60.6 %; x^2 = 101.00, p = 0.00).

Though only 26.7 % of fowl sellers would buy sick poultry, 70 % of fowl sellers at Kaura and Central market would buy sick poultry (x^2 = 27.7; p = 0.01). However, all those who buy sick poultry in Kaura LBM use them for consumption while all those in Sokoto road, Station, Kafachan, Gwantu and Zonkwa purchase sick poultry because they are cheap (x^2 = 108.4; p= 0.00).

When questioned on their sales, 54.5% (55), 96.0 % (97) and 36.6 % (37) sell to vendors, consumers and restaurants respectively. Weekly LBM were likely to sell poultry to Vendors than daily markets (x^2 = 38.0; p= 0.00).

Some fowl sellers in Gwantu (40%), Station (80 %), Kachia (90%), Kaura (70%), Kafanchan (27.3%), Kwoi (76.9 %), Zonkwa (81.8%) and Central market LBM (50.0%; x^2 = 38.00, p = 0.00) sold poultry to vendors while all those in Sokoto road, Station, Kachia, Kafanchan and Zonkwa LBMs with 90% of fowl sellers in Gwantu, Kaura, Central market LBMs with Kwoi (92.3 %; p >0.05) selling poultry directly to consumers. However, only station (80%), Kachia (10%), Kaura (50%), Kafanchan (18.2%), Kwoi (61.5%), Zonkwa (81.8 %) and Central market (40 %; x^2 = 41.77, p = 0.00) sell to restaurants.

When asked how often poultry are slaughtered in their LBM, 46.5% (47) and 53.5 % (54) reported very often and not often respectively. All fowl sellers in Sokoto road, Station, Kafanchan and Central market LBMs reported poultry are very often slaughtered in their markets (p = 0.00).

Eid-el Fitr was reported as high sales period by 45.5% of fowl sellers (Figure 4.8). However, fowl sellers in Sokoto road, Gwantu, Station and Central market reported Eid-el Fitr festival as period of high sales (x^2 = 101.00, p = 0.00) while Eid-el Kabir was period of high sales in Central market and Kwoi (x^2 = 101.00, p = 0.00). However, Maulud Na Bi was a period of high sales in Kachia and Kafanchan (x^2= 202.00, p = 0.00) with Easter reported as high sales period in all the LBMs except Sokoto road and Central market LBMs (x^2 = 101.00, p = 0.00). Similarly, except for Sokoto road LBM, all the other LBMs reported high sales during New Year festival (x^2 = 101.00, p = 0.00). All the LBMs had fowl sellers association (FSA) except Kachia and Zonkwa LBMs (x^2 = 101.00, p = 0.00).

All fowl sellers in Sokoto road and Kafanchan LBMs knew poultry diseases though not all fowl sellers in other LBMs such as Gwantu (80%), Station (50%), Kachia (20%), Kwoi (23.1%), Zonkwa (18.2%) and Central market (40%). However, none of the fowl

sellers in Kaura LBM knew about poultry diseases (x^2 = 52.81, p = 0.00). Amongst fowl sellers who know poultry diseases, all in Sokoto road, Gwantu, station and Central market with Kafanchan (27.3%) and Kwoi (66.7 % ; x^2 = 131.20, p = 0.00) knew Gumboro disease.

