EGG DROP SYNDROME-76, NEWCASTLE DISEASE AND GUMBORO DISEASE ANTIBODIES IN POULTRY IN ZARIA AND ENVIRONS, KADUNA STATE, NIGERIA

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BY

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DECLARATION

I declare that the work in this dissertation entitled, "Egg Drop Syndrome-76, Newcastle disease and Gumboro disease antibodies in Poultry in Zaria and Environs, Kaduna State, Nigeria" has been performed by me in the Department of Veterinary Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria under the supervision of Dr. A. M. Wakawa, Professors P. A. Abdu and S. B. Oladele. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Ussa DELIA _____ _____
Student Signature Date

CERTIFICATION

This dissertation entitled, "EGG DROP SYNDROME-76, NEWCASTLE DISEASE AND GUMBORO DISEASE ANTIBODIES IN POULTRY IN ZARIA AND ENVIRONS, KADUNA STATE, NIGERIA" by Ussa DELIA 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.

Dr. A. M. Wakawa Chairman Supervisory Committee	…….. Signature	……. Date
Prof. P. A. Abdu Member Supervisory Committee	…….. Signature	……. Date
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Prof. L. B. Tekdek Head, Department of Veterinary Medicine, Ahmadu Bello University Zaria	…….. Signature	……. Date
Prof. Kabir Bala Dean, School of Postgraduate Studies Ahmadu Bello University Zaria	…….. Signature	……. Date

DEDICATION

This project is dedicated to my late father Major A. Delia (Rtd) for his immense contributions to my life.

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ABSTRACT

This study was conducted to determine the sero-prevalence of egg drop syndrome-76 (EDS-76), Newcastle disease (ND) and infectious bursal disease (IBD) in poultry sampled from rural households, live bird markets (LBM) and commercial farms in sixteen different locations of Zaria and environs, Kaduna State, Nigeria. Haemagglutination inhibition (HI) test was used to detect EDS-76 and ND antibodies, while agar gel precipitation test (AGPT) was used to detect antibodies to IBD. The result indicates that out of the 679 sera analyzed, 470 (69.2%) tested positive for EDS-76 antibodies, with prevalence of 100% obtained in Samaru commercial farm, ABU Quarters and Wusasa, and the lowest prevalence in Samaru LBM (34%). Analysis of variance (ANOVA) revealed that there was a highly significant difference between the locations ($p < 0.001$). The mean EDS-76 antibody titre in poultry in different locations sampled in Zaria and environs of Kaduna State, Nigeria was $4.73 \pm 0.09 \log_2$. The highest mean EDS-76 antibody titre ($7.07 \pm 0.55 \log_2$) was obtained in poultry from Wusasa and the lowest mean EDS-76 antibody titre ($2.73 \pm 0.66 \log_2$) in poultry from Samaru LBM. Rural households had a sero-prevalence of 66.8% for EDS-76 and a mean antibody titre of $4.36 \pm 0.15 \log_2$. Geese, pigeons and turkeys had a sero-prevalence of 33.3%, 52.6% and 18.2%, respectively for EDS-76, while turkeys had the highest mean EDS-76 antibody titre ($7.50 \pm 0.50 \log_2$). Local chickens had a sero-prevalence of 68.2%, while broilers had a sero-prevalence of 100% for EDS-76. The overall ND sero-prevalence of 72.1% was obtained for 1, 028 sera samples analyzed. High prevalence of 100% were obtained in birds sampled from Graceland, Samaru commercial farm, Sakaru, Wusasa and Zango commercial farms, while the lowest sero-prevalence was obtained from Giwa LBM (24.5%). The ANOVA revealed a significant difference between the locations ($p <0.0001$). The overall ND mean antibody titre was $6.08 \pm 0.13 \log_2$. The highest titre was recorded in flocks in Zango commercial farms ($9.61 \pm 0.93 \log_2$) and the lowest titre ($2.32 \pm 0.38 \log_2$) and

very low percentage of protective ND antibodies (14.5%) was obtained in birds from Pan hauya. Among the three LGAs sampled, flocks in Zaria had the highest mean ND antibody titre (8.84 ± 0.17 log_2) and all birds sampled having protective antibody titre ≥4log_2, while flocks in Giwa had the lowest mean ND antibody titre (3.55 ± 0.32 log_2) with the least having protective antibody titre (15.2%). Rural households had a sero-prevalence of 69.8% with 67.2% of poultry birds at risk of being affected by ND in an outbreak. The overall IBD sero-prevalence obtained was (48.4%). The highest IBD sero-prevalence was obtained in poultry sampled from Biye (81.8%). The lowest sero-prevalence of 11.6% was recorded in poultry sampled from Suleiman commercial farms. Of the three LGAs sampled, the highest IBD sero-prevalence was observed in flocks in Giwa (60.7%), while the lowest (44.1%) was in flocks in SabonGari. The results obtained from this study showed that poultry from Zaria and environs have circulating antibodies to EDS-76, ND and IBD and could serve as sources of introduction and spread of EDS-76, ND and IBD to susceptible hosts. It was recommended that birds from rural households should be vaccinated against ND and IBD routinely by owners so as to protect poultry from ND and IBD.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	- per cent
µl	- microlitres
^{0}C	- degree centigrade
ABU	- Ahmadu Bello University
CI	- confidence interval
DAdv-1	- duck adenovirus-1
DID	- Direct Immuno Diffusion
DNA	- deoxyribonucleic acid
EDS-76	- egg drop syndrome-76
ELISA	- enzyme linked immuno sorbent assay
HA	- haemagglutination
HAU	- haemagglutination unit
HI	- haemagglutination inhibition
IBD	- infectious bursal disease
IBDV	- infectious bursal disease virus
IFA	- immuno fluorescent antibody
LBM	- live bird market
LBMs	- live bird markets
LGAs	- local government areas
ml	- millilitre
ND	- Newcastle disease
NDV	- Newcastle disease virus
NVRI	- National Veterinary Research Institute
OIE	- Office International des Epizootics
OR	- odds ratio
PBS	- phosphate buffered salin

PI	- post infection
RBC	- red blood cell
Rf	- reference factor
S.E	- standard error
SN	- serum neutralization
SPSS	- statistical package for social sciences

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The importance of poultry industry can be judged from the fact that every family in rural and every fifth family in urban areas are directly or indirectly associated with poultry production (Sadiq, 2004). Despite improved management methods and disease preventive measures adopted in the public and private sectors, overall poultry population always remained victim to a number of infectious and non infectious diseases. Among the infectious diseases, egg drop syndrome-76 is the one posing a serious threat to the layer industry worldwide (Van Eck *et al*.,1976).This disease is primarily of economic significance, as the affected birds do not appear sick (CFSPH, 2006).

A syndrome causing lower egg production associated with laying of soft-shelled and shell less eggaccompanied by a 10%- 30% drop in egg production (Castro and Heuschelle, 1992) was firstdescribed in the Netherlands in 1976 and the possible causative agent of the syndrome was reported to be fowladenovirus (Van Eck *et al*., 1976). Later, several haemagglutinating adenoviruses were isolated by McFerran *et al*.(1978b) from affected hens in Northern Ireland and the correlation between the syndrome and the isolate was demonstrated (McFerran and Adair, 1977; McFerran*et al*., 1978a). The disease is now called EDS-76 causedby EDS-76 virus is the foremost cause of loss of egg production in laying hens throughout the world (Alam *et al.*, 2009).

Egg drop syndrome-76,is postulated to have been introduced into Nigeria via the use of contaminated vaccines (Baba *et al*., 1998). The disease also poses a potential threat to the Nigerian poultry industry (Nawathe and Abegunde,1980).Since its initial recognition, it has become apparent that sporadic outbreaks of EDS-76 occur as a result of the chicken becoming infected through direct or indirect contact with infected wild or domestic waterfowl (CFSPH, 2006). These groups of scavenging birds may serve as reservoirs of theEDS-76 virus to commercial poultry farms, when they are kept together (Spradbrow, 1987; Baba *et al*., 1998). The EDS-76 virus circulates under conditions of natural exposure primarily in domestic ducks and geese as a latent infection but not in other domestic avian species (Kaleta *et al*., 2003). Domestic turkeys and racing pigeons can be infected experimentally with EDS-76 virus, to produce serum antibodies but will develop no clinical signs. Free living ducks and geese of several genera and species can be infected latently under natural conditions (Kaleta *et al.*, 2003).

If some birds have acquired antibody before latentEDS-76 virus is reactivated, an apparently different clinical syndrome is seen as there may be failure to achieve predicted egg production, and onset of lay may be delayed, affected birds remain otherwise healthy (Van Eck, 1980).

Newcastle disease (ND) was first reported in Nigeria in 1953 (Hill *et al*., 1953). The disease is most severe during the dry harmattan (November – March) (Sa'idu*et al*., 1994; Abdu *et al*., 2005). The stress induced by cold also worsens the condition of ND (Abdu *et al*., 1992). Newcastle disease affects both exotic and local chickens.Velogenic ND has been recognized as one major diseasewhich kills large populations of rural birds annually in Africa and Asia (Bell and Mouloudi, 1988; Echeonwu*et al.*, 1993).

