# ANTIBODIES AND SERUM PROTEIN LEVELS IN LOCAL AND EXOTIC ADULT CHICKENS SUFFERING FROM AVIAN INFLUENZA

BY

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**SEPTEMBER, 2007**

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


**DEPARTMENT OF VETERINARY SURGERY AND MEDICINE,**
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**SEPTEMBER, 2007**

## DECLARATION

I hereby declare the originality of this work carried out by me in the Department of Veterinary Surgery and Medicine, Ahmadu Bello University, Zaria under the supervision of Professors P. A. Abdu, J. U. Umoh and Dr. G. S. A. Muhammad. The work of other investigators referred to in this study was duly acknowledged. No part of this thesis has been previously submitted for a degree or diploma.

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Wakawa, Aliyu Mohammed Date

## CERTIFICATION

This thesis entitled "ANTIBODIES AND SERUM PROTEIN LEVELS IN LOCAL AND EXOTIC ADULT CHICKENS SUFFERING FROM AVIAN INFLUENZA" by Wakawa, Aliyu Mohammed 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 lovely wife Hauwa, and our twin daughters Zainab and Husseina, as well as my parents Mr. and Mrs. Aliyu Wakawa.

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## ABSTRACT

The objective of this study was to determine the levels of antibodies and serum proteins in local and exotic adult chickens suffering from avian Influenza (AI). A total of 1, 000 blood samples were collected from 500 clinically sick local and exotic adult chickens confirmed to be suffering from AI and 500 apparently healthy local and exotic adult chickens in some parts of Jigawa, Kaduna and Katsina States of Nigeria. The samples were analysed for antibodies to AI type A H5 and Newcastle disease (ND) antigens, serum total protein (TP), albumin and globulin levels, using haemagglutination inhibition (HI) test, Biuret technique, and Modified Batholomew and Delaney technique, respectively.

Seventy nine (31.6%) of the clinically sick local, 49 (19.6%) of the clinically sick exotic, and 6 (2.4%) of the apparently healthy local chickens tested positive for AI type A H5 antibodies with mean antibody titres of 5.6 ± 0.1 $\log_2$, 7.1 ± 0.1 $\log_2$ and 5.8 ± 0.1 $\log_2$ respectively. Thirteen (5.2%) clinically sick local chickens, 23 (9.2%) clinically sick exotic chickens, 97 (38.8%) apparently healthy local chickens, and 213 (85.2%) apparently healthy exotic chickens tested positive for ND antibodies with mean antibody titres of 3.9 ± 0.3 $\log_2$ ,4.4 ± 0.2 $\log_2$, 4.8 ± 0.1 $\log_2$ and 5.5 ± 0.1 $\log_2$ respectively. The values of TP obtained for clinically sick local and exotic chickens (3.6 ± 0.0 and 3.0 ± 0.0 g/dl respectively) were significantly ($p<0.001$) lower than those obtained for apparently healthy local and exotic chickens (6.5 ± 0.1 and 7.8 ± 0.1 g/dl respectively). The values of albumin levels obtained for clinically sick local and exotic chickens (2.1 ± 0.0 and 1.9 ± 0.0 g/dl respectively) were significantly ($p<0.001$) lower than those obtained for apparently healthy local and exotic chickens (3.3 ± 0.0 and 4.1 ± 0.1 g/dl respectively). A significant decrease ($p<0.001$) in globulin levels was observed

in clinically sick local and exotic chickens (1.5 ± 0.0 and 1.1 ± 0.0 g/dl respectively) below the values established for apparently healthy local and exotic chickens (3.2 ± 0.1 and 3.7 ± 0.1 g/dl respectively).

This study showed an overall prevalence rate of 13.4% for AI and 34.5% for ND in the area of study. The presence of antibodies to AI H5 antigen further confirmed the disease in the clinically sick chickens sampled. Local chickens might likely be source of AI virus and play significant roles in its transmission and spread to susceptible backyard and commercial poultry. Avian influenza resulted in a decrease in the concentration of serum TP, albumin and globulin in the affected local and exotic adult chickens. It was strongly recommended that a national active AI surveillance be conducted to define the true status of the disease in Nigeria. If vaccination against AI is to be carried out, there is the need to place birds on high level of protein supplements in order to augment their serum protein levels which are expected to drop post-vaccination.

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# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Introduction

Avian influenza (AI) is a disease caused by influenza type A virus, a member of the family *Orthomyxoviridae*, which may be transmitted by direct inhalation of aerosol and contaminated dust or indirectly via ingestion of contaminated water, food or infected carcasses. Birds of all ages and almost all avian species are susceptible to AI virus. Chickens and turkeys are the most adversely affected birds by AI. At present, AI is recognized in two forms: highly pathogenic AI (HPAI) and low pathogenic AI (LPAI). The incubation period of the disease is usually 3 to 7 days depending on the isolate, the dose of inoculum, species and age of birds (Abdu *et al.,* 2005; Hansen, 2005; Hanson, 2005).

Perroncito initially reported AI in 1878 in Italy (Bankowski and Samadieh, 1980; Easterday *et al*., 1997). The disease came to international attention in 1997 when it caused an outbreak of severe disease in poultry in Hong Kong, China, and for the first time a true AI virus caused serious human disease with 18 people clinically affected and six deaths (Morris and Jackson, 2005).

## 1.2 Statement of Research Problems

The reduction in egg production, high mortality and high cost of treatment and eradication due to AI result in loss of income and livelihoods. Production losses due to AI subsequently result in high cost of poultry and poultry by-products because of the resultant scarcity of poultry products. Countries and regions affected by AI stand the risk of losing the right to export poultry and poultry by–products to AI-free countries (Abdu *et al*., 2005). About 1.5 million chickens were killed (stamped out) in Hong Kong in order to get rid of the source of

infection in 1997 (Mubiru, 1998). About 100 million chickens were slaughtered in South Korea, Cambodia, China, Indonesia, Japan, Laos, Thailand and Vietnam in 2003 (WHO, 2005).

Between December 2003 and March 2004, an epidemic of AI in South East Asia involving South Korea, Cambodia, China, Indonesia, Japan, Laos, Thailand and Vietnam resulted in the infection of 34 and the death of 23 people (WHO, 2005). Between March and May 2003, an outbreak of AI in the Netherland caused the death of a veterinarian after exhibiting respiratory symptoms and mild illness of conjunctivitis (pink eye) in 89 other humans working directly with poultry (MDTH, 2005; WHO, 2005;). Between 2003 and 2007 a total of 251 persons working in close contact with poultry have been infected out of which 151 deaths have been recorded (WHO, 2007) (Table 2.1).

Avian influenza was reported in Zimbabwe in 1995 and South Africa in 1998 (OIE, 2004a: Pfitzer *et al.*, 2000). The outbreak of AI in Nigeria was the first outbreak of the H5N1 Asian strain on the African continent. It was discovered initially in Kaduna state in February, 2006 (Adene *et al.*, 2006). Despite immediate containment measures taken by the veterinary and public health authorities in conformity with standard operating procedures, the disease spread to other parts of the country (NADIS INFO, 2006a). A total of 122 cases have been officially reported by the Federal Government of Nigeria from 38 local governments in 14 states, and the disease is still spreading in some states (NADIS INFO, 2006b). However, prior to the outbreak of February 2006, in year 2002 a preliminary survey for antibodies against some selected viruses in village chickens of various age groups was carried out in Borno state, Nigeria. The results showed that 26.5% of 320 samples tested positive for influenza type A virus antibodies (El- Yuguda and Baba, 2002).

Confirmed outbreaks of AI have been reported in Niger Republic, Egypt, Burkina Faso, Sudan, Cote d'ivore, Djibouti, and Cameroon. Six human deaths from 14 infected people have been recorded in Africa (NADIS INFO, 2006a). The concern of the scientific community today is that AI virus may mutate into a form that can easily be transmissible from human to human. With no existing immunity to such a mutant, this might prove devasting (Thanawat *et al*., 2005).

### 1.3 Objectives of research

The objectives of the research were to:

- Determine antibodies to AI and ND viruses in apparently healthy local and exotic adult chickens.
- Determine the normal baseline serum total proteins, albumin and globulin levels in apparently healthy local and exotic adult chickens.
- Determine antibodies to AI and ND viruses in local and exotic adult chickens confirmed to be suffering from AI.
- Determine the serum total proteins, albumin and globulin levels in local and exotic adult chickens confirmed to be suffering from AI.

### 1.4 Justification of research

Highly pathogenic avian influenza (HPAI) is a devastating disease in poultry. It is associated with a high mortality rate and disrupts poultry production and trade. It may be transmitted from birds to humans, and is a potential source of future human pandemics (Thanawat *et al*, 2004). There are a number of research activities which are crucial to achieving more effective control of HPAI infection. Without additional information in these various areas, control decisions will continue to be made without adequate scientific

basis, which entails a high risk of failure (Morris and Jackson, 2005). Avian influenza is likely to be a potentially global problem of serious proportions for some years to come and effective control is being seriously hampered by lack of a number of pieces of information which would not be costly or difficult to obtain (Morris and Jackson, 2005).

There are viruses that can be detected by their haemagglutination activity i.e. capacity to agglutinate red blood cells of certain animal species e.g influenza viruses (Rockborn *et al.*, 1990). Haemagglutination inhibition (HI) tests were used to screen Swiss fancy breed chicken flocks for antibodies against 12 avian infectious agents; out of which 40% prevalence for HPAI was recorded (Wunderwald and Hoop, 2002). Haemagglutination inhibition test is suited for influenza A antibody screening in species harbouring several influenza subtypes (de Boer *et al.*, 1990).

Newcastle disease antibodies were tested for in this study in view of the fact that AI viruses were reported to have caused more severe disease in Newcastle disease-infected chickens (Paul and Schrier, 2001).

Total protein (TP) has been known to play important roles in infections accompanied by invasion of the body by foreign materials, whether it is of bacterial, viral, protozoal or parasitic origin (Coles, 1974). It has also been established that a decrease or increase in TP is one of the important parameters used to assess the health status of animals. Therefore in avian species, the knowledge on blood parameters is valuable in the diagnosis of some diseases and the determination of the extent of damage to the blood cells (Hawkey and Dennett, 1989; Oladele and Ayo, 1999). The Biuret technique is the simplest chemical quantitative analytical method for determination of total serum protein concentration (Coles,

1974). Albumins prevent rapid excretion of drugs and are of assistance in detoxification and inactivation of materials which may be toxic to the animal body. Serum albumin can be determined by the modified Bartholomew and Delaney method (Coles, 1974).

Alteration in antibodies, total proteins, albumin and globulin concentrations existing within an animal may be indicative of disease (Coles, 1974). Studies on TP in indigenous chickens of northern Nigeria could be of value in developing efficient comprehensive flock health programmes for prophylaxis and therapy of local poultry disease (Oladele and Ayo, 1999). Further studies are still needed to establish normal baseline serum total proteins and albumins for healthy local chickens. The data that will be obtained from such studies could serve as standard for comparing with values obtained from clinically sick chickens (Oladele and Ayo, 1999).

Investigations into haematological values in apparently healthy and sick domestic animals have received increasing attention in recent years in the temperate and tropical countries. There are both scientific and economic reasons for attempting to establish normal haematological values in normal and clinically sick tropical animals (Oladele, 2000). In Veterinary Medicine, haematological values, especially total protein and haemaglobin are used in diagnosis, treatment and prognosis of many livestock diseases (Amand, 1986; Ogunsanimi *et al.*, 1994; Jordana and Cuenca, 1997).

It has been demonstrated that in avian species, knowledge of blood parameters is of value in the diagnosis of certain diseases (Onyeyili *et al.*, 1991). However studies on blood parameters such as total proteins, albumins and haemoglobin in local avian species in the northern Guinea Savannah zone of Nigeria are scanty and grossly inadequate (Oladele,

2000). Previous studies have shown that changes in normal values of blood parameters are pointers to various disease conditions, even at sub-clinical levels in birds (Hawkey *et al*., 1983). Changes in plasma globulins in birds may reflect the severity of disease processes and thus serve as basis for prognosis of avian diseases (Cole and Boyd, 1965; Hawkey *et al*., 1983).

Biological research with any species of animals is concerned in many instances with normal values for purposes of comparison with experimentally-induced diseases, and also with naturally induced diseases (Ross *et al*., 1976). Several authors have reported higher values of total protein in birds as a result of different diseases (Cole and Boyd, 1965; Hawkey *et al*., 1983; Robertson and Maxwell, 1995).