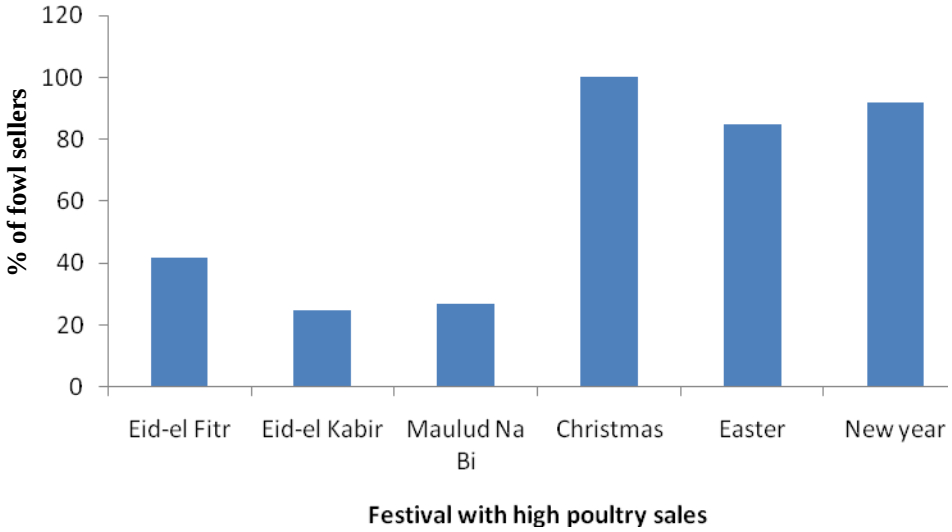


Figure 4.8: Festival fowl sellers reported as period of high sales

Newcastle disease was known by all fowl sellers who were knowledgeable on poultry diseases in Gwantu, station, Kafachan, Zonkwa and Central market LBMs but in Kwoi LBM, only 66.7% knew ND with none in Kaura and Sokoto road LBMs (x^2 = 137.70, p=0.00). However, all fowl sellers in Sokoto road and Central market LBMs knew coccidiosis unlike the other LBMs where none of the fowl sellers knew coccidiosis (x^2 = 165.96, p=0.00). None of the fowl sellers in Gwantu, Kachia and Kaura LBM knew fowl

pox. However, all fowl sellers in Sokoto road, station, central market and Zonkwa LBMs knew fowl pox unlike fowl sellers in Kafanchan (27.3%) and Kwoi (66.7 %; x^2 = 125.97, p=0.00).

All fowl sellers in Sokoto road LBM knew about AI but differ in other LBMs viz Gwantu (80%), Station (40%), Kachia (20%), Kaura (30%), Kafanchan (81.8%), Zonkwa (9.1%) and Central market (60%; x^2 = 50.37, p=0.00). However, all fowl sellers in Sokoto road accepted knowing AI signs, though only 80% in Gwantu, 10% in Station, 20% in Kaura, 40 % in Central market, 0% in Zonkwa, Kwoi and Kachia LBMs (x^2 = 159.17, p=0.00) accepted knowing AI. When asked to list the clinical signs of AI, all fowl sellers in Sokoto road and Gwantu LBMs listed the clinical signs correctly with only 25% of fowl sellers in Central market listing the signs but none from Station and Kaura LBMs (x^2 = 26.20, p=0.00).

Similarly, all fowl sellers in Sokoto road claimed to know the differential diagnosis for AI with 30 % in Gwantu, 63.6 % in Kafanchan, 40 % in Central market and 0 % in Station, Kachia, Kaura, Kwoi and Zonkwa (x^2 = 123.20, p = 0.00) though none of the fowl sellers in Sokoto road could list the differential diagnosis correctly unlike in Gwantu where 66.7 % listed correctly and 75% in Kafanchan and central market (x^2 = 18.25, p=0.00).

Most fowl sellers in daily markets (87.7 %) know where to report a suspected HPAI outbreak in their poultry, the fowl sellers from weekly markets (81.8 %) do not know (x^2 = 49.11; p = 0.00). However, daily market fowl sellers (93.0 %) trust to report HPAI outbreaks to veterinarians while weekly market fowl sellers (72.7%; x^2 = 46.74; p = 0.00) would prefer to report to their community leader. However, only in Sokoto road, Kachia and Kafanchan LBMs (Figure 9) do all fowl sellers know where to report HPAI outbreak

(x^2 = 61.26, p = 0.00). All fowl sellers in Kachia trust to report HPAI outbreak (x^2 = 50.64, p = 0.00) (Figure 4.10).

Fowl sellers in Kaura, Kwoi did not know that AI affects human with only 27.3 % knowing from Zonkwa (x^2 = 74.49, p=0.00) though none of the fowl sellers in Kwoi believe AI affects humans. However, all fowl sellers in Sokoto road, Kachia, Kaura believes AI affects humans while 70% in Gwantu, 80% in station, 18.2% in Zonkwa and 60 % in central market (x^2 = 64.63, p=0.00). Seventy (69.3%) and 89.5% (51) fowl sellers in daily markets believe HPAI affects humans (x^2 = 25.01; p = 0.00) though only 31.6 % (30) of fowl sellers know HPAI differential diagnosis (x^2 = 28.44; p = 0.00).

Most fowl sellers (54.5%) in weekly LBMs (x^2 = 27.00; p = 0.00) would sell their poultry when suspecting HPAI (Figure 4.11).