Infectious bursal disease (IBD) is a highly contagious viral disease of young birds, it's a disease that has high affinity to lymphoid tissues, with the bursa of Fabricius as its predilection site (Lukert and Saif, 2003).The causativeagent is infectious bursal disease virus (IBDV) whichbelongs to the genus *Avibirnavirus* within the family*Birnaviridae* (Dobos *et al*., 1979). The two serotypes, 1 and 2, ofthe virus are differentiated by virus neutralization (VN)test (McFerran *et al*., 1980). Serotype 1 contains thepathogenic strains to chicken and can be grouped intoclassical, antigenic variant and very virulent (vv) strains(Brown *et al*., 1994; Cereno, 2008).Gumboro disease is immunosuppressive in nature (Allan *et al*., 1972). Infection with IBDV reduces antibody response to other vaccinations (Faragher, 1974; Giambrone, 1976; Rosenberger and Gelb, 1977; Muller, 2003; Westbury 2008). Thedisease by itself usually causes mortality of 5-10% butthis rate can reach 30-40% (WOAH, OIE, 2004). The outcome ofIBD is largely dependent on the strain and the amount ofthe virus, age and breed of birds, the route of inoculation,the presence or absence of neutralizing antibodies,intercurrent primary and secondary pathogens andenvironmental and managerial factors (Muller *et al*., 2003).

1.2 Statement of Research Problems

Chickens of all ages are susceptible to EDS-76 virus infection. Once EDS-76 infection is established into a poultry breeding organization, it is often seen as a failure to achieve egg production targets, and eggshell changes (pale, thin-shelled or shell-less eggs) are less apparent. Egg drop syndrome-76 virus infection in birds can cause great economic losses in egg production (CFSPH, 2006).

Newcastle disease affects all ages of birds. It affects both domesticated and wild birds which could serve as carrier to other susceptible birds (CFSPH, 2008). It is an acute infectious and

highly contagious and devastating viral disease of poultry (Adu *et al*., 1985; Abdu and Garba, 1989; Awan *et al*.,1994;Sa'idu*et al*., 2006).

Infectious bursal disease virus is a major problem of commercial and rural chickens and constitutes a major threat to poultry production in Nigeria (Okoye and Uzoukwu, 2001; Musa *et al*., 2010). Infectious bursal disease virus has high affinity for lymphoid tissues which could lead to immunosuppression, which exposes birds to inter-current infections and also a reduced response to vaccination against other diseases (Shane, 1997).

1.3 Justification of the Research

Serological evidence of EDS-76 virus in turkeys and free-range chickens was observed in some areas in Nigeria (Oyeduntan and Durojaiye, 1999; Abubakar *et al.,* 2008;Ezeibe *et al.,* 2008; Ezema *et* al., 2008; Salihu*et al.,* 2010), and in commercial farms (Nawathe and Abegunde, 1980), however, to the best of our knowledgethere is no literature on EDS-76 virus in the study area.

In view of the fact that polyvalent vaccines which containEDS-76are currently being used for vaccination of chickens in Nigeria, there is a need to serologically screen flocks for the presence of EDS-76 antibodies and if positive, check the antibody titre in free-range chickens, poultry farms and live bird markets (LBMs) in some Local Government Areas in Kaduna State.

Newcastle disease is endemic in the country and is of great economic threat in Nigeria (Oladele *et al*., 2003). In an outbreak of ND the mortality and morbidity could be very high which would enormously affect production.

Infectious bursal disease affects chicks of young age (3 – 6 weeks of age) and is associated with serious economic losses (Lukert and Saif, 1997). The disease was also reported in older birds in some areas in Nigeria (Okoye and Uzokwu, 1981; Abdu, 1988; Owoade and Durojaiye, 1995; Mbuko *et al*., 2010; Musa *et al*., 2010).

1.4 Aim of the Study

The aim of this study was to determine the sero-prevalence of EDS-76, ND and IBD in Zaria and environs, Kaduna State, Nigeria.

1.5 Objective of the Study

The objectives of the study were to determine:

1. The sero-prevalence of EDS-76, ND and IBD in poultry in poultry in commercial farms, rural households and LBMs in Zaria and environs, Kaduna State.
2. Determine the levels of antibody titres to EDS-76 and ND in poultry in Zaria and environs, Kaduna State.
3. To determine the sero-prevalence of EDS-76, ND and IBDV in relation to location, type of poultry and specie of poultry in Zaria and environs, Kaduna State.

1.6 Research Questions

1. Do poultry in Zaria and environs have EDS-76, ND and IBD antibodies? If Yes
2. What are the antibody titre levels of EDS-76 and ND in poultry in Zaria and environs, Kaduna State?

CHAPTER TWO

LITERATURE REVIEW

2.1 Egg Drop Syndrome-76

2.1.1History of egg drop syndrome-76

Egg drop syndrome-76 is a disease of chickens that affect egg production, caused by haemagglutinating adenoviruses (McFerran and Adair, 1977). The syndrome first recognized in 1976 is caused by anadenovirus that is widely distributed in wild anddomestic geese and ducks (McFerran *et al*., 1977). Exotic and free-rangingflocks may be at a greater risk than conventional ones,as there is likely to be more contact with wild-fowl and geese or duck carriers which serve as reservoirs of the causative agent (Bartha and Meszaros, 1985).The virus is transmitted vertically through the egg. The virus often remained latent until birdswere approaching onset of lay (Calnek, 1978). Because of theabsence of antibody to the virus in chickens prior to 1974and the failure of the virus to grow in mammalian cells, aswell as its poor growth in turkey cells and optimal growthin duck cells, it was suggested that this was probably aduck adenovirus (Calnek, 1978). This suggestion was quickly confirmedby isolation of EDS-76 virus from normal ducks and demonstrationof antibody in many duck flocks (Baxendale, 1978; Calnek, 1978).

2.1.2 Incidence and distribution of egg drop syndrome-76

Egg drop syndrome-76 is worldwide in distribution, the virus has been isolated in France, Great Britain, Belgium, Israel, Australia, Japan,Hungary, Singapore, Taiwan, South Africa, India and China (Picault, 1978; Baxendale, 1978; Meulemans *et al*., 1979; Malkinson and Weisman, 1980; Firth *et al*., 1981; Yamaguchi *et al*., 1981; Zsak and Bartha 1979; Singh and Chew-Lim, 1981;

Lu*et al*., 1985; Bragg, *et al*., 1991; Kumar *et al*., 1992; Zhu and Wang, 1994). Antibody to the virus was demonstrated from chickens in Denmark, Brazil, Mexico, Nigeria, Iraq and New Zealand (Badstue and Smidt, 1978; Hwang *et al*., 1980;Rosales *et al*., 1980; Nawathe and Abegunde, 1980; Al-Hilly *et al*., 1982; Howell, 1982).

2.1.3 Aetiology of egg drop syndrome-76

Duck adenovirus A is the causative agent of EDS-76; it's a member of the genus *Atadenovirus* and family *Adenoviridae* (Hess *et al*., 1997; Benko *et al*., 2005). Synonyms to the virus includes, duck adenovirus type 1(DAdV-1), egg drop syndrome virus, EDS-76 virus and adenovirus 127 (Benko and Harrach, 1998).It is a non-enveloped, haemagglutinating DNA virus, with a diameter of 74-80 nm and replicates in the nucleus of the host cells (McFerran *et al*., 1978a; Jordan, 1990; Regenmortel *et al*., 2000).

2.1.4 Classification of egg drop syndrome-76 virus

Egg drop syndrome-76 virus is classified as an adenovirus on the basis of itsmorphology, replication, and chemical composition (McFerran *et al*., 1978b). Thevirus was not related to 11 fowl and 2 turkey prototypeaviadenoviruses using serum neutralization (SN) orhaemagglutination inhibition (HI) tests (McFerran *et al*., 1978b). Although theaviadenovirus group specific antigen is not detected byimmunodiffusion or immunofluorescence tests in EDS-76 virus preparations, presence of common antigenic determinantswere indicated in experiments in which aviadenovirusgroup specific antibodies, which had developed inchickens after inoculation with a subgroup I virus, wererestimulated following a subsequent inoculation with EDS-76 virus (McFerran *et al*., 1978b).Although initial isolates of EDS-76 virus were from

chickens (McFerran, 2003), the virus is now recognized as having originatedfrom ducks, and its species designation is as DAdV-1 (Benko *et al*., 2000).

2.1.5 Propagation of egg drop syndrome-76 virus

Egg drop syndrome-76 virus replicate to high titres in duck kidney, duckembryo liver, and duck embryo fibroblast cell cultures andgrew well in chick embryo liver cells, less well in chick kidney cells, and poorly in chicken embryo fibroblastcells (Adair *et al*., 1986). Growth in turkey cells was poor and no replicationwas detected in a range of mammalian cell cultures (Adair *et al*., 1979). There is also a high replication of titer in goose cell cultures (Zsak *et al*., 1982). In chick liver cell cultures, peak virus and intracellular haemagglutination (HA) titres were reached after 48 hours, with peak extracellular HA titres being achieved after 72 hours (Yamaguchi *et al*., 1981).

The virus was observed to have grown very well when inoculated into theallantoic sac of embryonated duck or goose eggs producing HA titres of 1/16,000-1/32,000. No growth wasdetected in embryonated chicken eggs (Adair *et al*., 1979; Zakharchuk *et al*., 1993).

2.1.6 Strain classification of egg drop syndrome-76 virus

Only one serotype of EDS-76 virus has been recognized (Darbyshire and Peters, 1980; Yamaguchi *et al*., 1981). With the use of restriction endonuclease analysis,however, it has been possible to divide a number of isolatesinto three genotypes- Strain 127, BC14 and D61 (Todd *et al*., 1988).

2.1.7 Susceptibility of egg drop syndrome-76 virus to chemical and physical agents

The EDS-76 virus was stable to treatment with chloroformand variations in pH between 3 and 10. The virus can be inactivated by heating for 30 minutes at 60°C, but survivedfor 3 hours at 56°C, and was stable in the presence ofmonovalent but not divalent cations (Adair *et al*., 1979, Yamaguchi *et al*., 1981). Treatment with 0.5% formaldehyde or 0.5% glutaraldehyde inactivates the virus (Takai *et al*., 1984).