At present, Nigeria does not practice vaccination against HPAI due to the fact that vaccines administration may interfere with the stamping-out control policies that is generally recommended in Nigeria. Vaccinated birds might continue to shed the virus in the environment and susceptible birds might come in contact with the virus and come down with the disease (NADIS INFO, 2006a). Therefore, additional information on the prevalence of HPAI in Nigeria and the level of immunity in birds suffering from the disease could be useful in prevention, control and eradication of the disease.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Introduction

Avian influenza is a viral disease affecting the digestive, nervous and respiratory systems of almost all domestic and wild birds. The disease is characterized by respiratory, digestive and/or nervous signs with high morbidity and mortality. The incubation period of AI is few hours to few days (Whiteman and Bickford, 1989; Easterday *et al.*, 1997; Defra, 2003; Abdu *et al.*, 2005). The synonyms for AI include avian influenza A, bird flu, fowl pest, fowl plague, and highly pathogenic avian influenza (HPAI) (Whiteman and Bickford, 1989; Easterday *et al.*, 1997; Larson, 1998; Alexander, 1999).

The trends in the current AI epidemic started with reports of outbreaks in South-East Asian poultry towards the end of 2003. Avian influenza continued to spread from Asia westwards to the Middle East and reached Eastern Europe with outbreaks in Croatia and Romania in 2005 (WHO, 2005).

## 2.2 History

Peroricito initially reported AI in 1878 in Italy (Bankowski and Samadieh, 1980; Easterday *et al.*, 1997). Thereafter, AI outbreaks were reported in many areas of the world during this century, including north and South America, north Africa, the middle and far east, Europe, Great Britain and the former Soviet Union (Easterday *et al.*, 1997).

Other reports of outbreaks include Oregon (1971), Australia (1975 and 1978), England (1979), the United States of America (USA) (1983 – 84), Ireland (1983 – 84), Mexico (1994), Pakistan (1994) (Easterday *et al.*, 1997; Abdu *et al.*, 2005).

Further outbreaks were reported in Hong Kong (1997 and 2003), Republic of Korea (2003), Thailand, Vietnam and Cambodia (2004); Turkey, Greece, Bulgaria, Romania, (2005) (Morris and Jackson, 2005).

In Africa, AI outbreaks have been reported in Zimbabwe (1995) and South Africa (1998) (Pfitzer *et al.,* 2000; OIE, 2004). Other outbreaks were recently reported in Nigeria, Niger Republic, Egypt, Burkina Faso, Sudan, Cote d'Ivoire and Cameroon in 2006. The first suspected case of AI in Nigeria was reported in Sambawa Farms, a semi commercial farm situated in Kaduna State, North central Nigeria on January 16, 2006 (Ahmed, 2006).

The disease was clinically diagnosed initially at the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria (NADIS INFO, 2006c; Adene *et al.*, 2006). The National Veterinary Research Institute (NVRI) Laboratory, Vom confirmed the disease to be an AI type A virus on February 6, 2006, and the OIE/FAO reference laboratory in Padova, Italy finally confirmed the disease as HPAI caused by a H5N1 virus (profile: PQGERRRKKRGLFG) on February 7$^{th}$, 2006 (NADIS INFO, 2006c).

**2.3 Incidence and Distribution**

Avian influenza is worldwide in distribution and has been reported in north, south, and West Africa; Australia, north and south America (Canada, USA, Mexico, Chile), Middle and East Asia (Pakistan, Hong Kong), and Europe (Italy, Germany, UK, the Netherlands, Belgium, Turkey) (Alexander, 1999; Defra, 2003).

## 2.4 Aetiology

Influenza type A is a member of the family *Orthomyxoviridae* which causes AI. It is an enveloped, spherical or filamentous (80 – 100 nm in diameter on cross section) single stranded segmented (8) negative-sense RNA virus (Alexander, 1999). It codes for 10 proteins (Haemagglutinin (H), neuraminidase (N), protein matrix (M), ribonucleoprotein (RNP), etc) with an M and a RNP, which give the virus its antigenic type specificity (Easterday, 1981; Abdu *et al*., 2005).

Avian influenza type A virus (found in humans, birds, pigs and horses), B or C (found in humans) have two surface (envelope) glycoproteins – H and N which gives the virus its antigenic subtype specificity (Swayne *et al*., 1998; Abdu *et al*., 2005). There are 16 H (H1 to 16) and 9 N (N1 to 9) subtypes (Swayne *et al*., 1998; Alexander, 1999; Swayne, 2003). There are therefore, 144 possible Al virus subtypes. There is no cross protection between subtypes. Subtypes H1, H5 and H7, N1, N2, N3, N7 and N8 are the most pathogenic AI viruses. Others are mildly pathogenic or avirulent although they could later mutate without warning and become virulent without a change in their H and N subtype antigens (Alexander, 1999; Eckroade *et al*., 2002; Suarez, 2003; Hansen, 2005). Apathogenic AI viruses are also able to cause severe disease in Newcastle disease-infected chickens (Paul and Schrier, 2001).

The time it takes for avirulent or mildly pathogenic AI virus to become virulent varies. For example, the low virulent H5N2 virus in the USA became virulent within six months, while low virulent H2N1 virus in Italy caused severe outbreak of AI within nine months (Easterday *et al*., 1997; WHO, 2005). Due to their segmented nature and the presence of different surface proteins, AI viruses are susceptible to both random mutations within the

genome segments (antigenic drift) as well as re-assortment (swapping) of gene segments (antigenic shift) between different AI viruses in mixing vessels, such as pigs and humans following concurrent infection in the hosts (Easterday, 1981; Beard, 1998; Shane, 2002; Suarez, 2003; Meredith, 2004; WHO, 2005). Therefore, thousands of avian influenza type A viruses have been isolated from domestic and wild birds (Alexander, 1995; Easterday *et al*., 1997; Alexander, 1999).

**2.5 Resistance to Chemical and Physical Agents**

Avian influenza viruses are enveloped viruses, and thus, are relatively sensitive to inactivation by lipid solvents such as detergents. Infectivity is also rapidly destroyed by formalin, β-propiolactone, oxidizing agents, dilute acids, ether, sodium desoxycholate, hydroxylamine, sodium dodecylsulfate, and ammonium ions. They are inactivated by heat, extremes of pH, non isotonic conditions, and dryness (Easterday *et al*., 1997).

**2.6 Transmission**

The Al virus is present in secretions from the eyes, mouth and nostrils and faeces from infected birds. Transmission may also be by direct inhalation of aerosol and contaminated dust or indirectly via ingestion of contaminated water (especially surface water), food or infected carcasses (Chalmers, 2005). Broken contaminated eggs may infect chicks in the incubator (Defra, 2003). The faeco-oral route is the most common route of spread from bird to bird (Umoh, 2005).

The AI virus is more frequently isolated from water fowls, domestic ducks, turkeys and chickens in descending order (Swayne *et al*., 1998). Domestic and wild ducks, migratory, sea and pet birds may harbour more than one virus subtype for long periods without

becoming clinically sick. Live-bird markets, contaminated poultry houses and equipment, clothing, shoes, vehicles and eggs are good sources of the AI virus (Mubiru, 1998). The virus can survive for four to 30 days in contaminated water, depending on its temperature, and remain viable for up to 44 days in faeces and for more than 3 months in poultry manure and litter (Alexander, 1999; Defra, 2003; Hanson, 2005). The AI virus has been recovered from poultry houses more than 100 days after the flock has been marketed (Hanson, 2005). Pigs and humans may also transmit AI to poultry (Shane, 2002).

**2.7 Hosts**

Birds of all ages and almost all avian species are susceptible to AI virus. Humans, pigs and horses can be infected with AI virus (Abdu *et al*., 2005). Chickens and turkeys are the most adversely affected birds by AI virus (Hanson, 2005). Chickens (in live-bird markets), turkeys and ducks are potential sources of AI. Domestic and wild ducks (particularly mallard ducks) have asymptomatic infection and may excrete AI virus for long periods, they are carriers and harbour more than one AI virus subtype and rarely produce precipitin antibodies. They are thus the natural reservoirs of AI viruses. Ratites (ostriches and rheas), quails, muscovy ducks, geese, guinea fowls, chukars, partridges and pheasants, pets, sea and shore birds are susceptible to AI to varying degree (Hansen, 2005).

Avian influenza is seasonal in high-risk areas due to migratory activity of water fowl, the only species of bird in which the AI virus was found to be present year round (Hansen, 2005). Avian influenza prevalence is highest in birds on free range due to continuous field exposure to the virus (Abdu *et al*., 2005). Interspecies (e.g. between chickens, turkeys, pigs and humans with H1N1 subtype; chickens, emus, geese, and water fowls with H5H1

subtype; and ducks, pigs and humans) infection with AI virus do occur (Alexander, 1995; Swayne *et al.,* 1998; Shane, 2002).

**2.8 Incubation Period**

The incubation period of AI ranges from few hours, 3 days to 1–2 weeks, depending on the virus dose, route of infection and species of bird infected (Easterday *et al.*, 1997; Abdu *et al*., 2005).

**2.9 Clinical Signs**

At present, AI is recognized in two forms, highly pathogenic (HPAI) and low pathogenic (LPAI) AI. The signs of AI in domestic birds vary, depending on the age, health status, species and sex of bird, the dose and strain of the virus and route of virus entry (Hanson, 2005). The signs may appear as respiratory, enteric or reproductive abnormalities (Easterday *et al*., 1997; Hansen, 2005).

The LPAI viruses cause drop in egg production and mild respiratory disease with low mortality. However, if there is secondary bacterial or viral infection there may be severe respiratory disease with high mortality (Paul and Schrier, 2001; Swayne, 2003). The most notable sign of illness in chickens is silence with lack of normal sounds (Halvorson *et al*., 1980). HPAI viruses such as H5N2 and H7N7 subtypes cause a disease that spreads rapidly among flocks and sudden onset of high morbidity (100%) and mortality (50-100%) with cessation to moderate or severe drop in egg production and the laying of eggs with abnormal shell and pigmentation (Easterday *et al*., 1997). Other signs include greenish diarrhoea, anorexia, depression and ruffled feathers, coughing, sneezing, rales, dyspnoea (due to edema of glottis), sinusitis (particularly in quails, ducks and turkeys) and mucoid

ocular and nasal (blood-tinged) discharges. Cyanosis and oedema of the eyes, head, comb and wattles; discolouration of the shanks and feet; nervous disorders, excitation, convulsion, circling and ataxia in birds that survive the acute phase of the disease have been reported (Whiteman and Bickford, 1989; Easterday *et al*., 1997; Aiello, 1998; Swayne *et al.*, 1998; Alexander, 1999; Defra, 2003).

**2.10 Gross Lesions**

The organs involved and the lesions at postmortem vary with location and severity depending on the specie of bird and the pathogenicity of the infecting AI virus (Abdu *et al*., 2005). Low pathogenic avian influenza (LPAI) cause catarrhal, fibrinous, serofibrinous, mucopurulent or caseous inflammation of the sinuses. Air sacs are thickened with fibrinous or caseous exudates. In the peritoneum there is catarrhal or fibrinous peritoritis and egg yolk peritonitis. In the intestines and caeca catarrhal and fibrinous enteritis are seen. Abnormalities in the oviduct include presence of exudates and oviduct involution. There is regression of ovarian follicles. In the heart, occasional fibrinous pericarditis may be seen (Abdu *et al*., 2005).

With some HPAI viruses, death is sudden and there are no lesions seen. Some viruses cause oedema of the head with swollen sinuses, cyanotic, congested and haemorrhagic wattles and combs, congestion and haemorrhages on the legs; severe congestion and oedema of the lungs, and enlargement and congestion of the kidneys with urates, and presence of necrotic foci on the wattles, skin, liver, spleen, kidneys and lungs. Others cause swelling of the comb and wattles with periorbital oedema. Vesicles to severe swellings and cyanosis, ecchymosis and frank necrosis of the comb are seen (Easterday *et al*., 1997). Sometimes there are swellings of the shanks and feet with ecchymotic discolouration. Petechial

haemorrhages of serosal and mucosal surfaces, especially of the heart and the junction between the ventriculus and the proventriculus, small intestine, mouth, muscles and abdominal fat are seen. The pancreas may have light yellow and dark red areas along its length and the spleen with mottled appearance (Bankowski and Samadieh, 1980; Aiello, 1989; Whiteman and Brickford, 1989; Easterday *et al*., 1997; Swayne *et al*., 1998; Alexander, 1999; Defra, 2003; Abdu *et al*., 2005).

**2.11 Microscopic Lesions**

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. In the trachea, there is necrosis of the epithelium, heterophil infiltraton, hyperplasia and mononuclear cell infiltration. In the lungs the bronchi, atria and air capillaries are filled with serofibrinous exudates mixed with macrophages, heterophils and epithelial cells and with some lobules completely necrotic, there is prominent cuboidalization of atrial epithelium, presence of fibrin thrombi in small arterioles and diffuse severe congestion of pulmonary vasculature. In the spleen, there is severe congestion, fibrin deposition and lymphocytes depletion. The blood vessels of the kidneys are severely congested (Easterday *et al*, 1997).