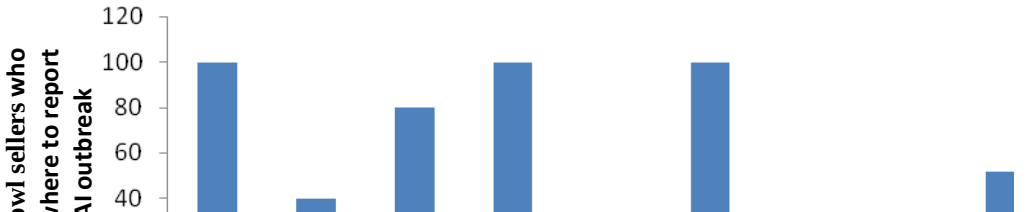


Figure 4.9: Fowl sellers' knowledge on where to report suspected highly pathogenic avian influenza in poultry

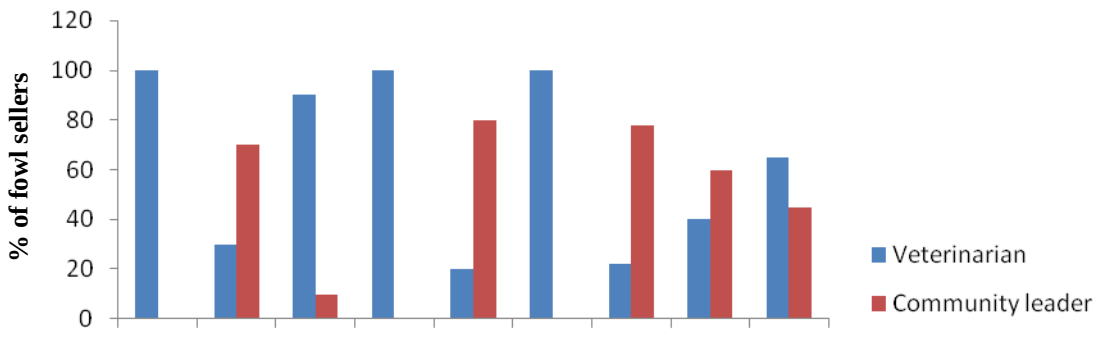


Figure 4.10: Authority fowl sellers trust to report highly pathogenic avian influenza outbreak

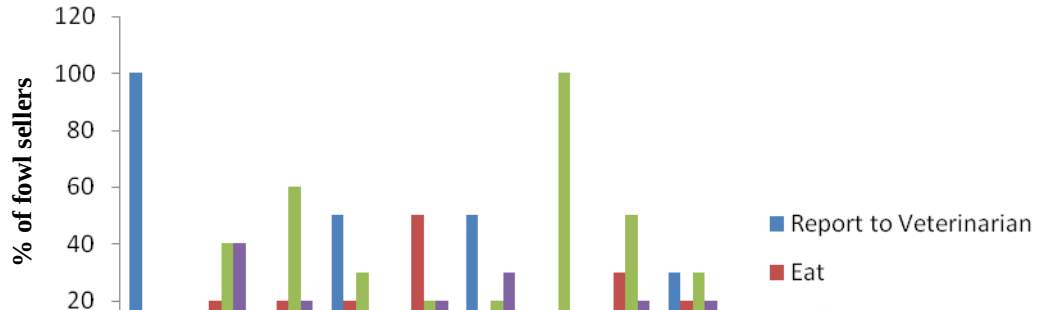


Figure 4.11: Fowl sellers' action when suspecting highly pathogenic avian influenza

When asked on fowl sellers action when suspecting HPAI, all fowl sellers in Sokoto road would report to the veterinarian while in Gwantu, fowl sellers either eat (20 %), sell (40%) or self medicate (40 %; x^2 = 90.25, p=0.00) with none reporting to a veterinarian (Figure 4.10).

All fowl sellers in Kachia and Sokoto road LBMs (x^2 = 61.26, p=0.00) had fear rate > 5 on a scale of 10 (Figure 4.12) and all fowl sellers in Station LBM family/colleagues'' fear rate for HPAI was < 5 (x^2 = 49.88, p=0.00).