2.1.8 Transmission of egg drop syndrome-76 virus

Duck adenovirus A can be transmitted vertically in eggs; as both the egg content and the egg shell contains the virus; it is probable that eggs from hens that fed on infected eggs, transmits the virus to progeny, seen in 18 days incubated eggs (Van Eck, 1980; Kumar *et al*., 2003; Dhama *et al*., 2011). Chicks hatched from infected eggs may excrete the virus immediately. More often, the virus remains latent until the bird becomes become sexually mature; it is then excreted in both eggs and droppings (Dhama, *et al*., 2011).

In EDS-76, horizontal transmission is thought to be mainly by the oral route; however, respiratory disease in goslings was reproduced by intra-tracheal administration of the virus (McFerran, 2003). Duck adenovirus A can also be spread on/in formites including water (McFerran, 2003). Some outbreaks have been attributed to contact with wild birds or water contaminated by feaces from wild birds. Iatrogenic transmission is possible by needles (McFerran, 2003, McFerran and Adair, 2003). Transmission via insects is possible but unproven (McFerran, 2003; McFerrran and Adair, 2003).

2.1.9 Species affected by egg drop syndrome-76

Ducks and geese appear to be the natural hosts for duck adenovirus A. The virus was also isolated from coots and grebes, and antibodies have been found in many avian species including gulls, owls, storks, swans, guinea fowls, and pigeons(Bidin *et al*., 2007). Clinical disease has been reported in chickens, quails, and geese. Turkeys can be infected experimentally but remain asymptomatic (Bidin *et al.,* 2007).

Although disease outbreaks have been recorded only in laying hens, it is reported that the natural hosts for EDS-76 virus are ducks and geese (Bidin *et al*., 2007). Egg drop syndrome-76 virus HI antibody is widespreadin domesticated ducks (Baxendale, 1978; Badstue and Smidt, 1978; Malkinson and Weisman, 1980; Firth *et al*., 1981; Bartha *et al*., 1982) and domestic geese (Bartha *et al*., 1982; Zsak *et al*., 1982). In a study of ducks in the Atlanticflyway in the United States, antibody was found in ruddy,ring-necked, wood, bufflehead, lesser scaup, mallard, northern shoveler and gadwall ducks and in mergansers, coots, and grebes (Schloer, 1980; Gulka *et al*., 1984). Antibody was also detected inMuscovy ducks and in cattle egrets, Canada geese, herring gulls, owls, a stork and a swan.The virus has been isolated from healthy domestic ducks (Baxendale, 1978; Schloer, 1980; Kaleta *et al*., 1980; Bartha *et al*., 1982).

Quails (*Coturnix coturnix japonica*) have also beenshown to be susceptible to infection and to develop classic signs of EDS-76 (Das and Pradhan, 1992). No evidence indicates naturallyoccurring infection of turkeys or pheasants, although theycan be infected experimentally (Parsons *et al*., 1980; Bartha *et al*., 1982). Guinea fowls may be infected naturally or experimentally, and soft-shelledeggs may be produced. However, in other studies,guinea fowls became infected but remained clinically normalfollowing exposure to a fowl isolate of EDS-76 virus (Watanabe and Ohmi, 1983).Because EDS-76 virus initially was transmitted verticallyin

chickens, there was often an apparent association withcertain chicken breeds (Van Eck *et al*., 1977). A wide range of breeds, however,have been shown to be equally susceptible to experimentalinfection, although analysis of naturally occurring outbreakssuggests that broiler breeders and heavy breeds producing brown eggs are more severely affected thanwhite-egg producers (Van Eck *et al*., 1977).

Chickens of all ages are susceptible to EDS-76 virus infection.Introduction of EDS-76 virus into a laying site affectedegg production in all ages of laying hens. However, theappearance of disease at around peak egg production may be due to reactivation of latent virus (McFerran *et al*., 1978a).

2.1.10 Incubation period of egg drop syndrome-76

The incubation period for EDS-76 is highly variable. Experimental infection of matured Rhode Island Red hens, produced abnormal eggs from 10-24 days post-infection (McCracken and McFerran, 1978). Birds infected vertically can remain asymptomatic until they begin laying eggs. In experimentally infected goslings, the incubation period for respiratory disease is three to four days (Ivanics *et al*., 2001).

2.1.11 Pathogenesis of egg drop syndrome-76

Experimental oral infection of adult hens with EDS-76 virusresulted in a viremia with limited virus replication in the nasal mucosa (Smyth *et al*., 1988). At three to four days postinfection (PI), virusreplication occurred in lymphoid tissue throughout thebody; particularly in the spleen and thymus (Taniguchi *et al*., 1981). In addition, theinfundibulum of the oviduct was consistently affected. At 7-20 days PI, substantial levels of virus replication weredetected in the

pouch shell gland with lower levelsof replication in other parts of the oviduct (Taniguchi *et al*., 1981). This replicationwas associated with a pronounced inflammatoryresponse in the pouch shell gland, and production of eggswith abnormal shells (Taniguchi *et al*., 1981; Yamaguchi *et al*., 1981; Smyth *et al*., 1988).

Unlike subgroup I and II adenoviruses, EDS-76 virus doesnot appear to replicate in the intestinal mucosa and the presenceof virus in the faeces is probably due to contamination with oviduct exudate (Van Eck *et al*., 1976).

2.1.12 Clinical signs of egg drop syndrome-76

First signs were noticed seven to nine days post experimental infection with EDS-76 virus (McCracken and McFerran, 1978; Cook and Darbyshire, 1981), but in some experiments, clinical signs did not appear until 17 days PI (McFerran, 2003). Loss of shell colour in pigmented eggs was among the first signs noticed, which was followed by production of thin-shelled, soft-shelled, or shell-less eggs (McFerran *et al*., 1978; McCraken and McFerran, 1978). Thin-shelled eggs were observed to be roughened, with a sandpaper-like texture or had a granular roughening of the shell at one end of the egg (McFerran *et al*., 1978; McCraken and McFerran, 1978). If the abnormal eggs were discarded, there was no effect on fertility or hatchability and no long-term effect on egg quality. If birds were infected in the late stages of egg production, forced molting of the flock appeared to restore egg production to normal (McFerran *et al*., 1978; McCraken and McFerran, 1978). The fall in egg production was very rapid or extended over several weeks. Egg drop syndrome-76 outbreaks usually lasted four to ten weeks, and egg production was reduced by up to 40%; however, there was often compensation later in lay, so that the total number of eggs lost was typically 10-16/bird (McFerran *et al*., 1978;

McCraken and McFerran, 1978). If the disease resulted from reactivation of latent virus, the fall usually occurred when production was between 50% and peak level. Small eggs have been described in naturally occurring outbreaks, but no effect on egg size was found in experimental infections (McFerran *et al*., 1978; McCraken and McFerran, 1978). Watery albumen has been described (Van Eck *et al*., 1976; McFerran, 2003). Age at time of infection may be important, however; birds infected at 1 day of age produced apparently normal eggs except for impaired albumen quality and smaller size (Cook and Darbyshire, 1981).

If some birds have acquired antibody before latent virus is reactivated, an apparently different clinical syndrome is seen. There may be failure to achieve predicted egg production, and onset of lay may be delayed (OIE, 2000). If a careful examination is made, it usually can be established that there has been a series of small clinical episodes of classic EDS-76 (OIE, 2000). Presumably, presence of EDS-76-specific antibodies slows down the spread of the virus in the bird. A similar picture has often been observed in birds housed in caged units, where spread of the virus can be slow and EDS-76 not suspected (OIE, 2000).

Affected birds remain otherwise healthy. Although inappetence and dullness have been described in some affected flocks, these are not consistent findings(Smyth *et al*., 1988). Transient diarrhoea described by some authors is probably due to the exudate from the oviduct (Smyth *et al*., 1988). Egg drop syndrome-76 virus does not cause clinical disease in growing chickens in the field(Cook and Darbyshire, 1981).

Oral infection of susceptible day-old chicks resulted in increased mortality in the first week of life, but there was no increase in mortality in many flocks of chickens produced by virus-infected parent flocks(Cook and Darbyshire, 1981).

2.1.13 Diagnosis of egg drop syndrome-76

2.1.13.1Isolation and identification of egg drop syndrome-76 virus

The most sensitive medium for virus isolation is eitherembryonated duck or goose eggs derived from a flock freeof EDS-76 virus infection or duck or goose cell cultures. Ifthese are unavailable, chicken cells should be used.Chicken embryo liver cells are more sensitive thanchicken kidney cells, and chicken embryo fibroblasts were insensitive. Embryonated chicken eggs are not suitable (Adair *et al*., 1986).

Not only are duck or goose cells or embryonatedduck or goose eggs more sensitive, they also have theadvantage that they do not support the growth of manychicken viruses (Adair *et al*., 1979).

It is not sufficient to rely on embryo death or cytopathiceffect to indicate isolation of EDS-76 virus. Allantoic fluid frominoculated goose or duck eggs or supernatant from infectedcell cultures should be checked after each passage for presenceof EDS-76 virus HA, using avian erythrocytes (0.8% chickerythrocyte suspension is suitable) (Adair *et al*., 1986). Alternatively, immunofluorescence,using a labeled EDS-76 virus antiserum, can beused to detect the presence of the virus in the cells. Conjugatedantiserum to subgroup I avian adenoviruses will notdetect EDS-76 virus (Adair *et al*., 1986). If duck cells are used for isolation, a minimum of two passages are required, and with chick cells, two to five passages are necessary before declaring a specimen negative (Adair *et al*., 1986).The need for extensive passage is partly due to poorgrowth of the virus on primary isolation in chick cells, andpartly because virus titres in the tissues can vary particularlyif the bird submitted to the laboratory is not at thestage of the disease process where virus titres are maximal (Adair *et al*., 1986).