Highly pathogenic avian influenza viruses cause widespread lesions (Alexander, 1999). In the comb the following lesions are seen; oedema of the dermis and vesicles in epidermis, microvesicles in the epithelium, larger vesicles or focal necrosis underneath the epithelium, surrounded or invaded by heterophils, lymphocytes and accompanied by a marked lymphocytic perivascular cuffing in the wattles. In affected heart, there is severe

myocarditis and myocardial necrosis, oedema, hyperemia, haemorrhages and foci of lymphoid cuffing. Focal areas of necrotic muscles and necrotizing myositis in ocular and skeletal muscles characterized by focal necrosis of fibres and infiltration of mononuclear cells are observed (Easterday *et al*., 1997; Abdu *et al*., 2005)).

In the pancreas the lesions vary from multifocal, to wide spread oedema, hyperemia, haemorrhages and necrosis and vacuolation of acinar cells and necrosis of exocrine cells and loss of normal structure. Scattered heterophil infiltration is present and eosinophilic intranuclear inclusion bodies can be seen in the islets of Langerhans. In the brain there is widespread perivascular cuffing with mononuclear cells, focal gliosis, focal degeneration and necrosis of neurons, neuronophagia, oedema and necrosis of nervous tissue and vascular proliferation and some haemorrhages in the brain and vascular endothelial swelling, along with some infiltration of mononuclear cells in the meninges (Easterday *et al*., 1997).

Some AI virus isolates induce severe lymphoid necrosis in the spleen, thymus, bursa, intestine and lungs with fibrinoid degeneration of sheathed arteries in the spleen. Haemorrhages associated with necrosis of lymphoid cells is present in the proventriculus; oedema, hyperemia, haemorrhages and necrotic foci in the intestine; oedema, hyperemia, haemorrhages and foci of perivascular lymphoid cuffing in the spleen, lungs, liver and kidneys and parenchyma degeneration and necrosis in the spleen, liver and kidneys are observed (Riddell, 1987; Ficken *et al.*, 1989; Easterday *et al*., 1997).

**2.12 Differential Diagnosis**

Differential diagnosis for AI include acute fowl cholera, avian adenoviruses causing respiratory disease or drop in egg production (e.g. EDS76) and viscerotropic velogenic

Newcastle disease as well as infectious laryngotracheitis, infectious bronchitis, mycoplasmosis, infectious coryza, chlamydiosis and pulmonary aspergillosis (Whiteman and Brickford, 1989; Easterday *et al.*, 1997; Aiello, 1998; Alexander, 1999; Defra, 2003).

### 2.13 Diagnosis

Tentative diagnosis is based on clinical signs and gross lesions. Definitive diagnosis requires (a) virus isolation and/or identification from cloacal swabs in embryonated chicken eggs, (b) detection of viral (proteins) antigenic type using agar gel immuno-diffusion (AGID) test, solid phase enzyme linked immunosurbent assay (ELISA) or indirect fluorescent antibody tests or viral nucleic acid using reverse transcriptase polymerase chain reaction (PCR) and (c) serology (which is useful in surveillance for AI) using AGID (OIE, 2004). AGID is the preferred serological test and it is used for detecting antigens type A, B or C, with antigens from Office International des Epizootis Reference Laboratory, Ames Iowa USA or antigen in ground chorioallantoic membrane or yolk sac. Indirect ELISA or HIT using control sera and antigens representing H1 to H16 subtypes and allantoic fluid as antigen and virus neutralizaion test could be used to detect the neutralizing character (OIE, 2004b; Hansen, 2005).

#### 2.13.1 Isolation and Identification of causative agent

Avian influenza viruses are recovered commonly from the trachea and/or cloaca of either live or dead birds, because the viruses typically replicate in the respiratory and/or intestinal tracts. Tissues, secretions or excretions from these tracts are appropriate for virus isolation. In the case of systemic infections produced by HPAI virus, virtually every organ can yield virus because of the high levels of viraemia (Easterday *et al.,* 1997). Embryonated chicken eggs are most commonly used for virus isolation because AI viruses grow very well in them (Easterday *et al.*, 1997).

### 2.13.2 Serology

Serological tests are used to demonstrate the presence of antibodies which may be detected as early as 7–10 days after infection. Several techniques are used for serological surveillance and diagnosis. The most commonly used are the haemagglutination inhibition (HI) test to detect antibodies to the HA, and double immunodiffusion to detect antibodies to the NP (OIE, 2004b). Other serologic tests to detect antibodies include virus neutralization, complement fixation (generally unsatisfactory for avian serum) and single radial haemolysis. (Easterday *et al.*, 1997). Haemagglutination inhibition tests have been employed in routine diagnostic serology, but it is possible that this technique may miss some particular infections because the haemagglutin is subtype specific (OIE, 2004). The detection of antibodies to the nucleoprotein, HA or NA of influenza virus in poultry is considered indicative of a recent disease (OIE, 2004).

## 2.14 Treatment

Amantadine hydrochloride reduces mortality due to AI in some avian species. However, its use is not economical in commercial birds because of cost and shortage in supply (Aiello, 1998). Resistance to amantadine and/or rimantadine frequently develops (Aiello, 1998; Mungall *et al*., 2003; CDCm, 2005; WHO, 2005). ). For example, the influenza A (H5N1) viruses identified in human patients in Asia in 2004 and 2005 have been resistant to amantadine and rimantadine (CDC, 2005). Zanamivir (Relenze), oceltamivir and determivir has been approved in several countries for the treatment and prophylaxis of influenza infections (Mungall *et al.*, 2003; MDTH, 200). Treatment with broad-spectrum antibiotics, proper nutrition and increasing house temperatures may also control secondary bacterial

infection, relieve respiratory distress and reduce mortality (Whiteman and Brickford, 1989; Aiello, 1998).

## 2.15 Prevention, Control and Eradication

### 2.15.1 Prevention

Prevention, control and eradication are the methods used in controlling AI. Prevention is the preferred method of dealing with AI and is the first line of defense. This is best practiced by biosecurity to prevent the exposure of poultry to the AI virus introduced through contaminated equipment, clothing, shoes and infected bird. Prevention includes avoidance of flock additions and exclusion of wild, water and migratory birds from poultry houses. Locating poultry farms away from migratory bird routes and wet lands. Encouraging good husbandry practices (confinement, reduce movement of people and equipment in and out of the farm and institute flock biosecurity). It also includes educating farmers about the disease and ban on importation of poultry and poultry products from affected areas and countries. Prevention also includes mandatory reporting of outbreaks of acute respiratory diseases to local, state and federal veterinary authorities. Countries that have control policies for AI usually consist of setting up embargoes on trade with countries experiencing it (Alexander, 1995; Easterday *et al*, 1997; Alexander, 1999; Abdu, 2006).

In countries with developing poultry industries, good management should begin at the planning stage, ensuring that farms are situated away from migratory waterfowl routes and not developed in clusters frequently seen in countries with established industries (Abdu, 2006). Where possible birds should be reared in confinement in wild bird-proofed houses (Abdu, 2006). Movement of people between farms and flocks should be discouraged and when it must take place, actual contact with birds should be kept to a minimum and

disinfectant baths used for vehicles and other equipment (Easterday *et al*, 1997). Locating poultry farms away from migratory bird routes, encouraging good husbandry practices, prevention of stress, confinement, reducing movement of people and equipment in and out of the farm and institution of flock bio-security measures through keeping birds of different species and age separately, etc (Abdu *et al*., 2005).

### 2.15.2 Control

Control of LPAI includes the use of inactivated oil emulsion polyvalent vaccines, containing the subtypes isolated and identified in affected areas. Vaccines have been found to be useful in the USA. The major protective antigen on the AI virus particle is the H and to a lesser extent N i.e. it will only provide practical protection. Vaccines do not completely prevent infection, but will protect birds from clinical signs and mortality and decrease the environmental AI virus load by reducing virus replication and shedding in vaccinated birds (Alexander, 1995; Aiello, 1998; Defra, 2003; Swayne, 2003).

Reduction in AI virus shedding significantly reduces the chance that an infected farm will infect another farm and people (Anonymous, 2005a). A higher dose of the virus is necessary to infect vaccinated birds (Swayne *et al*., 2001; Anonymous, 2005b). Vaccination at the face of an outbreak also stops virus shedding and transmission (Anonymous, 2005b). However, live conventional influenza vaccines against any subtype are generally not recommended (OIE, 2004b).

### 2.15.3 Eradication

Eradication is used for HPAI and this includes active and passive serological testing of serum and yolk for antibodies to AI virus, quarantine of flocks found to be affected through clinical signs, serology and virology, the destruction, burying or burning of infected poultry

and faeces on the site (stamping out policy) and restocking of farm three weeks after cleaning and disinfecting the houses. The AI virus is sensitive to heat, desiccation, extreme pH, formalin (2%) and sodium hypochlorite (Hanson, 2000). Dead or destroyed birds should not be fed to other animals nor their carcasses be sold. Farmers should be compensated based on prevailing market prices. It also includes banning the importation of poultry and poultry products from affected areas and mandatory reporting of outbreaks of acute respiratory diseases to local, state and federal veterinary authorities (Defra, 2003; Swayne, 2003; Anonymous, 2005a).

**2.16 Public Health Importance of AI**

Under normal conditions, human strains of AI belong to the H1, H2 and H3 subtypes and poultry strains have been of the H5 and H7 subtypes (Hanson, 2005). Until 1997, AI viruses have not been known to be transmitted directly from birds to humans. That year, out of the 18 people that become sick with severe respiratory disease after contact with poultry that were sick or died of HPAI caused by H5N1 subtype of Al, six died in Hong Kong, China (Larson, 1998; WHO, 2005). All the 1.5 million chickens in Hong Kong were killed in order to get rid of the source of infection (Mubiru, 1998). Human deaths were again reported from AI virus subtype H5N1 in Hong Kong in 2003 and March 2004. An epidemic of AI in South East Asia, involving South Korea, Cambodia, China, Indonesia, Japan, Laos, Thailand and Vietnam resulted in the infection of 34 and death of 23 people, and millions of birds. This resulted in the emergency destruction of about 100 million birds (WHO, 2005). Some health workers that took care of the sick also became severely ill (WHO, 2005).

Between March and May, 2003 an outbreak of HPAI caused by H7N7 virus in the Netherlands resulted in the death of a veterinarian, after exhibiting respiratory symptoms,

and mild illness of conjunctivitis (pink eye) in 89 other humans working directly with poultry (Anonymous, 2005c; MDTH, 2005; WHO, 2005). In July, 2004, a second wave of disease began in poultry in Thailand and Vietnam, and continues extension to Cambodia. There have also been human cases in all three countries, with one case of likely human to human transmission (Morris and Jackson, 2005; Ungchusak, 2005). Most or possibly all of other human cases can be reasonably attributed to poultry exposure, although in some cases human to human transmission cannot be excluded. Avian influenza has also caused the death of wild cats that were fed with dead AI infected birds (Abdu *et al*., 2005).

Recently, a family of three died of AI in Indonesia without evidence of having contact with infected poultry (Morris and Jackson, 2005). On the 1st of August 2005 an outbreak of AI was reported in ducks and geese in Siberia, Russia. In this case, Serotype H5N1 was isolated. This shows clearly that HPAI in humans and poultry is gradually spreading and may soon become a global problem. Over the last decade the virus has shown a high rate of evolution, and unusually wide and expanding host range. Its future behaviour is of continuing concern both as an animal pathogen and as a potential source of human pandemic virus (Ungchusak *et al*., 2005). It has to be considered as such also because the human influenza pandemic of 1918 killed about 21 million people worldwide when the means of world transportation was not as easy, cheap and fast as it is today and there is the possibility that the Al virus could one day be easily transmitted from human to human (Larson, 1998; WHO, 2005).

Today, travelling is faster and therefore the AI virus can be disseminated easily and faster around the world. An indication of the possibility of human to human transmission was shown in the Netherlands, where 60% of the people that had no contact with H7N3 AI virus

infected poultry, but were in close contact to an infected poultry worker had antibodies to the virus (Anonymous, 2005c). Between 2003 and 2007, a total of 152 persons have died out of the 258 persons that were infected with confirmed HPAI H5N1 (WHO, 2007) (Table 2.1).

**2.17 Economic Importance of AI**

The reduction in growth rate, egg production and high mortality and high cost of treatment, control and eradication due to AI results in loss of income and livelihoods. Production loss due to AI subsequently results in high cost of poultry and poultry by-products, because of the resultant scarcity of the products. Countries and regions affected by AI stand the risk of losing the right to export poultry and poultry by-products to free countries. Thus, AI has been described as an international problem that requires international efforts and cooperation to solve (Swayne, 2003).