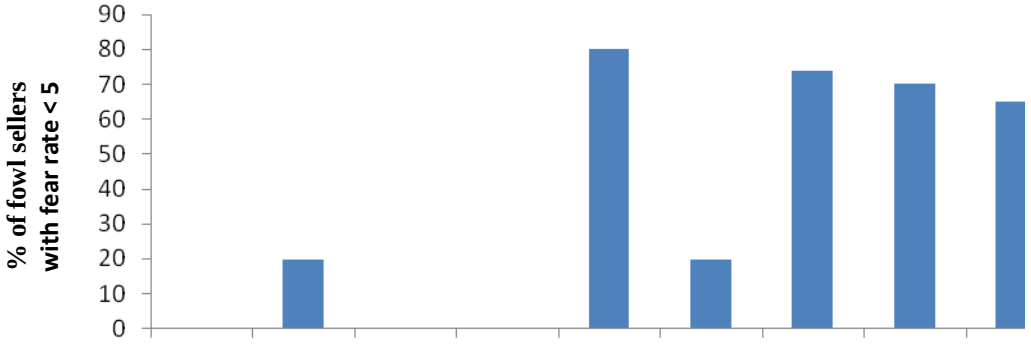

% of fowl sellers with fear rate < 5
90
80
70
60
50
40
30
20
10
0

Figure 4.12: Percentage of fowl sellers with fear <5 in different live bird markets

4.3 Newcastle, Gumboro Diseases and Avian Influenza (H5 Subtype) Surveillance Study

The mean Newcastle disease (ND) titre of the 230 poultry sampled was 4.46 ± 0.25 $\log_2$ with a prevalence of 60.4% (139) though 41.3% (95) had titre < 4 $\log_2$ with 23.0% (53) and 35.7% (82) had 4 – 6 $\log_{10}$ and > 7 $\log_2$ respectively. However, the mean H5 HI titre was 3.90 ± 0.415 $\log_2$ with a prevalence 35.7% (56) and 50.9% (112) for H5 AI and Gumboro antibodies respectively.

All exotic cocks sampled in the study had Gumboro antibodies (x^2 = 19.48; p=0.01) though prevalence of ND (x^2 = 14.62; p=0.06) and H5 AI (x^2 = 11.00; p=0.03) antibodies

among other chicken types varied (Figure 13). Poultry in Sokoto road LBM had 47.5% (19), 56.1% (23) and 42.9% (15) prevalence of Gumboro (x^2 = 23.74; p = 0.003), Newcastle disease (p>0.05) and H5 AI (x^2 = 27.50; p=0.024) antibodies respectively (Figure 4.14).

Among the poultry species sampled, 61.9% (138) chickens were sero-positive for ND antibodies (x^2 = 10.02; p=0.007) while none of the six guinea fowls sampled had ND antibodies. The only turkey sampled had ND antibodies. However, though not statistically significant (p>0.05), the prevalence of H5 AI antibodies in chickens and guinea fowls were 34.9% (53) and 60% (3) respectively. None of the guinea fowls and turkeys sampled had antibodies against Gumboro with a chicken prevalence of 52.6% (n=112; x^2 = 7.5; p= 0.024).

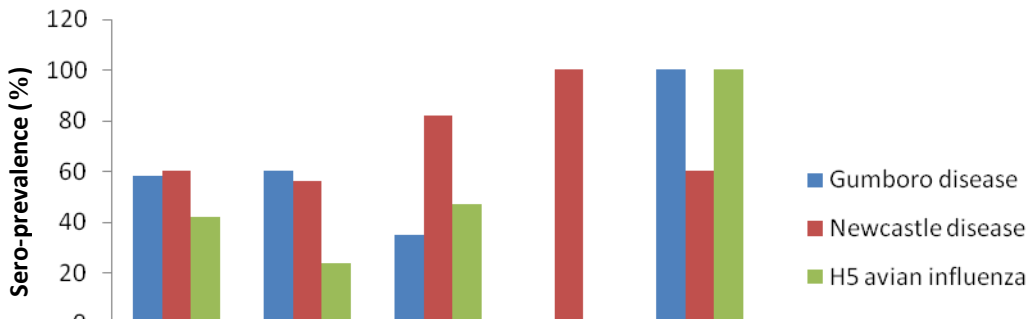


Figure 4.13: Sero-prevalence of H5 avian influenza, Gumboro and Newcastle diseases among chicken types