2.1.13.2Selection of specimens

Because of the absence of obvious clinical signs and theoften slow spread of infection, it can be very difficult toselect infected birds for either virus isolation or serology (Smyth and Adair, 1988). The finding that abnormal eggs contained virus and thatthese eggs were produced after the bird had developed EDS-76 antibodies has allowed a rational approach to diagnosis (Smyth and Adair, 1988). To isolate the virus, abnormal egg has been fed toantibody-negative adult laying hens, which produced antibodies after some weeks, where these birds also produce abnormal eggs too. EDS-76 virus was detectable in organs of young chicks up to five weeks post-infection and in their feaces up to two weeks PI (Ursula *et al*., 1982). In adult birds EDS-76 virus persisted in tissues for about three weeks and was excreted in faeces for only one week PI (Ursula *et al*., 1982). Examination of randomely selected cloacal swabs has been successful in some cases (OIE, 2000).

For serological diagnosis, all the birds in cages whereabnormal eggs are being produced should be sampled (OIE, 2000).If the birds are housed on litter, care must be taken to select samples throughout the house, as it is usually notpossible to determine which birds are producing abnormaleggs in these circumstances (OIE, 2000).

2.1.13.3Serology

The HI, enzyme linked immuno sorbent assay (ELISA), SN, direct immuno diffusion (DID) and immuno fluorescent antibody (IFA) tests are of similar sensitivity (Adair *et al*., 1986). When birds have been infected with a numberof adenovirus serotypes, however, with consequent stimulationof high levels of cross-reactive antibodies, therewere positive reactions in the ELISA, IFA, or DID tests but not in the HI or SN tests (Adair *et al*., 1986). Haemagglutination inhibition

is the test of choice forserological diagnosis (OIE, 2000). Antigen for HI test can be preparedin either embryonated duck eggs or in cell culture (OIE, 2000). HigherHA titers are obtained if duck eggs are used, but high HAtiters can also be obtained using chick embryo liver cellcultures (OIE, 2000). A suitable HI test uses 4 HA units (HAU) of antigen, aninitial 1:4 serum dilution, and 0.8% chicken erythrocytes (OIE, 2000).

The EDS-76 virus agglutinates erythrocytes from chickens,geese, turkeys, and ducks but not mammalian erythrocytes (OIE, 2000). There is no hemolysin. If nonspecific haemagglutininsare present in the serum, they can be removed bypre-adsorption of the serum with a 10% erythrocyte suspension (OIE, 2000).

The SN test, using 100 $TCID_{50}$ of EDS-76 virus, 1hour at 37°C reaction time, and duck or chick cell culturesas the indicator system, is sensitive and specific (Adair *et al*., 1986). Whenusing chick cell cultures, it often helps if the end-pointsare read by presence of haemagglutinins in the supernatantfluid rather than by cytopathology (Adair *et al*., 1986). The SN test is reallyrequired only in diagnostic situations to confirm anunusual HI test result as in an eradication program or toconfirm a positive HI result in species in which EDS-76 hasnot previously been recognized (Adair *et al*., 1986).Many flocks containing birds that had been infectedwith EDS-76 virus *in ovo* did not develop antibodies duringthe growing period; it only became apparent immediatelyfollowing the development of clinical signs of the disease (Adair *et al*., 1986).Therefore, even a negative serological test of all birds in aflock, at around 20 weeks of age, gives no guarantee offreedom from infection (Adair *et al*., 1986; OIE, 2000).

2.1.14 Treatment of egg drop syndrome-76

Various treatments have been tried (for example, vitaminsand increasing calcium or protein in the ration), but incontrolled trials, no effect could be demonstrated. Therefore,no successful treatment is available (Alam *et al*., 2009).

2.1.15 Eradication of egg drop syndrome-76

Egg drop syndrome was eradicated successfully from abreeding organization in Northern Ireland. The methodwas based on a number of presumptions (McFerran, 1979):

(i) chickens produced from EDS-76 virus-infected eggs may be latently infected and fail to develop antibody;

(ii) the virus will become reactivated and will be excreted at around the time of peak egg production, and EDS-76 antibody will develop, which will prevent or reduce further excretion;

(iii) Lateral spread is poor.

The eradication program was based on the elite andgrandparent flocks aged 40 weeks or more by Dr. M. Pattisson in 1978. At this stage, these flocks had produced abnormal eggs and had EDS-76 HIantibody. Chicks hatched from these eggs were dividedinto small groups of about 100 (separated by nettingwire). Ten to twenty-five percent of the chicks were tested for EDS-76 antibodies by HI test at about 6-week intervals (McFerran, 1979).If one or two reactors were found, they wereremoved; 100% of the birds in the pen and 100% ofadjoining pens were then tested twice at weekly intervals.If HI test positive reactors were found or if positive reactorskept appearing within a single pen, the whole penwas then removed and the in-contact pens of birds weretested (McFerran, 1979). At 40 weeks, 100% of the birds were tested by HItest, and eggs

were collected for the next generation. Thisprogram was successful, and subsequently, the grandparentand parent flocks were found to be free of infection (McFerran, 1979).

2.1.16 Public health significance of egg drop syndrome-76

Egg drop syndrome-76 virus affects only avian species and, therefore, has nopublic health significance (CFSPH, 2008).

2.2 Newcastle Disease

2.2.1 Incidence and distribution of Newcastle disease

Newcastle disease is endemic in many countries of the world. However, some European countries have been free of the disease for years (OIE, 2000).The first outbreaks of ND are thought to have occurred in 1926 in Java, Indonesia and in Newcastle-upon-Tyne, UK (Alexander, 1997). Different clinical representations of the disease gave rise to different names, such as pseudo-fowl pest, avian distemper or avian pneumo-encephalitis, until serological evaluations revealed that these syndromes were due to the same causative agent (Alexander, 1997). It is speculated that the first panzootic spread of ND virus started in the 1920s in Southeast Asia, and took about 30 years to spread around the globe. A second panzootic might have begun in the Middle East in the late 1960s and global spread occurred much more rapidly due to the intensification of the poultry industry worldwide, reaching most countries in about a decade (Alexander, 1997). Imported NDV-carrying psittacine birds captured in the wild are associated with this second panzootic of ND (Alexander, 1997). A third panzootic spread of ND, around the late 1970s, has been related to pigeons and doves (*Columba livia*) that were kept for leisure (Alexander, 1997).It reached Europe in the 1980s (Alexander, 1997). Newcastle disease

was first reported in Nigeria in 1953 (Hill *et al*., 1953) and since then, there have been several reports from different parts of the country (Ezeokoli *et al*., 1984;; Abdu *et al*., 1992; Echeonwu *et al*., 1993; Halle *et al*., 1999; Orajara *et al*., 1999 and Saidu *et al*., 2006).

2.2.2 Aetiology of Newcastle disease

It is caused by a linear, non-segmented single stranded,enveloped, negative sense RNA virus. Newcastle disease virus belongs to the order Mononegavirales, genus Avula virus of sub-family *Paramyxovirinae* andfamily *Paramyxoviridae* (Mayo, 2002; Barbezange and Jestin, 2005).

2.2.3 Transmission of Newcastle disease virus

Infection with NDV takes place either by inhalation or by ingestion and this mode of infection is also used for mass application of live vaccines by spray and aerosol generators (Alexander, 1997). In aerosols, particles of <5 microns disperse in the entire respiratory tract including the air-sacs. Particles > 5 microns are caught in the conjunctivae, nose and tracheae down to the bifurcation (OIE,2000). Horizontal transmission of NDV between birds depends on the availability of NDV in infectious form which may occur through inhalation of fine aerosols or large droplets containing the virus (OIE,2000). These droplets originate from the exhaled air of birds in which the virus has replicated in the respiratory tract (OIE,2000). The NDV may be transmitted by dust and other particles, including faeces. Spread of avirulent NDV may predominantly occur through ingestion of infected faeces; in this case respiratory signs are absent (OIE,2000). Virus is shed during the incubation period and for a limited period during convalescence. As an exception, some psittacine birds have been demonstrated to shed NDV intermittently for over one year (OIE, 2000).

Vertical transmission is not important for transmission of NDV, and it has not been clearly demonstrated. Experimental infection of laying hens frequently stops them from laying, and chick embryos die very soon after infection with NDV(Alexander, 1997). Furthermore, the virus may penetrate the shell of infected eggs, complicating the assessment of true vertical transmission (Alexander, 1997).

Outbreaks of ND may occur as a result of movement of infected live birds (Clavijo *et al*., 2000), from infected water fowl (Takakuwa *et al*., 1998), contaminated persons or equipment, infected poultry products (meat, feathers, blood, offal, poultry scraps and bones), airborne spread, contaminated poultry feed, drinking water (surface contamination or by seepage from infective faeces), and vaccines (Kouwenhoven, 1993; Alexander, 1997). However, the greatest risk of spread of NDV comes from the movement of people and equipments. Due to centralization of many processes in the poultry industry there is intensive traffic of personnel and vehicles (feed and chicken trucks, egg collectors, advisors, helpers, veterinarians, neighbors) moving from one flock to another (Kouwenhoven, 1993). Newcastle disease virus is easily spread by mechanical transfer, by people carrying the virus or transporting contaminated equipment, or by infective material (most probably faeces). Personnel involved in vaccination have also been implicated in the spread of NDV, as have incompletely inactivated and contaminated vaccines (Alexander, 1997).

2.2.4 Species affected by Newcastle disease virus

Many species of bird are susceptible to NDV. In addition to the domestic avian species, natural or experimental infection with NDV has been demonstrated in at least 236 species from 27 of the 50 orders of birds (Bolte *et al*., 2001). It is generally agreed that aquatic birds such as ducks and

geese are least susceptible to NDV (Bolte *et al*., 2001), while the most susceptible are chickens, and gregarious birds forming temporary or permanent flocks (Kaleta and Baldauf, 1988; Kouwenhoven, 1993). The breeds used in the poultry industry are equally susceptible, but some indigenous breeds are less susceptible to the virus (Kouwenhoven, 1993).