A total of 915,650 birds (mortality 321,812 and depopulated 593,838) have been lost since the beginning of the outbreaks of AI in Nigeria. The sum of N107, 639,866 has so far been paid as compensation to 728 farmers (Ahmed, 2006; NADIS INFO, 2006d).

**Table 2.1:** Cumulative number of confirmed human cases of Avian Influenza (H5N1) reported to the World Health Organization.

| Year | 2003 | 2004 | 2005 | 2006 | 2007 | Total |
|---|---|---|---|---|---|---|
| Country | Cases (Deaths) | Cases (Deaths) | Cases (Deaths) | Cases (Deaths) | Cases (Deaths) | |
| Azerbaijan | 0 (0) | 0 (0) | 0 (0) | 8 (5) | 0 (0) | 8 (5) |
| Cambodia | 0 (0) | 0 (0) | 4 (4) | 2 (2) | 0 (0) | 6 (6) |
| China | 1 (1) | 0 (0) | 8 (5) | 12 (8) | 0 (0) | 21 (14) |
| Djibouti | 0 (0) | 0 (0) | 0 (0) | 1 (0) | 0 (0) | 1 (0) |
| Egypt | 0 (0) | 0 (0) | 0 (0) | 14 (6) | 3 (3) | 17 (9) |
| Indonesia | 0 (0) | 0 (0) | 17 (11) | 43 (35) | 6 (6) | 66 (56) |
| Iraq | 0 (0) | 0 (0) | 0 (0) | 2 (2) | 0 (0) | 2 (2) |
| Nigeria | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (1) | 1 (1) |
| Thailand | 0 (0) | 17 (12) | 5 (2) | 2 (2) | 0 (0) | 24 (16) |
| Turkey | 0 (0) | 0 (0) | 0 (0) | 12 (4) | 0 (0) | 12 (4) |
| Viet Nam | 3 (3) | 29 (20) | 61 (19) | 0 (0) | 0 (0) | 93 (42) |
| Total | 4 (4) | 46 (32) | 95 (41) | 96 (64) | 10 (10) | 251 (151) |

Source: www.who.int/csr/avian influenza/country/en/.28/02/2007.

### 2.18.1 Situation of AI in Nigeria

Nigeria has an estimated poultry population of over 140 million, comprising 25% commercial, 15% small holder and 60% free range rural poultry (Ahmed, 2006). Poultry is one of the major sources of animal protein in Nigeria, and the industry has the potential to employ the highest labour apart from crop farming. It is currently the means of livelihood for over one million families. An estimated one billion table eggs and over 500,000 metric tonnes of poultry meat are produced for human consumption annually (Ahmed, 2006; NADIS INFO, 2006c).

Prior to January, 2006, there was no report of HPAI in Nigeria and there was no evidence to suggest the presence of the disease in the country (NADIS INFO, 2006c). Early results of strategic virologic sampling during which 1, 200 samples collected at wetlands, surrounding rural poultry and selected large scale farms in Jigawa and Yobe States showed that all samples tested negative. The survey was carried out by the National Veterinary Research Institute, Vom, Nigeria (Ahmed, 2006).

Nigeria has many wet lands (about 24) and lies along two important wild birds migratory routes – the East Atlantic flyway and the East Africa West Asia flyway. The country's veterinary services are inadequate. Risk analysis carried out in November, 2005 showed that the risk of introduction of AI infection into the country was moderate, while the risk of spreading the disease was high (Ahmed, 2006).

The first suspected case of AI was reported in Sambawa Farms, a semi commercial farm situated in Kaduna State, North central Nigeria on January 16th, 2006. The disease was clinically diagnosed at the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria,

Nigeria (Adene *et al*., 2006; NADIS INFO, 2006c). Since the first confirmation of AI in Nigeria, 498 suspected cases were reported out of which 122 cases have been confirmed over a period of eight months in 42 local Government Areas in 15 states and the Federal Capital Territory (Ahmed, 2006). The current status of AI in Nigeria derives from passive and active surveillance from National Animal Disease Surveillance (NADIS) network of Pan-African Control of Epizootics (PACE). The possibility of under-reporting particularly in small scale backyard poultry has been reported (Obi, 2006).

### 2.18.2 Strategies for prevention of introduction of AI into Nigeria

Strategies for the prevention of introduction of bird flu into Nigeria were recommended by FDL&PCS in 2005. The total ban on importation of poultry and poultry products from countries known to be infected by AI into Nigeria. An effective animal disease surveillance involving high risk areas such as poultry markets, wet lands and poultry located along known migratory birds routes, poultry abattoirs, borders and targeted farms should be carried out. Results obtained may then be used to assess the need for a complete nationwide AI active disease, virus and sero-surveillance to determine epidemiological status of the disease in the Nigeria. National Veterinary Quarantine Services (NVQS) including immediate rehabilitation and revitalization of existing veterinary quarantine infrastructure, control posts and enhanced manpower capabilities should be improved. This is to enable a sustained surveillance of animal disease at the ports of entry in the course of animal/animal products, biologics and germplasm trade. The NVQS service staff should maintain continuous presence at the ports of entry into the country and constitute part of the Joint Intelligence Board (JIB), which should play an active role in AI surveillance at the ports. Targeted community based training of rural backyard poultry farmers in various aspects of AI recognition and control including biosecurity procedures applicable to rural small scale

poultry enterprises and the role of animal disease vigilante in the control of AI should be organized.

Tractability mechanism for animals and strict monitoring of movement of poultry and poultry products through registration and licensing of poultry farms, hatcheries and other poultry enterprises should be developed. Participatory rural livestock and poultry disease surveillance system and integrating it into the existing epidemio-surveillance network should be organized. Veterinarians, auxiliaries and other categories of poultry farmers should be trained on AI prevention and control strategies including aspects of biosecurity. Effective public enlightenment and awareness programmes on AI should be organized (FDL&PCS, 2005).

### 2.18.3 Overall Policy for AI emergency in Nigeria

The present epidemiological features of AI disease coupled with the fact that the risk of introduction can be considered as moderate to high and the risk of sustenance and maintenance may be ranked as very high. Therefore, the overall policy for bird flu emergency Nigeria should be to restrict the disease to the primary foci, eradicate the disease in the shortest possible period and limit the economic and public health impact using a combination of measures (FDL&PCS, 2005). Where the disease is discovered in limited populations and has not spread beyond the immediate vicinity, the recommended actions should be modified stamping-out which involves quarantine and destruction of infected poultry with full compensation, sanitary disposal of destroyed poultry and contaminated poultry products according to standard operating procedures, quarantine of farms and movement control on poultry and poultry products in the infected areas or zone. Other measures should include; decontamination (cleaning and disinfection) of facilities, products

and equipment to eliminate the virus on infected premises and prevent spread to other areas, active and passive AI surveillance to determine the source and extent of the infection, and effective public awareness campaign to elicit cooperation from large scale commercial and backyard poultry owners.

Where modified stamping-out failed and AI becomes established in the country, a different policy would be adopted (FDL&PCS, 2005). These should include ring or mass vaccination of poultry. However, vaccination is subtype specific and vaccinated birds will be protected form dying and shedding a lot of virus in the environment but may be infected without showing clinical signs (carriers). It should only be used during an outbreak with the subtype isolated in the area. The marketing of vaccinated birds should be controlled. Movement of poultry and poultry products should be restricted, clinically infected birds should be eliminated with payment of full compensation to prevent transmission between farms. There should be sanitary disposal of dead and destroyed poultry and contaminated poultry products. Affected premises should be cleaned and disinfected according to standard operating procedures. Active and passive AI surveillance (by serological testing of serum and yolk for antibodies to AI virus) to determine the source and extent of the infection should be conducted.

Other steps include enforcement of legal provisions with regards to registration of poultry premises, farms and hatcheries in Nigeria. Hatchable eggs and day-old parent and grand parent chicks should be imported from certified disease-free countries only and with duly issued import permit. There should be mandatory reporting of outbreaks of acute respiratory diseases to local, state and Federal Veterinary authorities.

**2.19 Total Proteins**

The plasma of birds, like other vertebrates contain a variety of proteins, which could be arbitrarily grouped into pre-albumins, albumins, post-albumins and globulins (Amand, 1986). Several authours have reported higher values of TP in birds as a result of different diseases (Coles and Boyd, 1965; Hawkey *et al*., 1983; Robertson and Maxwell, 1996). In studies conducted by Oladele (2000), higher TP values were observed in clinically sick birds suffering from unspecified diseases than in apparently healthy birds. Viral and rickettsial diseases do not significantly increase the absolute (relative) amount of proteins (Bush, 1991). Factors that can cause decrease in TP levels include relative decrease (over hydration), age (young animals), decreased protein synthesis (caused by protein starvation, small intestinal malabsorption, liver disorders, congestive heart failure, etc). Factors that can cause increased TP levels include dehydration, increased globulin level caused by acute inflammation, chronic inflammation, liver disease, neoplasia, viral and rickettsial diseases (unusual) , etc (Bush, 1991). Alterations of TP within an animal's body may be indicative of a disease (Coles, 1974).

It was observed that daily mean TP concentration of Newcastle disease infected chickens dropped below the normal established value of 3.0-6.0 g/dl for healthy birds from days 4 to 21 post- infection ((Campbell and Coles, 1986; Oladele *et al*., 2005). The reduction in daily mean TP during this period was attributed to decrease in albumin content, resulting from tissue pathology, such as necrosis of the liver and renal tubular and glomerular necrosis of the kidneys observed in the infected chickens (Oladele *et al*,. 2005). Total protein concentrations may decrease in birds having so much liver damage that the albumin is not synthesized in the right quantity (Kaneko, 1989). This is because the liver is primarily responsible for albumin synthesis which constitutes 40-60% of TP concentrations in serum

of birds (Campbell and Coles, 1986). Therefore, any disease condition that interferes with normal functioning of the liver may be reflected as a decrease in concentration of albumin, and ultimately TP concentration (Duncan, 1998; Oladele *et al*., 2005).

**2.20 Albumin**

Albumins and all other proteins except the immunoglobulins are synthesized in the liver (Bush, 1991). It is the main storage form of protein and source of amino acids to the tissues and is chiefly responsible for the colloid osmotic pressure of the blood. It binds and transports many substances including unconjugated bilirubin, free fatty acids and thyroxin (in part), and many drugs. Calcium is also transported bound to albumin, and hypoalbuminaemia results in hypocalcaemia (Coles, 1974; Bush, 1991).

Factors that cause decrease in albumin levels include relative decrease in protein synthesis (due to protein starvation, small intestine malabsorption, liver disorders, congestive heart failure, severe trauma, etc), increased protein loss and poor diet. Absolute increases in albumin are generally not to arise, although there have been reports of increases following anabolic steroid therapy, dehydration and drug interference (Bush, 1991).

**2.21 Globulins**

Globulins produce antibodies which essentially act against disease agents in the body of birds (Lloyd, 1989). The globulin fraction comprises alpha globulin (which increases in concentration in acute inflammatory disorders), beta globulin (which increases in concentration when there is severe antigenic stimulation and neoplasia), and gamma globulin (which increases in concentration with antigenic stimulation, especially with chronic infections and autoimmune disorders) (Bush, 1991).

Gamma globulins have been primarily associated with antibodies. In general, an increase in concentration of gamma globulins accompanies a rise in antibody titre. However, there is some experimental evidence that this does not always occur (Coles, 1974). Decreases in concentration of individual globulin fractions may be encountered, although there is seldom a decrease in total globulin concentration (Coles, 1974).

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Areas of study

The areas of study include areas where AI outbreaks have been confirmed in Jigawa State (Auyo, Gagarawa, Gumel, and Maigatari towns), Kaduna State (Kaduna north metropolis, Hanwa-Zaria, and Shika-Giwa towns), and Katsina State (Katsina metropolis and Dutsinma town) (Figure 3.1). The outbreaks were confirmed by the National Veterinary Research Institute (NVRI), Vom, Nigeria.