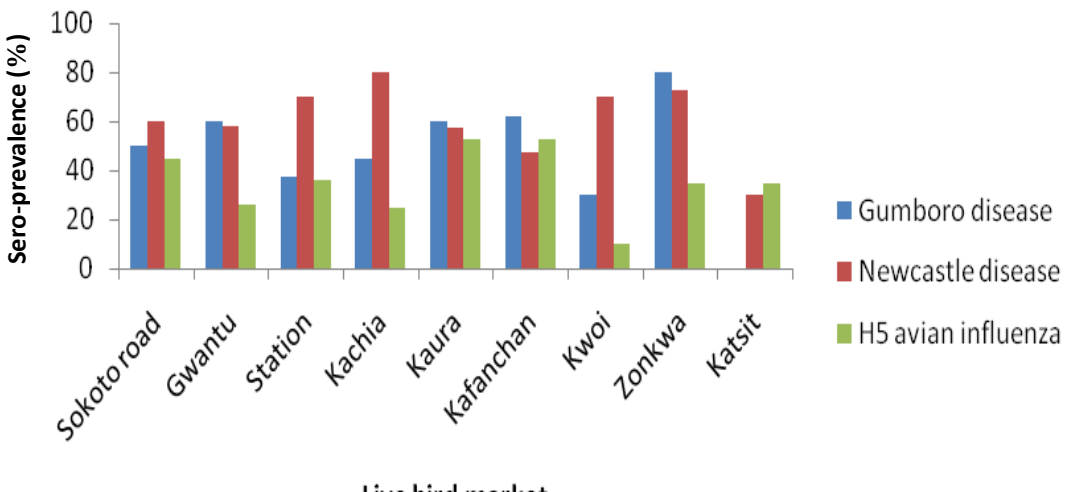


Figure 4.14: Sero-prevalence of H5 avian influenza, Gumboro and Newcastle diseases in live bird markets

CHAPTER 5

DISCUSSION

The study revealed that there are more weekly markets in the rural parts of the study area of Kaduna state than daily markets which were found in the urban and sub-urban areas. However, the LBM located close to residential areas increases human, domestic pets and house pest exposure to avian influenza in case of an outbreak thereby making it difficult to control the outbreak.

Since most weekly markets operate on Thursdays, Fridays or Saturdays movement restriction in case of outbreak of avian influenza would reduce the chances of spreading

the infection by movement of infected poultry between markets. However, the lack of fence in most LBMs implies movement restriction into and out of the LBMs would be difficult with easy access by unauthorized persons and animals which increases the probability of introduction and exposure to the HPAI virus.

The practice of using wooden cages makes cleaning and disinfection of the cages difficult with likelihood of contaminated cages not being properly disinfected and acting as the focus of infection to newly introduced poultry thereby maintaining the virus in the LBM. However, the improvement seen in the use of metallic cages is probably due the intervention of the Federal Government of Nigeria (FGN) in some markets. The separation of poultry based on age, breed, species and type needs to improve (Muhammed, 2010). The better practice of separating poultry by age, breed and species in LBMs which experienced HPAI outbreak highlights the success of FGN intervention in the area of training live bird marketers on biosecurity with respect to HPAI which was revealed in this study. The practice of separation by breed, species and type was common in LBMs that experienced AI outbreak than in LBMs without HPAI outbreak history. This is likely because government intervention in terms of infrastructure has been mainly in the LBMs situated in local governments that experienced outbreaks. These LBMs are situated in urban areas and have increased access to avian influenza information. The fencing of LBM might have play a role in the LBMs not having outbreak. The sales of other animals in some LBMs is likely to expose these other animal species to avian influenza as cross species infections has been reported (Shane, 2002). Moreover, sales of pigs in the LBMs are not recommended as they have been reported as the likely mixing vessel of avian influenza virus (Shane, 2002).

This study revealed that live bird marketers engaged in risky practices similar to those reported by previous studies in Nigeria and would enhance human exposure and HPAI

spread among poultry (Assam, 2010; Aye, 2010). These risky practices are probably due to the low level of HPAI risk perception amongst live bird marketers. Risky practices of selling sick poultry would spread the infection since most rural farmers increase their flock size through purchases from LBMs (Assam, 2010). The purchase of sick poultry by live bird marketers because it is cheap without considering the risk indicates that more needs to be done on marketers education to increase their risk perception with respect to the effect of avian influenza on their health and business. Also, refusal of marketers to purchase sick birds will discourage the sales of sick birds by poultry farmers especially local poultry farmers (Assam, 2010).

The study further confirms previous reports of self medicating sick poultry in an attempt to treat infected poultry which would likely increase environmental contamination with the AI virus if poultry is infected (Assam, 2010).

Throwing dead poultry away also increases the spread of H5N1 virus and environmental contamination while increasing the likelihood of infection of wild birds which serves as reservoirs of avian influenza (Alexander and Brown, 2000). These dead poultry thrown away could be carried away by scavengers which mechanically spread the virus to uninfected areas.