2.2.5 Incubation period of Newcastle disease virus

The incubation period of ND varies from 2 to 15 days (average five to six days). The variation depends on the strain, the host species, its age and immune status, intercurrent infections, environmental conditions, the route of exposure and the dose (Parede and Young, 1990; Alexander, 1997).

2.2.6 Pathogenesis of Newcastle disease

The virus enters the body via the respiratory or intestinal tract. In the trachea, the virus is spread by ciliary activity and by cell-to-cell spread(Kouwenhoven, 1993; Brown *et al*., 1994). After initial multiplication at the introduction site, the virulent virus is carried by viraemia to the spleen, liver, kidneys and lungs (Kouwenhoven, 1993; Brown *et al*., 1994). While lentogenic NDV strains are present only at low titres in the circulation, mesogenic NDV strains spread rapidly to the kidneys, lungs, bursa and spleen(Kouwenhoven, 1993; Brown *et al*., 1994). Virulent virus can therefore be found virtually within 22-24 hours in practically all tissues, titres being most elevated in the thymus (Kouwenhoven, 1993; Brown *et al*., 1994). The virus multiplication is then usually interrupted for 12-24 hours and the virus titres drop. During the second multiplication of the virus, after the arrest period, the virus is once again released into the circulation. This release is associated with the appearance of general disease signs, and the virus is released into the environment by exhaled air and faeces (Kouwenhoven, 1993). Virus

invadesthe brain after multiplication in non-nervous tissue has stopped, whereupon death is imminent (Kouwenhoven, 1993).

2.2.7 Clinical signs of Newcastle disease

Clinical signs of ND include loss of appetite, listlessness, decreased thirst, huddling, weakness and somnolence (Kouwenhoven, 1993). There is a sudden decrease in egg production (40% to occasionally 100%) together with de-pigmentation, and loss of the eggshell and albumen quality in layers (Kouwenhoven, 1993). The severity of the disease in chickens depends largely on the strain and host immune status. Some NDV strains may kill fully susceptible, unvaccinated chickens within three to four days, whereas the low virulence virus may circulate without clinical signs in unvaccinated birds (Kouwenhoven, 1993). The signs may affect the respiratory (gasping and coughing), circulatory (cyanosis of comb and wattles), gastrointestinal (crop dilation, catarrh, and foamy mucus in the pharynx) and nervous systems (drooping wings, dragging legs, circling, ataxia, paralysis, and torticollis). Egg production may be reduced or cessation altogether (Awan *et al*., 1994; Alexander, 2001).

Consequently, NDVs have been classified into pathotypes, referring to their pathogenicity in chickens (Lopaticki *et al*., 1998).Infections of chickens with viscerotropic velogenic NDV strains may lead to listlessness, increased respiration and weakness, ending in prostration and death. Mortality may be up to 100% in fully susceptible flocks (Spradbrow, 2000; Sa'idu and Abdu, 2008). In the 1970s, viscerotropic velogenic NDV outbreaks induced marked respiratory distress in the UK and Northern Irish flocks (Alexander, 2001). Oedema around the eyes and head, and greenish diarrhoea may also be present, before death muscular tremors, torticollis, opisthotonus and paralysis of the legs may be apparent (Alexander, 1993).

Infections of chickens with neurotropic velogenic NDV strains may lead to sudden onset of severe respiratory distress followed by neurological signs one to two days later. Egg production drops dramatically, but mortality is much lower than in flocks infected by vicerotropic velogenic NDV infections, and usually reaches a maximum of 50% in adult chickens (Alexander, 1997).

Infection of chickens with mesogenic strains of NDV usually causes respiratory disease. In adult chickens, egg production will drop greatly, which may last for several weeks. Nervous signs are uncommon. Mortality is low and only marked in young birds (Alexander, 1997).

Infection with lentogenic strains of NDV does not usually cause disease in adult birds. In young birds infections with LaSota strains may cause respiratory disease, often resulting in mortality (Alexander, 1997).

Infection with Asymptomatic strains of NDV usually consists of a subclinical enteric infection (Alexander and Senne, 2008).

Egg production may return to normal after three to four months, except after infection with velogenic NDV strains (Kouwenhoven, 1993). Embryos from acutely infected flocks often die within five days of hatching and hatchability is reduced (Kouwenhoven, 1993). In turkeys, the signs of disease are usually less severe than in chickens. Ducks and geese are even more resistant to NDV infections than turkeys (Kouwenhoven, 1993). The signs of the disease in turkeys are predominantly respiratory with airsacculitis and nervous signs (Kouwenhoven, 1993).

2.2.8 Public health significance of Newcastle disease

Newcastle disease virushas a zoonotic potential and the most common sign of infection in humans is conjunctivitis that develops within24 hours of NDV exposure to the eye (Swayne

andKing, 2003). It was reported to have been non-life threateningand usually not debilitating for more than a day or two (Chang, 1981). The most frequently reported and bestsubstantiatedclinical signs in human infections have been eye infections, usually consisting of unilateral or bilateral reddening, excessive lachrymation, oedema of the eyelids, conjunctivitis and sub-conjunctivalhemorrhages (OIE, 2012). Although the effect on the eye may be quite severe, infections are usually transient and the corneais not affected. There is no evidence of human-to-human spread (OIE, 2012). There is one report of the isolation of a pigeon-likeAPMV-1 from lung tissue, urine and faeces of an immunocompromised patient who died of pneumonia(Goebel *et al.*, 2007).

2.3 Infectious Bursal Disease

2.3.1 History of infectious bursal disease virus

The first report of a specific disease affecting the bursa ofFabricius in chickens was made by Cosgrove in 1962 (Cosgrove, 1962).The first cases were observed in the area of Gumboro, inDelaware (United States of America [USA]), which is theorigin of the name- "Gumboro". The disease is also known as 'Infectious Bursal Disease', 'infectiousbursitis', 'Gumboro disease', 'Infectious Avian Nephrosis'. Infectious bursal disease is worldwide in occurrence (Ojo *et al*., 1973). In Nigeria, the first report of IBD was by Ojo*et al*. (1973).

2.3.2 Aetiology of infectious bursal disease

Infectious bursal disease is a highly contagious viral disease of young chickens caused by a virus, IBDV, which is a member of the genus, *Avibirnavirus* of the family *Birnaviridae* (Delmas *et al.,* 2004). The virus is a double stranded RNA virus that has bi-segmented genome (Wu *et al.,*

2007; Zhu *et al.*, 2008). Avibirnaviruses to which IBDV belongs are long recognized as being immunosuppressive predisposing chickens to intercurrent infections and lack of adequate responses to vaccinations against other diseases responsible for greatest economic losses in the affected flocks (Shane, 1997).

2.3.3 Transmission of infectious bursal disease virus

Infectious bursal disease virus is highly contagious (Benton *et al*., 1967). Transmission of IBDV predominantly takes place via the faecal-oral route, because the virus is shed in the faeces for up to two weeks post infection in high amounts (Benton *et al*., 1967). The high resistance of the virus contributes to this mode of transmission as it could survive for over 52 days (Benton *et al*., 1967). Airborne spread is not important and there is no evidence for egg-transmission or virus carriers (McFerran, 1993). Wild birds or rodents might transport the virus and act as mechanical vectors (McFerran, 1993). It has been demonstrated that the lesser mealworm (*Alphitobius diaperinus*) that was taken from a house eight weeks after an outbreak could act as a vector for IBDV when fed as a ground-suspension to susceptible chickens (Lukert and Saif, 1991). Also, insects such as mites and mosquitoes can play a role in the transmission of IBDV (Howie and Thorsen, 1981).

2.3.4 Species affected by infectious bursal disease virus

Chickens, turkeys, ducks, guinea fowls and ostriches may be infected with IBDV, but clinical disease only occurs in chickens. Mortality is higher in lighter breedsof birds than heavier breeds (van den Berg, 2000). Serotype 1 viruses mainly infect chickens less than 10 weeks, and turkeys to a lesser extent, although the widespread use of serotype 1 vaccines makes it difficult to determine the true prevalence of IBD (AU-IBAR, 2013).

Infectious bursal disease virusserotype 2 viruses are widely distributed in turkeys (McFerran, 1993). In several other species, IBDV or IBDV-specific antibodies have been detected, such as in coturnix quails, pigeons, pheasants, village weavers (*Ploceus cuncullatus*), pied cordon bleus (*Uraeginthus bengalus*), magpie geese (*Anseranus semipalmata*), shearwaters (*Puffineus carneips*, *P. pacificus*), soothy terns (*Sterna fuscata*), common noddy (*Anous stolidus*), silver gulls (*Larus novaehollandiae*), and black ducks (*Anas superciliosa*) (Wilcox *et al*., 1983; McFerran, 1993). Reports of IBD antibodies have been demonstrated in wild rats (Okoye and Uche, 1986).

2.3.5 Incubation period of infectious bursal disease

The incubation period of infectious bursal disease is very short. After an incubation period of two to four days, young chickens show symptoms of apathy and distress, and death may follow one to three days later. Mortality will peak and recede usually in a period of five to seven days (Lukert and Saif, 1991; Becht, 1994). Morbidity approaches 100% and mortality may be up to 20-30%, but there may also be no mortality (Lukert and Saif, 1991; McFerran, 1993). Characteristically, chickens may peck at their own vents in the early stage of the disease (Lukert and Saif, 1991).