## 3.2 Sample size

A total of 1,000 blood samples, comprising 250 samples from clinically sick local and 250 from clinically sick exotic confirmed to be suffering from HPAI, 250 from apparently healthy local, and 250 from apparently healthy exotic adult chickens were collected for this study (Table 3.1). The sample size was defined using the formular:

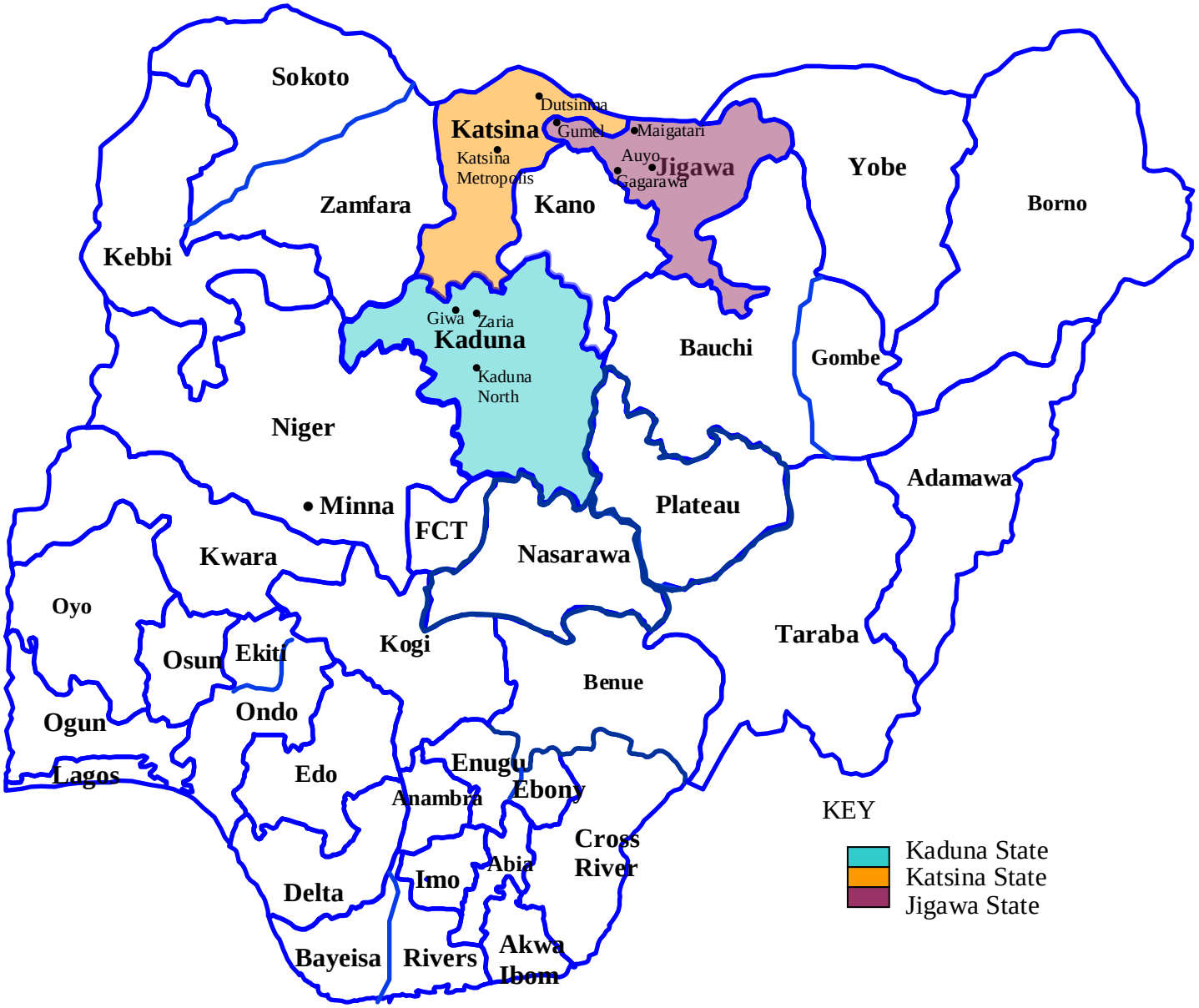
$$n = \frac{Z^2P\ (1- d)}{d}$$

Where n = sample size, Z = 95% confidence interval, P = prevalence, d = 5% desired precision. The blood samples from the apparently healthy local and exotic chickens were collected 3 months after the disease outbreaks from the same areas (Table 3.2).

## 3.3 Sample collection

Two millilitres (ml) of blood samples were collected from adult local and exotic chickens through venipuncture (brachial vein) using 21 gauge needles and syringes by simple random sampling (Niederman, 1973). The samples were collected in the month of March, 2006. All the birds sampled had no history of vaccination against AI. The blood was allowed to clot at room temperature. Sera were separated by centrifugation at

**Figure 3.1:** Areas of the study




Source: Microsoft Encarta 2006

**Table 3.1:** Sample size and groupings of clinically and apparently healthy chickens.

| | Group | Abbreviation | No. of samples |
|---|---|---|---|
| 1 | Clinically sick local chickens | CSL | 250 |
| 2 | Clinically sick exotic chickens | CSE | 250 |
| 3 | Apparently healthy local chickens | AHL | 250 |
| 4 | Apparently healthy exotic chickens | AHE | 250 |
| | Total | | 1, 000 |

about 447.2 g for 5 minutes (Collee *et al*., 1982). The sera were stored in the refrigerator at -4$^0$C until used.

### 3.4 AI H5 antigen and AI H5 positive serum

AI H5 antigen and AI H5 positive serum were obtained from China via the NVRI, Vom, Nigeria. The reagents were used for the determination of antibodies to AI H5 in the sera collected.

### 3.5 Preparation of red blood cells (RBCs)

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

### 3.6 Determination of antibodies to AI

The titre for the H5 AI antigen was determined by haemagglutination test (HAT), while the antibody titres in the sera collected were determined by haemagglutination inhibition test (HI) using the methods described by OIE (2004b). Positive and negative control antigen and antiserum were ran with each test.

#### 3.6.1 Determination of titre of AI type A H5 antigen

Haemagglutination test (HA) was carried out according to the method described by OIE (2004b). Twenty five microlitres of PBS was dispensed into each well of micro titre plates. Twenty five microlitres of antigen suspension was placed into the first well.

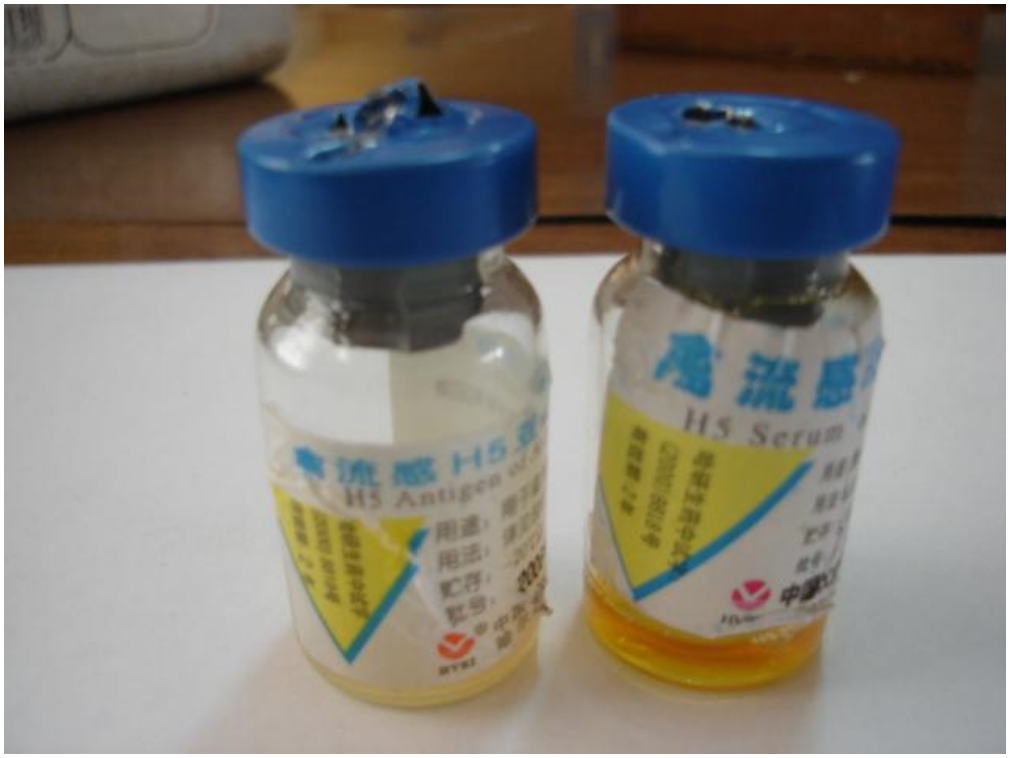



**Plate I:** The Avian influenza type A H5 antigen (left) and AI type A H5 positive serum (right) used for the haemagglutination inhibition (HI) test.

Twofold dilutions of 25 µl of the antigen suspension were made across the plate. A further 25 µl of PBS was dispensed to each well. Twenty five microlitres of 1% chicken RBCs was dispensed to each well. This was mixed by tapping the plate gently and then the RBCs were allowed to settle for about 40 minutes at room temperature. HA was determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration was read to the highest dilution giving complete HA (no streaming). This represents 1 HA unit (HAU) and was calculated accurately from the initial range of dilutions. The titre of the antigen was determined as 7.0 $\log_2$ and this was used in the haemagglutination inhibition test.

### 3.6.2 Determination of antibodies to AI type A H5 antigens

Haemagglutination inhibition test (HI) was carried out according to the method described by OIE (2004b). Twenty five microlites of PBS was dispensed into each well of a micro titre plate. Twenty five microlitres of serum was placed into the first well of the plate. Twofold dilutions of 25 µl of the serum were made across the plate. Four HAU of the virus/antigen in 25 µl was added to each well and left for a minimum of 30 minutes at room temperature. Twenty five microlitres of 1% chicken RBCs was added to each well, mixed gently, and allowed to settle for about 40 minutes at room temperature. The HI titre was the highest dilution of serum causing complete inhibition of 4 HAU of antigen. The agglutination was assessed by tilting the plates. Only those wells in which the RBCs streamed at the same rate as the control wells (containing 25 µl RBCs and 50 µl PBS only) were considered to show inhibition. The validity of the test was assessed against a negative control serum, which gave a titre >4 $\log_2$ when and a positive control serum for which the titre was >12 $\log_2$.

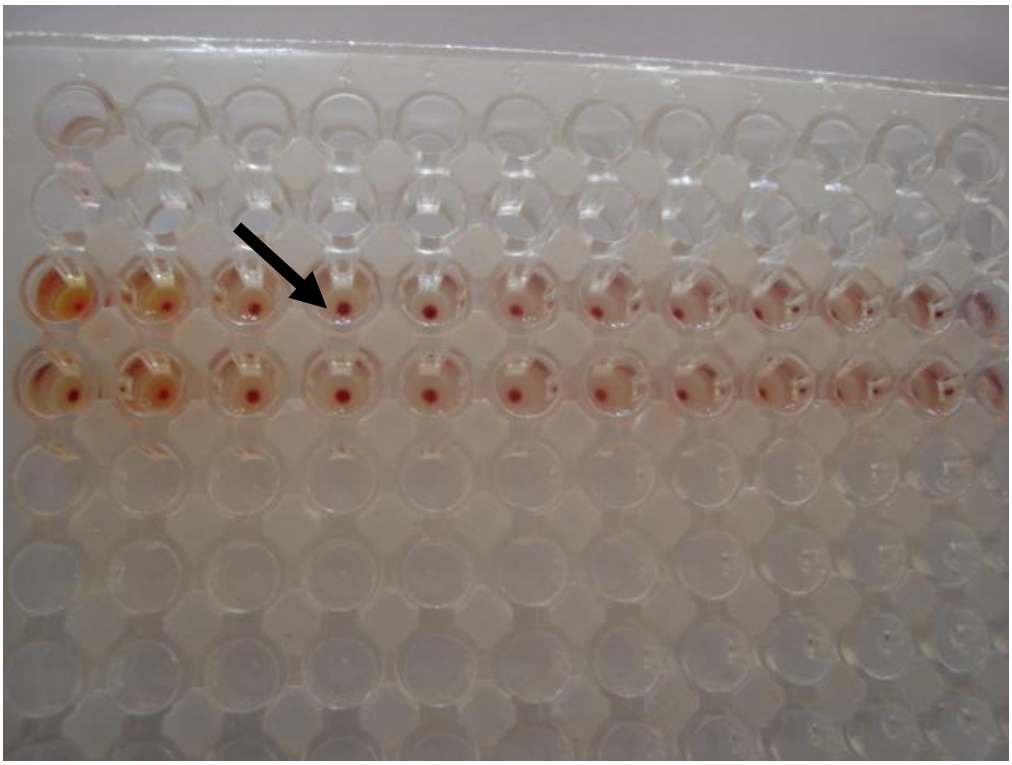


**Plate II:** Positive reaction by Avian influenza type A H5 antiserum (arrow)

### 3.6.3 Screening for Newcastle disease antibodies

All the serum samples were screened for the presence of ND antibodies using the method of haemagglutination inhibition (HI) test described by OIE (2004b). Newcastle disease vaccine La Sota strain obtained from NVRI, Vom, Nigeria was used as antigen for the test. The titre of the antigen was also determined using haemagglutination test (HA).