Preparing of sick poultry for food exposes the processors to the virus during processing and contaminates the environment and LBM.

The marketers in daily markets would report sick poultry to veterinarians unlike those in weekly markets because government intervention are mostly in the daily markets which have a Fowl Sellers Association and a biosecurity team which includes veterinary services personnel which they can readily access (Muhammed, 2010). However, marketers in the

weekly markets would likely either sell, eat or self medicate their sick poultry compared to daily markets as marketers in daily markets were more informed on avian influenza risks from the training workshops they have attended (Muhammed, 2010).

Consumption of poultry offal increases risk of exposure of consumers to the HPAI (H5N1) virus hence increase in chances of human infection. However, marketers not burning offal rather either throw away or use it in farms increases access of these offal to scavengers like eagles, dogs, pigs and vultures which might either act as reservoirs or transfer to distant uninfected areas.

Though marketers are likely to engage in the risky practice of throwing away feathers than either burning or burying might be as a result of the cost involved in these forms of disposal hence the need to explore other forms of disposal such as composing of feathers, offal and dead birds which might serve as manure for farms while inactivating the HPAI (H5N1) virus (Birnbaum and O'Brien, 2008).

The practice of throwing dead poultry by marketers in Kafanchan highlights the need for education of these marketers on the risk of avian influenza and how proper biosecurity practices could protect their investment in the event of HPAI outbreak. However, the practice of burying dead poultry by marketers in Kachia is probably based on their religious beliefs which does not encourage eating of dead animals and the marketers in Kwoi are more likely to be exposed to HPAI virus in the event of an HPAI outbreak since they do not believe avian influenza is zoonotic. Similarly, marketers in Sokoto road burn all their feathers because of the availability of an incinerator in the market though the disposal of feathers by marketers in Kachia would expose both humans and environment to virus in an HPAI outbreak. The use of poultry faeces by marketers without compositing would spread the virus and expose both human and animals especially local poultry to HPAI virus.

The study revealed that marketers do not wear protective clothing nor do they wash their hands during and after handling poultry which is likely to expose them to HPAI virus if poultry is infected.

The study further revealed that there is need to upgrade some of the LBM with processing section in terms of provision of tap water and proper drainage as these are essential for ensuring biosecurity of the LBM. Also, provision of water will ease daily cleaning and disinfection of the market especially the processing sections.

The study further revealed that more of the LBM studied were managed by Local Governments which made it a little difficult from benefiting from Federal Government intervention as the marketers are supposed to proof ownership of the market as a requirement for partnership with Federal Government of Nigeria. The local government ownership of markets might reduce participation of marketers in the management and cleaning of the market which is the responsibility of government officials who do not have any interest at stake if markets are not properly cleaned. Also, the sustainability of the fumigation and other intervention measures would not be guarantee in the eventual withdrawal of the Federal Government of Nigeria support.

Marketers in Kafanchan could discourage contact of children to poultry by refusing to purchase poultry from children as children are more susceptible to avian influenza than adults (Rutz, 2007). Though few marketers purchase sick poultry which is a risky practice, marketers in Kaura would be increasing human exposure since they consume the sick poultry while those in Sokoto road, Station, Kafachan, Gwantu and Zonkwa would encourage viral spread as they sell sick poultry to maximize profit.

The study revealed that most of the poultry is sold to consumers who are end users though a reasonable sales is to vendors who take the poultry to other markets with the possibility

of dissemination of poultry infection to other poultry and markets. Weekly markets are mostly rural markets which serve as the primary markets where vendors purchase poultry to be sold in daily markets which are mostly located in the urban centers.

The study revealed that Eid-el Fitr and Easter are a high sale periods which might be associated with outbreaks of Newcastle disease and avian influenza. Hence surveillance should be increased and veterinary service should be on the alert during these periods (Ahlers *et al*., 2009). The different markets have different periods of high sales which could be utilized by surveillance teams in preparing a timetable for HPAI surveillance.

The marketers in Kachia and Zonkwa LBMs should be encouraged to form FSA so that they could benefit from FGN intervention programs. Marketers in Kachia, Kaura, Kwoi, Zonkwa and Kaduna central market have poor knowledge of poultry diseases which might affect their disease reporting ability though this lack of knowledge might be as result of marketers buying only healthy poultry. However, though Gumboro disease was known by marketers in some LBMs, ND was known by all marketers. The knowledge on ND might be because ND outbreaks are common but this could be built on by encouraging them to report ND outbreaks as it is a differential to HPAI. Coccidiosis and fowl pox are other diseases noticed by live bird marketers.