2.3.6 Pathogenesis of infectious bursal disease

Initial outbreaks on farms are the most acute and recurrent outbreaks in succeeding flocks are less severe (Van den Berg, 2000).For vvIBDV infections, the virus primarily replicates in the lymphocytes and macrophages of the gut-associated tissues after oral infection or inhalation (Van den Berg, 2000). The virus then travels to the bursa via the blood stream, where replication

will occur. After 13hours PI, most follicles are positive for virus and by 16hours PI a second viremia occurs leading to disease and death (van den Berg, 2000).

2.3.7 Clinical signs of infectious bursal disease

Clinical signs in the first three days of exposure include vent pecking, soiled vent, watery diarrhoea, anorexia, depression, ruffled feathers, trembling, severe prostration, dehydration and hypothermia, the severity of clinical signs is dependent on thevirus strain(Cosgrove, 1962; Saif, 1998). Airsacculitis and colisepticaemia have also been observed in the affected birds, possibly caused by secondary bacterial infection (Muller*et al*., 2003).

2.3.8 Diagnosis of infectious bursal disease

Clinical signs alone are not sufficient to make adiagnosis of IBD, but when combined with post mortem lesions, it ispossible to arrive at a tentative diagnosis, then detection of specific antibodies to IBDV. Confirmatory diagnosis is arrived at by isolating the IBD virus or demonstrating the presence of IBD viral RNA by RT polymerase chain reaction (Saif, 1998).

2.3.8.1Virus isolation

Confirmation of diagnosis should beaccomplished by virus isolation or detection of viral antigens in tissues (bursa of Fabricius) from suspect cases (Saif, 1998). Isolation is commonly done in embryonated chicken eggs inoculatedvia the chorioallantoic membrane route. A variety ofprimary and established cell lines have been used forisolation and propagation of the virus. Once the virus isisolated, it could be identified by reacting the isolate with a knownanti-IBDV serum using any of a number of antigen-antibodytests such as VN, fluorescenceantibody (FA) test,ELISA, or agar gel precipitation(AGP).The viral antigens can be detected in tissues by a

varietyof tests including FA, antigen-capture ELISA, AGP,nucleic acid probes and polymerase chain reaction (PCR)and its derivatives.

2.3.8.2Serology

Acute and convalescent sera can be tested by VN, ELISA or AGP tests. With the exception of the VNtest, all the commonly used test procedures for viralantigens or antibodies detect group specific antigenswithout differentiating serotypes. The lack of serotype specificity should be considered when results of antibodyand virus assays are interpreted (Saif, 1998).

2.3.9 Control of infectious bursal disease

A combination of sanitary precautionary measures and vaccination are recommended for adequate prevention and control of IBD (Tsukamoto*et al*., 1995; Saif *et al*., 2008). Infectious bursal disease virus is resistant to ether, chloroform, pH extremes, high temperature,and phenol or quaternary ammonium disinfectants (Benton *et al*., 1967; Mandeville*et al*., 2000). Adequate sanitation of premise between flocks especially using formaldehyde and iodophors for disinfection is recommended forthe control of IBD (Pattison *et al*., 2008). However, being a robust virus in the environment coupled with its widedistribution, hygienic control measuresalone might not be sufficient forits control (Van den Berg,2000). Combination of thorough hygiene, biosecurity, and vaccination of up to 7 day-old chicks using live vaccine in the drinking water can prevent flocks from the disease (Balamurugan and Kataria, 2006; Pattison *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Zaria and its environs, Kaduna State- it included Giwa, SabonGari and Zaria LGAs. Specific locations where this study was carried out included: Bomo, Basawa, Zango, Samaru, ABU Quarters (Area A, C and G), Hanwa, SabonGari market in SabonGari LGA; Giwa Live Bird Market, Pan Hauya, and Biye in Giwa LGA and Wusasa and Sakaru in Zaria LGA.

Zaria is a cosmopolitan city which comprises two LGAs; namely, Zaria and SabonGari LGA's. It is the largest urban center in Kaduna State (RIM, 1993). Zaria is in the Northern part of Kaduna State, with coordinates 11º11´N and 7º38´E and 686m above sea level in northern guinea savannah ecological zone of Nigeria. Zaria is a medium sized city with an estimated population of over 408, 198 and SabonGari LGA have 286, 871 while Giwa LGA has a population of 286, 427 (NPC, 2006). The seasons in Zaria have been categorized as dry season (January to March), pre-rainy season (April to June), rainy season (July to September) and pre-dry season (October to December) (Abdu *et al*., 1992). The hottest period in the State is between March and April (43ºC) while the coldest period is between December and January (13ºC). The mean annual temperature is 34ºC while the mean annual rainfall is about 1,000 mm (RIM, 1993). It has an estimated land area of about 300 square kilometres and it is approximated that about 40-75% of its working population derives their principal means of livelihood from agriculture: crop farming, livestock rearing, fishing and trading (RIM, 1993; ABU, 2000).

Figure 2.1: Map of Kaduna State: showing the 23 LGAs of the State and the study areas (shaded).

Source: Mamman *et al.*,2000.

3.2 Sample Size

Sample size was determined using the formula by Thrusfield (1995) at 95% confidence level:

$$N = \frac{Z^2 pq}{d^2}$$

Where Z = 1.96; q = 1 – p; p =15% (Salihu *et al*., 2010).

$$N = \frac{(1.96)^2 \times 0.15 \times 0.85}{0.02^2}$$

$$N = \frac{3.8416 \times 0.15 \times 0.85}{0.0004}$$

$$N = \frac{0.4898}{0.0004}$$

N = 1, 224

N ≈ 1, 230; 1230 ÷ 3 sampling units

= 410 blood samples/sample unit

3.3 Sampling Units

A total of 1,028 poultry comprising 301 commercial chickens from Basawa, Hanwa GRA, Samaru, ABU quarters (Area A, C and G), Sakaru and Wusasa; 338 rural poultry from Biye, Bomo, Basawa, Zango, Pan-hauya and Samaru; and 389 poultry from LBMs at Samaru, Giwa and SabonGari markets were sampled.

3.4 Sampling Technique

Birds from commercial farms were selected by stratified random sampling. Samples from rural households were collected by convenient sampling and LBMs were collected from slaughter slabs based on convenient sampling.

3.5 Blood Collection

About 0.5 – 2ml of blood was collected from each bird through the brachial vein, using a 23G sterile hypodermic needles and 2 ml syringes. The blood was collected into sample bottles and allowed to clot at room temperature from which sera were obtained and stored at -20ºC until used for serology. The blood samples were collected between August and September, 2014.

3.6 Source of Egg Drop Syndrome -76 Antigen

Standard EDS-76 antigen and antiserum were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria, and used for the detection of EDS-76 antibodies. The validity and titre of the EDS-76 antigen was determined using haemagglutination (HA) test as described by OIE (2000). The sera were assayed by haemagglutination inhibition (HI) test as described by (OIE, 2000).

3.7 Source of Newcastle Disease Virus Antigen

Newcastle disease vaccine (La Sota strain) obtained from the NVRI, Vom, Plateau State, Nigeria was used as antigen for the test, while the positive sera used as control was also obtained from the NVRI Virology Laboratory.

3.8 Source of Infectious Bursal Disease Virus Antigen

The IBDV antigen was obtained from Professor P. A. Abdu of the Department of Veterinary Medicine, Ahmadu Bello University, Zaria.

3.9 Preparation of Red Blood Cells

Two millilitres of blood was collected from a local cock and it was pooled in an equal volume of Alsever's anticoagulant solution. The red blood cells (RBC) were washed three times in phosphate buffered saline (PBS) (pH 7.2) by centrifuging at 447.2 g for 5 minutes (Collee *et al*., 1982). One percent RBC suspension was made by adding 1 ml of washed RBC to 99 ml of PBS.

3.10 Determination of Titre ofEgg Drop Syndrome -76 and Newcastle Disease Antigens

Haemagglutination test was carried out according to the method described by OIE (2009). Twenty-five microlitres of PBS was dispensed into each well of "V" bottom microtitres plates except the first well. Twenty-five microlitres of antigen suspension in PBS was placed into the first well. A two-fold dilution done by taking 25 µl from the first well into the second well and mixed the 25 µl from 2nd well transferred to the 3rdwell and continued till 11th well. A 25 µl of 1% chicken RBC was dispensed into each well. These were mixed by gently tapping the plate and then allowing the RBC to settle for about 40 minutes at room temperature. The HA was determined by tilting the plate and observing for the presence or absence of tear- shaped streaming of the RBC. The titration was read to the highest dilution giving complete HA (no streaming); this represents 1 HAU.

3.11 Determination of Titre of Egg Drop Syndrome -76 and Newcastle Disease Antibodies

The antibody titre was determined by HI test as described by Allan and Gough, (1974). Twenty five microlitres of PBS was dispensed into each well of a plastic "V"-bottom microtitre plate. Twenty-five microlitres of serum was dispensed into the first well to the eleventh well. Two-fold dilutions of the 25 µl volumes of the serum were made across the plate from the first well. Four HAU of the antigen suspension in 25 µl was added to each well and left for a minimum of 30 minutes at room temperature. A 25 µl of washed chicken RBC was added to each well and after gentle mixing was allowed to settle for about 40 minutes at room temperature. The HI titre was the highest dilution of the serum causing complete inhibition of RBC agglutination by the 4 HAU antigen. Wells considered positive to HI were wells in which RBC stream at the same rate as positive control wells (wells A12 - H12) and the results were expressed in $\log_2$(OIE, 2012).

3.12 Agar Gel Preparation and Agar Gel Immunodiffusion Test for Detection of infectious bursal disease Virus Antibodies

Gel diffusion agar was prepared by adding 8 g of sodium chloride, 0.02 g of sodium azide and 1.25g of Difco agar to 100 ml of distilled water and heating the mixture slowly to boiling. The agar plates were made by dispensing 25 ml agar into 9 x 9.5 cm Perspex dishes. This gave the agar depth of 3 mm (OIE, 2008).