### 3.6.4 Results interpretation for AI and ND antibody titres:

HI titres were regarded as being positive, if there was inhibition at a serum dilution of 4 $\log_2$ or more against 4 HAU of both antigens.

### 3.7 Determination of serum total proteins

Biuret technique was used according to the method described by Coles (1974). Protein reacts with the alkaline copper sulphate to give a violet colour which is proportional to the concentration of protein present in the sample. Serum sample (0.1 ml) was mixed with 5 ml of Biuret reagent. Distilled water (0.1 ml) was mixed with 5 ml Biuret reagent (as blank). About 0.1ml of BSA (0.6%) was mixed with 5 ml of Biuret reagent (as standard). All the mixtures above were incubated at 37$^{o}$C 30 minutes before the absorbances (optical densities or OD) were read at 540 nm. The spectrophotometer (Ultraspec model 4054 made in England) was set to zero with the blank solution. Then the results were calculated by using the formula: $T/5$ x concentration of standard, where T is the absorbance of the mixture.

### 3.8 Determination of serum albumins

Modified Batholomew and Delaney technique was used according to the method described by Bush (1991). Albumin reacts with Bromocresol green (BCG) at pH 4.2 ± 0.05 to give a coloured complex which absorbs light at 630 nm. The intensity of the colour is proportional to the amount of albumin present in the sample. Serum sample (0.02 ml) was mixed with 4

ml of BCG. Distilled water (0.02) was mixed with 4 ml of BCG (as blank). 0.02 ml of BSA (0.3%) solution was mixed with 4 ml of BCG (as standard). After 10 minutes, the mixtures were incubated at $37^{O}C$ for 30 minutes. The spectrophotometer (Ultraspec model 4054 made in England) was set to zero using the blank solution. The absorbance was read at 630 nm. Results were calculated by using the formula: $T/5$ x concentration of standard, where T is the absorbance of THE mixture.

### 3.9 Determination of serum globulins

Serum globulins were determined by subtracting the values obtained for albumins from the values obtained for total proteins (Coles, 1974; Bush, 1991).

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### 3.10 Data analysis

Data generated on antibodies, total proteins, albumins and globulins were expressed as mean ± standard error of the mean (x ± S.E. M.) and reduced into tables. The data were also analysed using Student's t-test analysis. Values of ($p<0.05$) were considered significant (Kleinbaum and Kupper, 1978).

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1 Avian influenza type A H5 antibodies

The overall prevalence of AI in the birds sampled in the areas of the study was 13.4%. Of the 250 serum samples from clinically sick local chickens analysed for the presence of antibodies to AI type A H5 subtype, 79 birds (31.6%) were positive. Forty nine (19.6%) of the clinically sick exotic chickens tested positive. Six (2.4%) of the apparently healthy local chickens tested positive. None of the apparently healthy exotic chickens tested positive for the presence of antibodies to AI H5 subtype antigen (Table 4.1). The mean antibody titre of the 79 clinically sick local chickens was $5.6 \pm 0.1$ $\log_2$. The mean antibody titre of the 49 clinically sick exotic chickens was $7.1 \pm 0.1$ $\log_2$. The mean antibody titre of the 6 apparently healthy local chickens was $5.8 \pm 0.1$ $\log_2$ (Table 4.2).

### 4.2 Antibodies to Newcastle disease

The overall prevalence of ND in the birds sampled was 34.6%. Thirteen (5.2%) of the clinically sick local chickens tested positive for ND antibodies with a mean titre of $3.9 \pm 0.3$ $\log_2$ . Twenty three (9.2%) of the clinically sick exotic chickens tested positive for the presence of ND antibodies with a mean titre of $4.4 \pm 0.2$ $\log_2$. Ninety seven (38.8%) of the apparently healthy local chickens tested positive for the presence of ND antibodies with a mean titre of $4.8 \pm 0.1$ $\log_2$. while, 213 (85.2%) of the apparently healthy exotic chickens tested positive for ND antibodies with a mean titre of $5.5 \pm 0.1$ $\log_2$ (Tables 4.3 and 4.4 ).

### 4.3 Serum total proteins

Table 4.5 shows the mean of TP value for clinically sick local, clinically sick exotic, apparently healthy local and apparently healthy exotic adult chickens as $3.6 \pm 0.0$, $3.0 \pm 0.0$,

6.5 ± 0.1 and 7.8 ± 0.1 g/dl respectively. The values of TP in clinically sick chickens were significantly lower than those of apparently healthy chickens (P<0.001). Significantly higher TP values were obtained in clinically sick local chickens than in the clinically sick exotic chickens (P<0.001). The values of TP obtained in apparently healthy local chickens were significantly lower than those of apparently healthy exotic chickens (P<0.001).

### 4.4 Serum albumins

Table 4.5 shows the mean values of albumin (2.1 ± 0.0, 1.9 ± 0.0, 3.3 ± 0.0 and 4.1 ± 0.1 g/dl) for clinically sick local, clinically sick exotic, apparently healthy local and apparently healthy exotic chickens respectively. The values of albumin in clinically sick chicken were significantly lower than those of apparently healthy chickens (P<0.001). Significantly higher values of albumin were obtained in clinically sick local chickens than in clinically sick exotic chickens (P<0.001). The values of albumins obtained in apparently healthy local chickens were significantly lower than those of apparently healthy exotic chickens (P<0.001).

### 4.5 Serum globulins

Table 4.5 shows the mean values of globulins (1.5 ± 0.0, 1.1 ± 0.0, 3.2 ± 0.1 and 3.7 ± 0.1 g/dl) for clinically sick local, clinically sick exotic, apparently healthy local, apparently healthy exotic chickens respectively. The values of globulins obtained in the clinically sick chickens were significantly lower than those of apparently healthy chickens (P<0.001). Significantly higher values of globulin were obtained in clinically sick local chickens than in clinically sick exotic chickens (P<0.001). The values of globulins in apparently healthy local chickens were significantly lower than those of apparently healthy exotic chickens (P<0.001).

**Table 4.1:** Haemagglutination inhibition antibody response of clinically sick and apparently healthy local and exotic chickens to Avian influenza type A H5 antigen.

| Group | No. positive (%) | No. negative (%) | Total no. sampled |
|---|---|---|---|
| CSL[a] | 79 (31.6) | 171 (68.4) | 250 |
| CSE[b] | 49 (19.6) | 201 (80.4) | 250 |
| AHL[c] | 6 (2.4) | 244 (97.6) | 250 |
| AHE[d] | 0 (0.0) | 250 (100.0) | 250 |
| Total | 134 (13.4% ) | 866 (86.6% ) | 1, 000 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Table 4.2:** Avian influenza antibody titres of clinically sick and apparently healthy local and exotic adult chickens ($log_2$).

| | Antibody titre ($Log_2$ ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Mean titre ± SE | Min. | Max. |
| CSL[a] | 0 | 0 | 8 | 18 | 9 | 19 | 9 | 14 | 0 | 0 | 0 | 5.6 ± 0.2 | 3 | 8 |
| CSE[b] | 0 | 0 | 0 | 0 | 4 | 11 | 12 | 22 | 0 | 0 | 0 | 7.1 ± 0.1 | 5 | 8 |
| AHL[c] | 0 | 0 | 0 | 2 | 0 | 2 | 1 | 1 | 0 | 0 | 0 | 5.8 ± 0.7 | 4 | 8 |
| AHE[d] | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0 | 0 | 8 | 20 | 13 | 32 | 22 | 37 | 0 | 0 | 0 | | 3 | 8 |

a, CSL = sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Table 4.3:** Newcastle disease antibody responses of clinically sick and apparently healthy local and exotic adult chickens.

| Group | No. positive (%) | No. negative (%) | No. sampled |
|---|---|---|---|
| CSL[a] | 13 (5.2) | 237 (94.8) | 250 |
| CSE[b] | 23 (9.2) | 227 (90.8) | 250 |
| AHL[c] | 97 (38.8) | 153 (61.2) | 250 |
| AHE[d] | 213 (85.2) | 37 (14.8) | 250 |
| TOTAL | 346 (34.6) | 654 (65.4%) | 1, 000 |

a, CSL = sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Table 4.4:** Newcastle disease antibody titres of clinically sick and apparently healthy local and exotic adult chickens ($log_2$).

| | Antibody titre ($Log_2$ ) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | Mean titre ± SE | Min. | Max. |
| CSL[a] | 0 | 0 | 5 | 6 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3.9 ± 0.3 | 3 | 6 |
| CSE[b] | 0 | 0 | 4 | 10 | 5 | 4 | 0 | 0 | 0 | 0 | 0 | 4.4 ± 0.2 | 3 | 6 |
| AHL[c] | 0 | 0 | 15 | 27 | 23 | 23 | 8 | 1 | 0 | 0 | 0 | 4.8 ± 0.1 | 3 | 8 |
| AHE[d] | 0 | 0 | 0 | 68 | 37 | 57 | 22 | 25 | 4 | 0 | 0 | 5.5 ± 0.1 | 4 | 9 |
| Total | 0 | 0 | 24 | 111 | 66 | 85 | 30 | 26 | 4 | 0 | 0 | 5.2 ± 0.2 | 3 | 9 |

a, CSL = sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d,AHE=apparentlyhealthylocalchickens

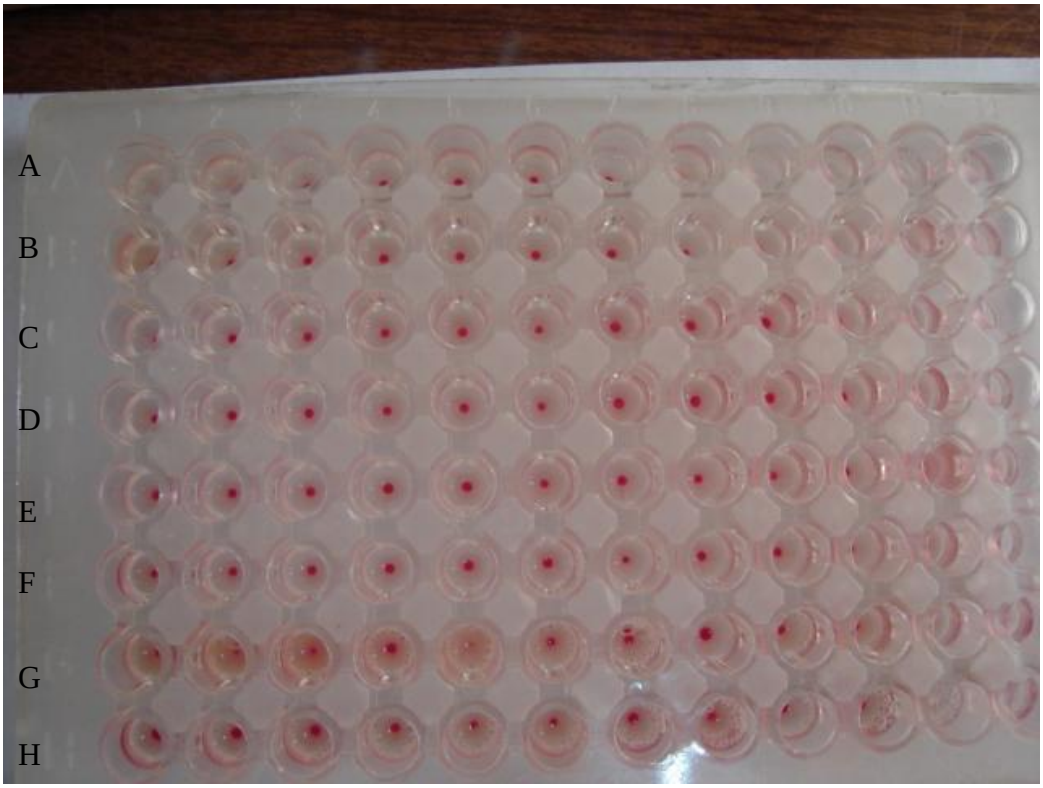


**Plate III:** Positive HI reaction for Avian influenza antibodies (with minimum titre of 6 $log_2$ and maximum titre of 8 $log_2$) in clinically sick local adult chickens.