Poor awareness of HPAI in Kachia, Kaura and Zonkwa will hamper disease recognition and reporting though the high awareness in the other LBMs reaffirms the success of the AICP in creating awareness on HPAI among stakeholders. The study revealed that marketers in Sokoto road and Gwantu LBMs would recognize HPAI in case of an outbreak enhancing the likelihood of swift reporting. However, the poor knowledge of the clinical signs of avian influenza might be as a result of the radio being the media of HPAI knowledge as reported in previous studies and calls for the need to enlighten these

marketers on HPAI recognition which will improve on HPAI disease reporting (Assam, 2010). This can be achieved by using audio-visual aids. The knowledge of marketers on HPAI differential was low which may result in poor reporting hence late intervention.

The marketers in daily market would easily report outbreak as they know where to report HPAI outbreak. In these LBMs, the desk officers are members of the biosecurity team which justifies their willingness to report outbreak to veterinarians. However, the poor knowledge of marketers in weekly markets on where to report HPAI outbreak might be due to the lack of organization of their markets. This will reduce disease reporting by these groups of marketers which could be ameliorated by engaging community leaders in HPAI outbreak reporting.

Knowledge on the public health significance of HPAI was very poor among marketers which leads to poor risk perception which is reflected in the believe that HPAI does not infect humans. Marketers in urban and semi-urban areas were afraid of HPAI which indicate their high risk perception due their exposure to more information on HPAI unlike marketers from LBMs in rural areas who have poor access to HPAI information. The study revealed that the trend of fear rate of marketers' family and colleagues was similar to those of local poultry farmers in a previous study (Assam, 2010).

The study confirmed earlier reports that ND is endemic in chickens (Adu *et al.,* 1986) though the prevalence is lower than previous prevalence in local chickens in Kaduna State (Nwanta *et al*., 2006; Assam, 2010). Though the exotic chickens were vaccinated, the source of the antibodies in local chickens might be due to either infection by field ND virus or from contact with vaccinal virus in the faeces of the exotic chicken since poultry where not separated by type as reflected by the high proportion of chickens with titre >7 $\log_2$. The lack of protective ND antibodies in chickens could result in high mortality in

these chickens upon infection with virulent field ND virus which can be confused with HPAI (Ahlers *et al*., 2009).

The study confirmed previous report that H5 subtype viruses are circulating in LBMs in Kaduna State (Aye, 2010). The antibodies might either be due to vaccination which is practiced illegally in commercial poultry or from challenge by field viruses. The antibodies due to vaccination would complicate surveillance as it will require the DIVA method thereby increasing cost of surveillance. However, antibodies might be due to infection with low pathogenic avian influenza (LPAI) since no clinical signs nor mortality were reported. Infection with LPAI in LBM calls for concern as these viruses have the potential of becoming HPAI after circulating in LBM as was the case in other outbreaks (Gracia *et al*., 1996)

The prevalence of Gumboro disease (GD) among poultry confirms previous reports (Assam, 2010; Aye, 2010). However, the absence of GD in broilers raises concern on the GD vaccination program practiced by commercial poultry farmers.

The prevalence of H5 in guinea fowls calls for concern as they act as bridge species and has a propensity of spreading the virus to distant unaffected areas and birds (Alexander, 2000).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

From the findings of this study, it could be concluded that:

1. Antibodies against H5 influenza virus were detected in various species and breeds in the LBMs in the study area while Newcastle and Gumboro Diseases had a seroprevalence of 64% and 60% respectively.
2. Generally in most of the live bird markets, biosecurity measures are not practiced despite government intervention, sensitization and trainings.
3. Risk perception of live bird marketers is low because they eat, buy and sell sick birds.
4. Live bird marketers are aware of avian influenza, but majority of them cannot recognize the disease and do not know where to report it.
5. Christmas and New year are periods of high sales of poultry and poultry products and this could be utilized by surveillance teams in preparing timetable for HPAI surveillance.

6.2 RECOMMENDATIONS

Based on the findings of the study, it is recommended that a virology study should be conducted to determine if the avian influenza virus is circulating in poultry in LBMs. Furthermore, there is need for continuous education of live bird marketers and processors on biosecurity practices in LBMs and the health implications of eating, buying and selling sick birds. There is need for government assistance toward the sustainable development of live bird markets in Kaduna State.

During festive seasons such as Christmas and the New Year, massive disinfection of live bird markets should be intensified.