Wells of 6 mm with 3 mm separation were cut into the already set agar. An offset linear pattern of 12 parallel rows was used with the middle row of every three having every hole cut. The middle row was for antigen while the two complete rows to either side were for antiserum dilutions. Thus, one antigen well served the four adjacent antisera wells. The agar plugs were removed from the wells with a hypodermic needle (OIE, 2008). Positive samples showed a precipitin line of identity to the bursa antigen (Harai *et al*., 1972).

3.13 DateAnalyses

The data obtained from serology for Newcastle disease antibodies and EDS-76 antibodies, were analyzed by cross tabulations and descriptive statistics using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA). The frequency, mean, standard error of mean and chi square values of the cross tabulations were calculated. Values of $p < 0.05$ were considered significant.

The prevalence of antibodies to IBDV was calculated using the formula outlined by Bennett*et al*. (1991):

Prevalence (%) = number of serum positive/total numberof serum examined× 100.

CHAPTER FOUR

RESULTS

4.1 Sero-prevalence of EDS-76 in relation to location, sampling units, type of poultry and specie of poultry

A total of 679 sera samples were tested for EDS-76 antibodies and an overall sero-prevalence of 69.2% was obtained. The overall mean EDS-76 antibody titre in poultry in different locations sampled in three LGAs of Kaduna State, Nigeria was $4.73 \pm 0.09\log_2$. The highest mean EDS-76 antibody titre was$7.07 \pm 0.55\log_2$ in poultry from commercial farm in Wusasa. The lowest mean EDS-76 antibody titre was$2.73 \pm 0.66\log_2$ in poultry from Samaru LBM (Table 4.1). From the three LGAs sampled, the highest EDS-76 mean antibody titre of $6.23 \pm 0.31\log_2$ was recorded in flocks in Zaria LGA, while poultry in Giwa LGA had the lowest mean antibody titre of$4.31 \pm 0.16\log_2$ (Table 4.1). Rural household had a sero-prevalence of 66.8%. The highest mean EDS-76 mean antibody titre by sampling units was$5.69 \pm 0.14\log_2$recorded from commercial farms and the lowest mean antibody titre was $4.02 \pm 0.18\log_2$which was recorded from LBM (Table 4.2). Goose had a sero-prevalence of 33.3% with the highest mean EDS-76 antibody titre by specie of poultry sampled been $7.50 \pm 0.50\log_2$ recorded in turkeys and no EDS-76 antibodies were detected in guinea fowls and ducks (Table 4.3). Of the different type of chickens sera tested, local chicken had a sero-prevalence of 68.2%, layers had the highest mean antibody titre of $5.55 \pm 0.15\log_2$, while local chicken sera had the lowest mean EDS-76 antibody titre of ($4.17 \pm 0.12\log_2$) (Table 4.4).

Table 4.1:Distribution of egg drop syndrome-76 antibodies in poultry located in Giwa, SabonGari and Zaria Local Government Areas in Kaduna State, Nigeria.

Local Government Area	Location	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E (log_2)
Sabon gari	Zango rural household	57	33 (57.9)	3.64 ± 0.31
	Bomo	48	36 (75.0)	3.25 ± 0.25
	Basawa	59	48 (81.4)	5.56 ± 0.26
	Samaru	21	5 (23.8)	3.00 ± 0.71
	Suleiman farm	14	11 (78.6)	4.45 ± 0.47
	Graceland	15	12 (80.0)	4.75 ± 0.43
	Zango commercial farm	38	35 (92.1)	5.64 ± 0.31
	Samaru commercial farm	14	14 (100.0)	6.64 ± 0.46
	ABU quarters	55	55 (100.0)	5.46 ± 0.19
	Samaru live bird market	32	11 (34.4)	2.73 ± 0.66
	Sabongari live bird market	83	46 (55.4)	4.3 ± 0.33
Sub total		436	306 (70.2)	4.73 ± 0.12
Zaria	Wusasa	14	14 (100.0)	7.07 ± 0.55
	Sakaru	30	29 (96.7)	5.83 ± 0.36
Sub total		44	43 (97.7)	6.23 ± 0.31
Giwa	Biye	29	26 (89.7)	5.04 ± 0.26
	Pan Hauya	54	31 (57.4)	5.04 ± 0.39
	Giwa live bird market	116	64 (55.2)	4.05 ± 0.22
Sub total		199	121(60.8)	4.31 ± 0.16
		679	470 (69.2)	4.73 ± 0.09

$\chi^2 = 23.59$; $p < 0.0001$

Sabon gari LGA vs Zaria LGA $p < 0.001$

Zaria LGA vs Giwa LGA $p < 0.001$

Table 4.2: **Distribution of egg drop syndrome-76 antibodies in poultry based on sampling unit located in Giwa, SabonGari and Zaria Local Government Areas in Kaduna State, Nigeria.**

Sampling unit	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E(log_2)	OR	95% CI
Live bird market	231	121 (52.4)	4.02 ± 0.18	Rf	
Rural household	268	179 (66.8)	4.36 ± 0.15	1.828	1.273 - 2.627
Commercial farm	180	170 (95.0)	5.69 ± 0.14	15.45	7.765 - 30.76
	679	470 (69.2)	4.73 ± 0.09		

$\chi^2 = 85.24$; $p < 0.0001$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

Rural household vs Commercial farm $p < 0.001$

Live bird market vs Commercial farm $p < 0.001$

Table 4.3: Distribution of egg drop syndrome-76 antibodies based on specie of poultry sampled in Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.

Specie of poultry	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E(log_2)	OR	95% CI
Chicken	604	447 (74.0)	4.73 ± 0.10	Rf	
Goose	3	1 (33.3)	5.00 ± 0	0.1756	0.0158-1.951
Pigeon	38	20 (52.6)	5.00 ± 0.48	0.3903	0.2012-0.7569
Turkey	11	2 (18.2)	7.50 ± 0.50	0.0781	0.1668-0.3653
	679	470 (69.2)	4.73 ± 0.09		

$\chi^2 = 26.07$; $p < 0.0001$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

Table 4.4: Distribution of egg drop syndrome-76 antibodies based on type of chickens sampled in Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.

Type of chicken	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E (log_2)	OR	95% CI
Local chicken	384	262 (68.2)	4.17 ± 0.12	Rf	
Broiler	3	3 (100.0)	5.00 ± 0.58	0.0667	0.0034-1.302
Layer	199	165 (82.9)	5.55 ± 0.15	2.260	1.474-3.464
Breeder	18	17 (94.4)	5.41 ± 0.47	7.916	1.041-60.19
	604	447 (74.0)	4.73 ± 0.10		

$\chi^2 = 19.83$; $p < 0.0002$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

Local chickens vs Layers $p < 0.001$

4.2 Sero-prevalence of ND in relation to location, sampling units, type of poultry and specie of poultry

A total of 1,028 sera samples were tested for Newcastle disease (ND) antibodies out of which 741 (72.1%) tested positive. The overall mean ND antibody titre was 6.08 ± 0.13log_2. The highest titre was recorded in poultry from commercial farms in Zango (9.61 ± 0.93log_2) and the lowest titre of 2.32 ± 0.38log_2 was obtained in Pan hauya. The sero-prevalence of ND antibodies recorded in this study ranged between 24.5% - 100%. The overall ND sero-prevalence recorded was 72.1% (Table 4.5). From the three LGAs sampled, poultry in Zaria had the highest mean ND antibody titre of 8.84 ± 0.17log_2, while poultry in Giwa had the lowest titre of3.55 ± 0.32log_2. Giwa LGA had the highest percentage of poultry at risk of being affected by ND (84.8%) (Table 4.5).

Rural households had a sero-prevalence of 69.8%, with 67.2% of poultry at risk of being affected by ND. The highest mean ND antibody titre by sampling units was 8.48 ± 0.12log_2 recorded in commercial farms while the lowest mean ND antibody titre was4.45 ± 0.23log_2 obtained in flocks in rural households (Table 4.6).

Chicken had a sero-prevalence of 76.6% based on the specie of poultry sampled, the highest mean ND antibody titre by specie of poultry sampled was 6.13 ± 0.13log_2 obtained from chicken sera, antibodies were not detected in geese (Table 4.7). Local chicken had a sero-prevalence of 61.8% with 73.0% of poultry at risk of being affected by ND. The highest mean ND antibody titre of 8.30 ± 0.12log_2 by type of chicken sampled was in layers. The lowest of 4.22 ± 0.20log_2 was in local chickens (Table 4.8).