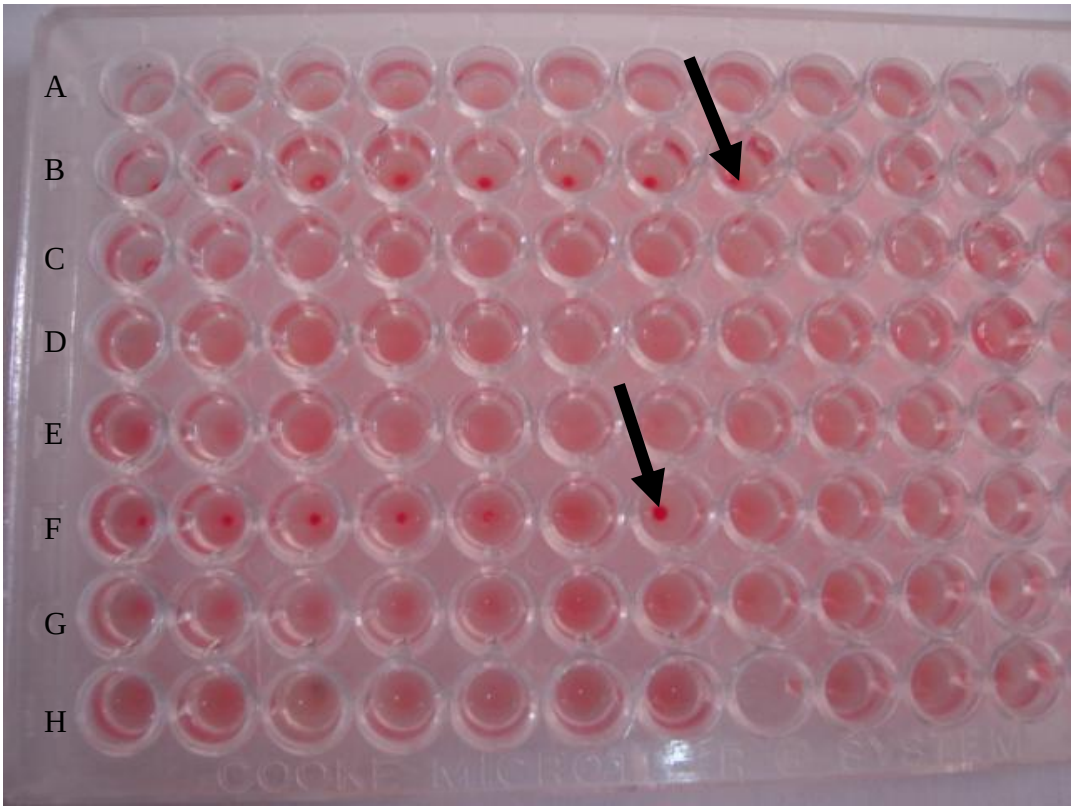


**Plate IV**: Positive HI reaction for Avian influenza antibodies in clinically sick local chicken in wells B and F (with antibody titres of 7 $\log_2$ and 6 $\log_2$ respectively).

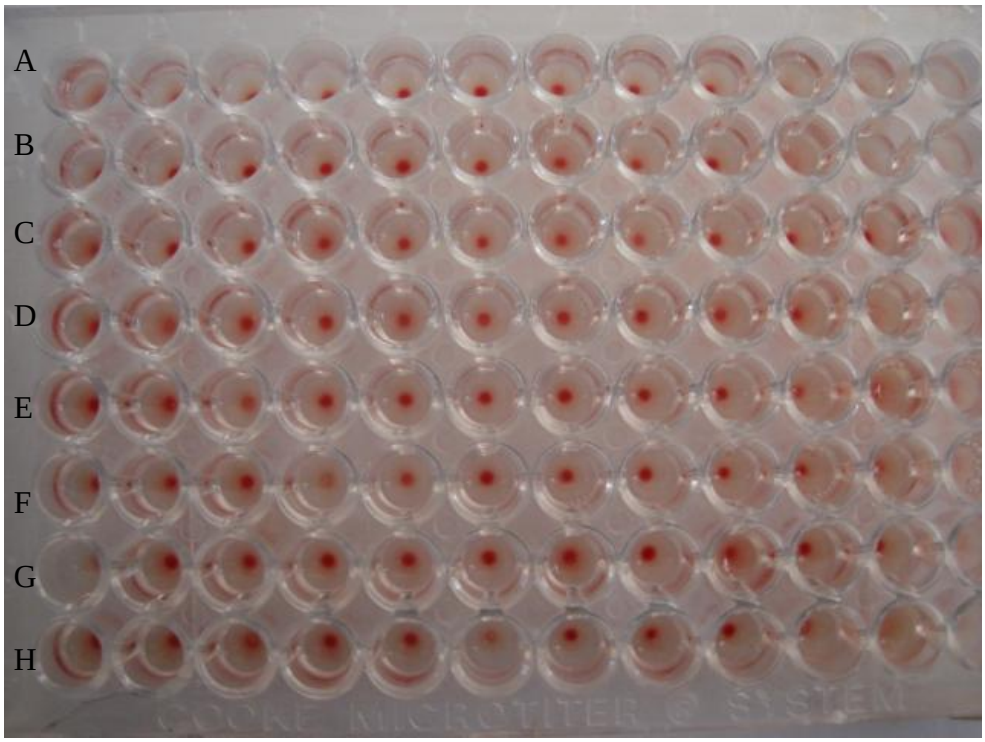


**Plate V:** Positive HI reactions for Avian influenza antibodies in clinically sick exotic chickens.

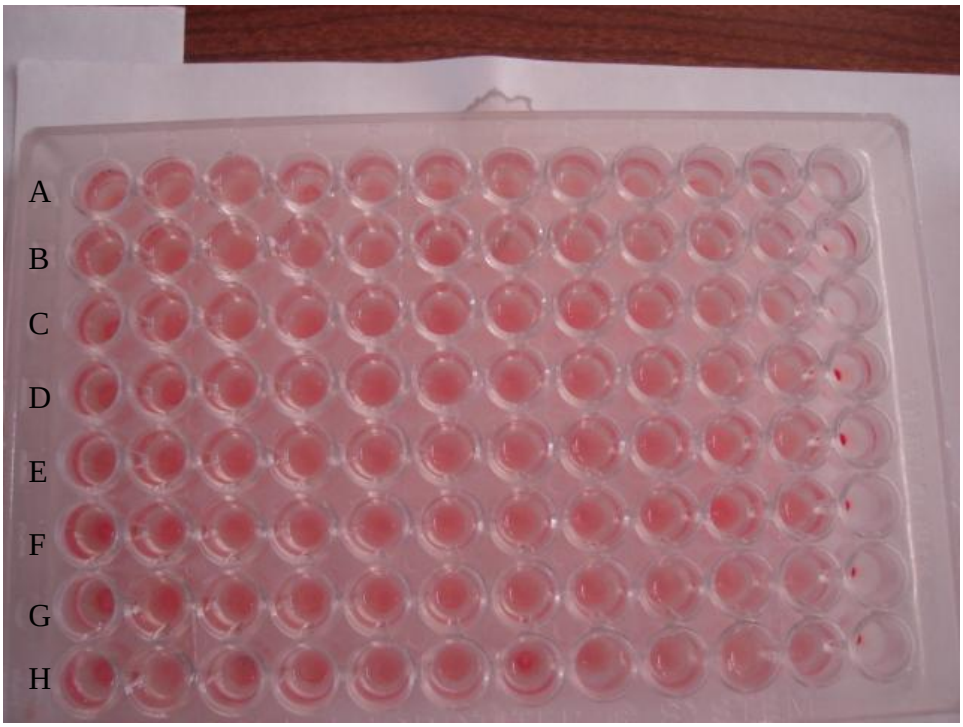


**Plate VI:** Negative Haemagglutination inhibition reaction for Avian influenza antibodies in apparently healthy exotic chickens.

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**Table 4.5:** Mean values of total proteins, albumin and globulin in clinically sick and apparently healthy local and exotic chickens (mean ± SE).

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| Group | Total proteins (g/dl) | Albumins (g/dl) | Globulins (g/dl) |
|---|---|---|---|
| CSL[a] | 3.6 ± 0.0 | 2.1 ± 0.0 | 1.5 ± 0.0 |
| CSE[b] | 3.0 ± 0.0 | 1.9 ± 0.0 | 1.1 ± 0.0 |
| AHL[c] | 6.5 ± 0.1 | 3.3 ± 0.0 | 3.2 ± 0.1 |
| AHE[d] | 7.8 ± 0.1 | 4.1 ± 0.1 | 3.7 ± 0.1 |

a, CSL = sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

# CHAPTER FIVE

## 5.0 DISCUSSION

In this study, the overall prevalence of AI antibodies was 13.4%. The highest prevalence rate of AI (31.6%) was observed in the clinically sick local chickens. This finding is in agreement with what was reported by previous authors that AI prevalence is highest in birds on range (Abdu *et al*., 2005). These birds might have been infected naturally as a result field exposure to the AI virus (El-Yuguda and Baba, 2002). Only 79 (31.6%) of the clinically sick local and 49 (19.6%) of the clinically sick exotic adult chickens demonstrated the presence of antibodies to AI type A H5 antigen out of 500 sick birds in this study. Using HI technique, it is believed that not all birds may develop demonstrable antibodies to AI because the course of the disease is short. As a result, some birds may die before antibodies are produced (OIE, 2004). Furthermore, the results obtained in this study may be due to the fact that it is possible to collect blood during the middle of AI disease outbreak and not be able to detect any antibodies to AI type A H5 antigen in many of the birds (Wunderwald and Hoop, 2002). However, the detection of antibodies to AI virus in clinically sick birds is considered indicative of a recent disease (OIE, 2004).

The demonstration of antibodies to AI by the apparently healthy local chickens might be as a result of previous field exposure to AI type A H5 virus (Abegunde and Mbibu, 1987; El-Yuguda and Baba, 2002). The implication of this is that, the apparently healthy local chickens might likely be source of AI infection and could play significant roles in its transmission to the more susceptible backyard and commercial poultry. This could cause serious setbacks to the control of HPAI in Nigeria (Abdu *et al*., 1985; Elssa, 1985; Adeniyi *et al*., 1993; El-Yuguda and Baba, 2002). The absence of antibodies to AI in the apparently

healthy exotic chickens may probably be due to non exposure to AI type A H5 virus and absence of previous vaccination (Wunderwald and Hoop, 2002).

The significantly higher ($P<0.05$) antibody titre observed in the clinically sick exotic adult chickens than that of clinically sick local adult chickens is in agreement with the findings of previous authors that higher titres may be observed following challenge in birds maintained on high quality diets (confined birds) than in birds exposed to field challenge with viruses under poor quality diets and environmental stress (Zander *et al*., 1997; Alders and Spradbrow, 2001).

The non significant difference ($P<0.05$) observed between the mean AI antibody titres of clinically sick local and apparently healthy local chickens may be due to subclinical infection in the apparently healthy local birds as a result of field exposure to the AI virus which resulted into development of protective antibodies, just like those that were clinically sick (Adeniji *et al*, 1993; El-Yuguda and Baba, 2002).

The overall prevalence rate (34.6%) of ND antibodies observed in this study is in close agreement to the findings of previous authors who reported a prevalence rate of 31.2% for ND (Halle *et al*., 1999). The highest prevalence rate (85.2%) was observed in the apparently healthy exotic chickens. This could be attributed to routine vaccination that is being carried out for commercial chickens (Alders and Spradbrow, 2001).The positive ND antibody response observed in the clinically sick and apparently healthy local chickens might have been due to field exposure to the ND virus. This is because local chickens are rarely vaccinated couple with the fact that there was no history of vaccination against ND in the local chickens in this study (Alders and Spradbrow, 2001), . The low ND antibody titre

which fell below the minimum protective level of 4 $\log_2$ in the clinically sick local chickens implies that they are vulnerable to ND which is endemic in Nigeria (El-Yuguda and Baba, 2002; OIE, 2004). On the other hand, the positive antibody responses of clinically sick and apparently healthy exotic chickens to ND may be attributed to routine vaccinations which are conducted in commercial poultry (Alders and Spradbrow, 2001).

The significantly lower ($p<0.05$) values of total proteins in the clinically sick local and clinically sick exotic chickens than the normal values for apparently healthy local and exotic chickens might have been due to necrosis and degeneration of the liver, and renal and glomerular necrosis of the kidneys which lead to protein loss. Necrosis and degeneration of the liver and kidneys have been reported in AI (Riddell, 1987; Easterday *et al.*, 1997; Alexander, 1999; Abdu *et al.*, 2005). Total protein concentrations may decrease in birds having severe liver damage that the TP is not synthesized in the right quantity (Kaneko, 1989). This is because the liver is primarily responsible for protein synthesis, and any disease condition that interferes with the normal functioning of the liver may lead to a decrease in the concentration of TP (Duncan, 1998).