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APPENDIX

A structured questionnaire on avian influenza in live bird markets in eight southern local government areas of Kaduna State, Nigeria.

PLEASE, ANSWER YES OR NO, WHERE APPLICABLE
SECTION A: MARKET IDENTITY

1. Serial Number----------Date----------
2. State----------
3. Local Government Area----------
4. GPS coordinates – Latitude----------Longitude----------
5. Has there been any report of Bird Flu in the Local Government Yes
6. Name of live bird market----------
7. Market type: a).Daily b.) Weekly c). Informal d). Others Specify----------
8. Market days M ☐ T ☐ W ☐ Th ☐ F ☐ Sat ☐ Sun ☐
9. Market days M ☐ T ☐ W ☐ Th ☐ F ☐ Sat ☐ Sun ☐

SECTION B. BIOSECURITY IN THE MARKET

10. Is the market fenced? Yes☐ No☐
11. Which is the nearest big Town/Village----------

12. Are birds kept in cages? Yes☐ N☐ If yes : Type of cages Metallic Wooden Others(Specify)----------

13. Are the birds kept separately by: 1. Age Yes ☐ No ☐

    2. Bread? Yes ☐ No ☐
    3. SpeciesYes ☐ No ☐
    4.Type? Yes☐ No ☐

14. Are other animals sold in the Markets? Yes ☐ No ☐

15. If yes list the other animals sold 1) Cattle ☐ 2) Sheep ☐
    3)Goats☐ 4) Pigs ☐ 5) Donkeys,☐ 6).Horses☐ 7).Rabbits ☐
    8). Others (specify)----------
16. What do you do with:

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

17 Any biosecurity practices observed in the market like

a. Wearing overalls? Yes☐ No ☐

b. Wearing boots? Yes ☐ No ☐

c. Wearing facemasks? Yes ☐ No ☐

d. Wearing hand gloves? Yes ☐ No ☐

e. Wearing goggles? Yes ☐ No ☐

f. washing of hands? Yes☐ No ☐

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

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

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

21. If Yes, by who----------

22. How far (in Meters or kilometers) is the market from a residential area?----------
23. Where does the water run of from the processing area flow to?----------

24. Do you buy sick birds? Yes ☐No ☐

25. If yes why?----------

26. If no, why?----------

SECTION C TRADE IN THE MARKET

28. Is there, or has there been government intervention in the market? Yes ☐ No ☐

29. If yes. Specify----------
27. Who is in charge of the market----------

30. Who brings poultry to the market? A). Adult b)Children c).Males

d) Females.

31. From which town----------Village?----------Country--------

32. Who buys poultry? A). Vendor ☐ b). Consumers ☐ c).Restaurants ☐

33. Where are poultry taken to from the market----------

34. Which are the periods of high sales----------

a). Eid-el fitr	b). Eid-el kabir	c).Maulud Na bi
Yes ☐ No ☐	Yes ☐ No ☐	Yes ☐ No ☐

d) Christmas	c). Easter	New Year
Yes ☐ No ☐	Yes ☐ No ☐	Yes ☐ No ☐

35. Is there an association of the poultry sellers Yes ☐ No ☐
36. If there is , who is a). The Chairman?----------
b). The Secretary?----------

37. What is the number of a) Owners? Male ☐ Female ☐
b) Sellers? Male ☐ Female ☐
c) Buyers? Male ☐ Female ☐

38. What is the average prices of the following

	From the owners?	From the seller?	From the buyers
a.	Chickens----------	----------	----------
b.	Guinea fowls----------	----------	----------
c.	Turkeys----------	----------	----------
d.	Ducks----------	----------	----------
e.	Geese----------	----------	----------
f.	Pigeons----------	----------	----------
g.	Other specify----------	----------	----------
h.	Chicken eggs----------	----------	----------
i.	Turkey Eggs----------	----------	----------
j.	Ducks Eggs----------	----------	----------
k.	Geese Eggs----------	----------	----------
l.	Pigeons Eggs----------	----------	----------
m.	Others (specify)----	----------	----------

SECTION D. KNOWLEDGE OF POULTRY DISEASES

39. Do you know any poultry disease(es)? Yes ☐ No ☐
40. If yes, which one(s)?----------

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

42. Do you know the signs of bird flu?----------

43. If yes, list some signs of bird flu----------

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

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

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

47. If yes which one(s)?----------

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

49. If no why----------
50. If yes to whom----------

Sign----------