The TP values obtained from the apparently healthy local chickens were significantly lower ($p<0.05$) than those of apparently healthy exotic chickens. This may probably be due to poor nutrition in the local chickens, especially protein deficiency, which is known to have negative effects on immune response (Oladele, 2000). The finding in this study agrees with the fact that the higher TP concentration observed in exotic than local chickens may be attributed, at least in part, to high quality nutrition in exotic chickens (Onyeyili *et al.*, 1991; Agaie and Uko, 1998; Iyayi and Tewe, 1998; Abiola, 2000; Alders and Spradbrow, 2001; Oladele *et al.*, 2005).

The significantly lower ($p<0.05$) serum total protein values observed in the clinically sick exotic than in clinically sick local chickens might be partly attributed to multiple and subclinical infections occurring concurrently, with other infectious agents (other than AI virus) at various levels of their developments in the local chickens (El-Yuguda and Baba, 2002).

The values of albumins obtained from the clinically sick local and exotic chickens were significantly lower ($p<0.05$) than the normal values established for apparently healthy local and exotic chickens. This may also be due to necrosis and degeneration of the liver, and renal tubular and glomerular necrosis of the kidneys previously observed in AI (Easterday *et al.,* 1997). It has also been established that TP concentration may decrease in birds having so much liver damage that albumin is not synthesized because the liver is primarily responsible for albumin synthesis (Campbell and Coles, 1986). Therefore any disease condition that interferes with the normal functioning of the liver may also be reflected as a decrease in concentration of albumin (Bush, 1991; Duncan, 1998; Oladele, 2005).

It has been established that a decrease in total serum albumin may result from deficient synthesis of albumin, excessive protein breakdown, or loss of albumin (Coles, 1974). Since it is known that liver pathology may occur in birds suffering from AI (Riddell, 1987; Easterday *et al., 1997;* Alexander, 1999), the decreased in albumin levels in the clinically sick birds may be attributed to liver damage. Loss of albumin is probably the most common cause of decrease in serum albumin in domestic animals (Coles, 1974).

The implication of this decrease in serum albumin concentration in clinically sick birds is that, when AI infection is actively taking place, chemotherapy might be of little or no value,

since albumin level may not be sufficient enough to bind with drugs and transport them to tissues (Coles, 1974).

The albumin concentrations obtained from apparently healthy local chickens were significantly lower ($p<0.05$) than those of apparently healthy exotic chickens. This might also be explained by the fact that birds placed on a protein deficient diet would have low serum albumin, and this is a common feature of local chickens (Bush, 1991; Dipeolu, 1998).

The significantly lower ($P<0.001$) values of serum albumin in the clinically sick exotic than clinically sick local chicken observed in this study might be due to severity of AI in exotic chickens (which are usually confined) than in local chickens that are usually kept on free range or semi intensive system, which may have resulted in more severe tissue damage, and consequently, much decrease in albumin levels in the clinically sick exotic chickens (Bush, 1991). This might be explained by the fact that disease resulting from stress (in confined birds) is related to its severity and duration than in birds on free range (Zander *et al*., 1997).

The values of serum globulins obtained from the clinically sick local and exotic chickens were significantly lower ($p<0.05$) than the normal values established for apparently healthy local and exotic chickens. It is expected that clinically sick birds should have higher globulin levels than apparently healthy birds during infections (Bell and Sturkie, 1965). This is because the globulins (gamma globulins) essentially produce antibodies that act against disease agents in the body during infections and a decrease could arise due to antigen-antibody reaction which might cause a decrease in globulin levels (Bell and Sturkie, 1965).

The decrease in globulin levels that was observed in the clinically sick birds in this study may have been caused by conditions of enteropathies and nephropathies in which the levels of globulin may fall due to protein loss (Lloyd, 1981). Similarly, it was reported that some AI virus isolates induce the aforementioned conditions in the form of severe lymphoid necrosis in the spleen, thymus, bursa of Fabricius, intestine, liver and kidneys that can lead to destruction of lymphocytes which in turn could result into depletion of globulins (Riddell, 1987; Easterday *et al*., 1997).

The levels of globulin obtained from the apparently healthy local chickens were significantly lower ($p<0.05$) than those of apparently healthy exotic chickens, probably due to the fact that animals placed on a protein deficient diet would have low serum globulins (Bush, 1991).

## CONCLUSIONS

The presence of antibodies to AI type A H5 antigen in the sera of clinically sick local and exotic adult chickens in this study further confirmed disease in the flocks sampled.

This study also showed an overall prevalence rate of 13.4% for HPAI and 34.5% for ND in the area under study.

Local chickens might likely be a source of AI virus and could play significant roles in its transmission and spread to susceptible backyard and commercial poultry.

AI and ND could occur concurrently in a flock inducing more severe disease and more severe damage on serum proteins.

Marked decrease in TP, albumin and globulin (responsible for immunity) levels was observed in the chickens suffering from AI

The levels of TP, albumin and globulins in apparently healthy local chickens were significantly lower than in apparently healthy exotic chickens.

## RECOMMNENDATIONS

- It is highly imperative that a national active AI surveillance be conducted to define the true status of the disease in Nigeria, so as to enable the Government re-evaluate the current measures used in combating the disease.

- It is also necessary to conduct a survey for the presence of the virus in local chickens that have Survived AI outbreaks.

- If vaccination is to be carried out against AI, birds should be placed on high level of protein supplements in order to augment their serum protein levels which are expected to drop post-vaccination.

- The feed of local chickens should be supplemented with high protein source.

- Vaccination of local poultry against ND should be introduced and sustained, while vaccination of commercial and backyard poultry against ND should be intensified.

- Targeted community based training of rural backyard commercial poultry farmers and other stakeholders in the poultry industry on various aspects of HPAI recognition and control including Biosecurity procedures to prevent the disease becoming endemic.

- Effective public enlightenment and awareness programmes on HPAI.

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## APPENDICES

**Appendix I**: AI positive cases, range, minimum and maximum antibody titres.

| Group | Positive cases (N) | Range ($log_2$) | Minimum ($log_2$) | Maximum ($log_2$) | Sum |
|---|---|---|---|---|---|
| CSL | 79 | 5.00 | 3.00 | 8.00 | 441.00 |
| CSE | 49 | 3.00 | 5.00 | 8.00 | 347.00 |
| AHL | 6 | 4.00 | 4.00 | 8.00 | 35.00 |

a, CSL = clinically sick local chickens.
b, CSE = clinically sick exotic chickens.
c, AHL = apparently healthy local chickens

**Appendix II:** Mean ± standard error of AI antibody titres ($log_2$).

| Group | Mean | Positive cases | Standard. deviation | Standard. error mean |
|---|---|---|---|---|
| CSL[a] | 5.5306 | 79 | 1.6721 | 0.23887 |
| CSE[b] | 7.0816 | 49 | 0.9754 | 0.13935 |
| AHL[c] | 5.8333 | 6 | 1.60208 | 0.65405 |

a, CSL = clinically sick local chickens.
b, CSE = clinically sick exotic chickens.
c, AHL = apparently healthy local chickens.

**Appendix III**: T-test results of AI antibodies.

| | | Df(n-1) | Sig. (2-tailed) |
|---|---|---|---|
| Pair 1 | CSL[a] / CSE | 48 | 000 |
| Pair 2 | CSL /AHL[c] | 5 | 741 |
| Pair 3 | CSE[b] /AHL | 5 | 058 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens

**Appendix IV**: Newcastle disease positive cases, minimum and maximum antibody titres ($log_2$).

| Group | Positive cases | Range | Minimum | Maximum | Sum |
|---|---|---|---|---|---|
| CSL[a] | 13 | 3.00 | 3.00 | 6.00 | 50.00 |
| CSE[b] | 23 | 3.00 | 3.00 | 6.00 | 101.00 |
| AHL[c] | 97 | 5.00 | 3.00 | 8.00 | 467/00 |
| AHE[d] | 213 | 5.00 | 3.00 | 8.00 | 1169/00 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix V**: Mean ± Standard Error of Newcastle disease antibody titres ($log_2$).

| | Statistic | | Standard deviation. | Variance |
|---|---|---|---|---|
| Group | Mean | Standard error | | |
| CSL[a] | 3.8462 | 24926. | 89872 | 808.00 |
| CSE[b] | 4.3913 | 20603 | 98807 | 976.00 |
| AHL[c] | 4.8144 | 12464. | 1.23755 | 1.507 |
| AHE[d] | 5.4883 | 09355 | 1.36537 | 1.864 |

a, CSL =clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix VI:** Range, minimum and maximum values of total proteins (g/dl).

| Group | Sample size | Range | Minimum | Maximum | Sum |
|---|---|---|---|---|---|
| CSL[a] | 250 | 2.30 | 30 | 4.60 | 9.03.40 |
| CSE[b] | 250 | 2.30 | 2.00 | 4.30 | 753.90 |
| AHL[c] | 250 | 2.30 | 4.60 | 7.90 | 1633.00 |
| AHE[d] | 250 | 5.90 | 3.90 | 9.80 | 1957.00 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix VII:** Mean ± standard error of total protein values (g/dl).

| Group | Statistic | | Standard deviation | Variance |
|---|---|---|---|---|
| | Mean | Standard. error | | |
| CSL[a] | 3.6136 | .02916 | .46107 | .213 |
| CSE[b] | 3.0156 | .03733 | .59018 | .348 |
| AHL[c] | 6.5320 | .05635 | .89093 | .794 |
| AHE[d] | 7.8280 | .07351 | 1.16233 | 1.351 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix VIII:** T-test results for total protein values.

| | Df | Sig. (2.tailed) |
|---|---|---|
| Pair 1 CSL[a] & CSE[b] | 249 | .000 |

| | | |
|---|---|---|
| Pair 2 CSL[a] & AHL[c] | 249 | .000 |
| Pair 3 CSE[b] & AHE[d] | 249 | .000 |
| Pair 4 AHL[c] & AHE[d] | 249 | .000 |

**Appendix IX**: Range, minimum and maximum values of albumin (g/dl).

| Group | Sample size | Range | Minimum | Maximum | Sum |
|---|---|---|---|---|---|
| CSL[a] | 250 | 1.70 | 1.40 | 3.10 | 513.70 |
| CSE[b] | 250 | 1.60 | 1.30 | 2.90 | 482.50 |
| AHL[c] | 250 | 2.80 | 2.00 | 4.80 | 826.50 |
| AHE[d] | 250 | 3.60 | 2.30 | 5.90 | 1015.00 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix X**: Mean ± standard error of albumin values (g/dl).

| Group | Statistic | | Standard deviation. | Variance |
|---|---|---|---|---|
| | Mean | Standard error | | |
| CSL[a] | 2.0631 | .02439 | .38479 | .148 |
| CSE[b] | 1.9300 | .02472 | .39089 | .153 |
| AHL[c] | 3.3060 | .04371 | .69114 | .478 |
| AHE[d] | 4.0600 | .06225 | .98421 | .969 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix XI:** T-test results for albumin values.

| | Df | Sig. (2 – tailed) |
|---|---|---|

| | | | |
|---|---|---|---|
| Pair 1 | CSL[a] & CSE[b] | 249 | .000 |
| Pair 2 | CSL[a] & AHL[c] | 249 | .000 |
| Pair 3 | CSE[b] & AHE[d] | 249 | .000 |
| Pair 4 | AHL[c] & AHE[d] | 249 | .000 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix XII:** Range, minimum and maximum globulin values (g/dl).

| Group | Sample size (N) | Range | Minimum | Maximum | Sum |
|---|---|---|---|---|---|
| CSL[a] | 250 | 1.40 | .70 | 2.10 | 377.70 |
| CSE[b] | 250 | 2.50 | .10 | 2.60 | 274.60 |
| AHL[c] | 250 | 4.10 | 1.40 | 5.50 | 820.50 |
| AHE[d] | 250 | 4.30 | 1.40 | 5.70 | 933.00 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix XIII:** Mean ± standard error of globulin values (g/dl).

| Group | Statistic | | Standard deviation | Variance |
|---|---|---|---|---|
| | Mean | Standard. error | | |
| CSL[a] | 1.5108 | .02397 | .37908 | 1.444 |
| CSE[b] | 1.0984 | .03382 | .053496 | .286 |
| AHL[c] | 3.2100 | .05844 | .92408 | .854 |
| AHE[d] | 3.7320 | .07003 | 1.10719 | 1.226 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens

**Appendix XIV:** T-test results of globulin values.

| | Df | Sig. (2 – tailed) |
|---|---|---|
| Pair 1 CSL[a] – CSE[b] | 249 | .000 |
| Pair 2 CSL[a] – AHL[c] | 249 | .000 |
| Pair 3 CSE[b] – AHE[d] | 249 | .000 |
| Pair 4 AHL[c] – AHE[d] | 249 | .000 |

a, CSL = clinically sick local chickens
b, CSE = clinically sick exotic chickens
c, AHL = apparently healthy local chickens
d, AHE = apparently healthy exotic chickens