Table 4.5: Distribution of Newcastle disease antibodies in poultry sampled in Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Local Government Area	Location	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E (log_2)	No (%) of sera positive for ND antibodies		No (%) of poultry at risk of ND	
					No	%	No	%
Sabon gari	Zango rural household	77	51 (66.2)	4.24 ± 0.50	22	28.6	55	71.5
	Bomo	57	51 (89.5)	5.22 ± 0.49	29	50.9	28	49.1
	Basawa	60	49 (81.7)	5.43 ± 0.47	27	45.0	33	55.0
	Samaru	27	14 (51.9)	4.50 ± 0.80	6	22.2	21	77.8
	Suleiman farm	43	39 (90.7)	6.79 ± 0.26	36	83.7	7	16.3
	Graceland	30	30 (100.0)	9.53 ± 0.71	30	100	0	0
	Zango commercial farm	51	51 (100.0)	9.61 ± 0.93	51	100	0	0
	Samaru commercial farm	37	37 (100.0)	7.43 ± 0.47	29	78.4	8	21.6
	ABU quarters	77	76 (98.7)	8.46 ± 0.26	71	92.2	6	7.8
	Samaru live bird market	134	92 (68.7)	3.92 ±0.30	43	32.1	91	67.9
	Sabon gari live bird market	116	83 (71.6)	5.37 ± 0.33	58	50.0	58	50.0
Sub total		709	573 (80.8)	6.24 ± 0.14	405	57.1	304	42.9
Zaria	Wusasa	19	19 (100.0)	8.74 ± 0.32	19	100	0	0
	Sakaru	44	44 (100.0)	8.89 ± 0.20	44	100	0	0
Sub total		63	63 (100.0)	8.84 ±0.17	63	100	0	0
Giwa	Biye	55	33 (60.0)	4.61 ± 0.70	15	27.3	40	72.7
	Pan hauya	62	38 (61.3)	2.32 ± 0.38	9	14.5	53	85.5
	Giwa live bird market	139	34 (24.5)	3.91 ± 0.52	15	10.8	124	89.2
Sub total		256	105 (41.0)	3.55 ± 0.32	39	15.2	217	84.8
		1,028	741 (72.1)	6.08 ± 0.13	507	49.3	521	50.7

$\chi^2 = 174.1$; $p < 0.0001$Sabon gari LGA vs Zaria LGA $p < 0.001$

Sabon gari LGA vs Giwa LGA $p < 0.001$Zaria LGA vs Giwa LGA $p < 0.001$

Table 4.6: Distribution of Newcastle disease antibodies in poultry based on sampling units located in Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Sampling unit	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E (log_2)	No (%) of sera positive for ND antibodies		No (%) of poultry at risk of ND		OR	95% CI
				No	%	No	%		
Live bird market	389	208 (53.5)	4.50 ± 0.21.	116	29.8	273	70.2	Rf	
Rural household	338	236 (69.8)	4.45 ± 0.23	111	32.8	227	67.2	2.01	1.481 - 2.734
Commercial farm	301	297 (98.7)	8.48 ± 0.12	280	93.0	21	7.0	64.61	23.61 - 176.8
	1,028	741 (72.1)	6.08 ± 0.13	507	49.3	521	50.7		

$\chi^2 = 173.6$; $p < 0.0001$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

Live bird market vs Commercial farm $p < 0.001$

Rural Household vs Commercial farm $p < 0.001$

Table 4.7: Distribution of Newcastle disease antibodies based on specie of poultry sampled in Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Specie of poultry	Number of sera tested	Number of sera positive (%)	Mean titre ± S.E (log_2)	No (%) of sera positive for ND antibodies		No (%) of poultry at risk of ND	
				No	%	No	%
Chicken	952	729 (76.6)	6.13 ± 0.13	501	52.6	451	47.4
Guinea fowl	16	2 (12.5)	6.00 ± 0.00	2	12.5	14	87.5
Pigeon	38	4 (10.5)	2.25 ± 0.95	1	2.6	37	97.4
Geese	3	0 (0)	0 ± 0	0	0	0	0
Duck	8	1 (12.5)	5.00 ± 0.00	1	12.5	7	87.5
Turkey	12	5 (41.7)	2.80 ± 1.60	2	16.7	10	83.3
	1,028	741 (72.1)	6.08 ± 0.13	507	49.3	521	50.7

$p < 0.046$

Table 4.8: Distribution of Newcastle disease antibodies based on type of chicken sampled in Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.

Type of chicken	Number of sera tested	Number of sera tested positive (%)	Mean titre ± S.E (log_2)	No (%) of sera positive for ND antibodies		No (%) of poultry at risk of ND	
				No	%	No	%
Local chicken	474	293 (61.8)	4.22 ± 0.20	128	27.0	346	73.0
Broiler	139	104 (74.8)	4.60 ± 0.27	61	43.9	78	56.1
Layer	320	314 (98.1)	8.30 ± 0.12	294	91.9	26	8.1
Breeder	18	18 (100.0)	8.17 ± 0.45	18	100	0	0
	951	729 (76.7)	6.13 ± 0.13	501	52.7	450	47.3

$\chi^2 = 146.5$; $p < 0.0001$

Local chickens vs Layers $p < 0.001$

Local chickens vs Breeders $p < 0.001$

Broilers vs Layers $p < 0.001$

Broilers vs Breeders $p < 0.001$

4.3. Sero-prevalence of IBD in relation to location, sampling units, type of poultry and specie of poultry

A total of 1,022 sera were tested for IBD antibodies out of which 495 (48.4%) tested positive. The highest IBD sero-prevalence was recorded in poultry in Biye village in a rural area (81.8%). The lowest sero-prevalence of 11.6% was recorded in flocks from Suleiman commercial farms (Table 4.9).

Of the three (3) LGAs sampled, the highest IBD sero-prevalence was observed in poultry in Giwa (60.7%), while the lowest was 44.1% in poultry in SabonGari (Table 4.9). The highest sero-prevalence by sampling unit was 57.8% obtained in poultry in rural households. Poultry in commercial farms had the lowest value (38.0%) (Table 4.10). The highest sero-prevalence obtained in different specie of poultry was 52.1% in chickens. Geese, guinea fowls, ducks and turkeys had no IBD antibodies (Table 4.11). The highest sero-prevalence by type of chicken was 72.2% in breeders, while it was 37.0% in layers (Table 4.12).

Table 4.9: Distribution of infectious bursal disease antibodies in poultrysampled in Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Local Government Area	Location	Number of sera tested	Number of sera positive (%)	OR	95% CI
Sabon gari	Zango Rural household	76	41 (53.9)		
	Bomo	58	30 (51.7)		
	Basawa	61	31 (50.8)		
	Samaru	27	11 (40.7)		
	Suleiman farm	43	5 (11.6)		
	Graceland	29	22 (75.9)		
	Zango commercial farm	52	19 (35.8)		
	Samaru commercial farm	37	7 (18.9)		
	ABU quarters	77	30 (39.0)		
	Samaru live bird market	133	61 (45.9)		
	Sabon gari live bird market	114	55 (48.2)		
		707	312 (44.1)	Rf	
Zaria	Wusasa	20	12 (60.0)		
	Sakaru	41	18 (43.9)		
		61	30 (49.2)	1.225	0.726 - 2.068
Giwa	Biye	55	45 (81.8)		
	Pan hauya	57	35 (61.4)		
	Giwa live bird market	140	73 (52.1)		
		252	153 (60.7)	1.924	1.44 - 2.579
		1,022	495 (48.4)		

χ^2 =19.51; $p < 0.0001$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

Table 4.10:Distribution of infectious bursal disease antibodies in poultry flocks based on sampling units located in Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Sampling unit	Number of sera tested	Number of sera positive (%)	OR	95% CI
Live bird market	388	189 (48.7)	Rf	
Rural household	334	193 (57.8)	1.441	1.073-1.935
Commercial farm	300	114 (38.0)	0.6453	0.4749-0.8769
	1,022	496 (48.4)		

$\chi^2 = 24.77$; $p < 0.0001$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

Table 4.11:Distribution of infectious bursal disease antibodies based on specie of poultry sampledin Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Specie of poultry	Number of sera tested	Number of sera tested positive (%)
Chickens	945	492 (52.1)
Geese	3	0 (0)
Guinea fowls	16	0 (0)
Pigeons	38	3 (7.9)
Ducks	8	0 (0)
Turkeys	12	0 (0)
	1,022	495 (48.4)

$\chi^2 = 66.62$; $p < 0.0001$

Table 4.12: Distribution of infectious bursal disease antibodies based on type of chickens sampled in Giwa, SabonGari and Zaria Local Government Areas, Kaduna State, Nigeria.

Type of chicken	Number of sera tested	Number of sera positive (%)	OR	95% CI
Local chickens	471	293 (62.2)	Rf	
Broilers	137	68 (49.6)	0.5987	0.4081-0.8784
Layers	319	118 (37.0)	0.3566	0.2658-0.4785
Breeders	18	13 (72.2)	1.580	0.5537-4.506
	945	492 (52.1)		

χ^2 =51.72; $p < 0.001$

Key: OR= Odd ratio, CI= confidence interval, Rf= Risk factor

CHAPTER FIVE

DISCUSSION

The findings of this work has demonstrated the presence of EDS-76, ND and IBD antibodies in poultry in the study area. It is important to note that most poultry of commercial origin that are kept for commercial purpose are vaccinated against various diseases, while local poultry which are mostly kept in rural households areas are rarely vaccinated against any disease (Abdu *et al*., 1987; Abubakar *et al*., 2008). The commercial poultry farms that were tested in this study might have vaccinated their birds against EDS-76; because most commercial farms vaccinate their birds against the disease. The detection of antibodies in rural household poultry investigated might be as a result of natural infection. This is in agreement with findings of similar works carried out by various authors (Nawathe and Abegunde, 1980; Ezema *et al*., 2008; Sanda *et al*., 2008 and Salihu *et al.,* 2010) who reported the presence of EDS-76 antibodies in different parts of Nigeria.

High mean EDS-76 antibody titres were observed in poultry sampled from Zaria LGA. This is probably because the poultry sampled from this LGA were commercial laying birds which might have been vaccinated against most poultry diseases as a routine practice and might have vaccinated against EDS-76. Poultry from Sabon gari and Giwa LGAs had moderately high sero-prevalence and similar mean EDS-76 antibody titre. This could be because poultry from Sabon gari LGA are a pool of poultry from rural households, LBMs and commercial farm, while poultry from Giwa LGA were mostly local poultry from rural households and LBM. The high percentage of EDS-76 antibody positive sera obtained for commercial farms could be attributed

to vaccination while those obtained from rural household areas could be due to exposure to EDS-76 virus and natural infection by the field EDS-76 virus.

Most commercial farm owners are learned, as they are aware of most poultry diseases and are financially capable of purchasing vaccines for preventing various diseases. Based on sampling units, there was a significant difference ($p < 0.001$) in EDS-76 antibody titres between poultry sampled from rural households and commercial farms, which could be associated to routine vaccination against EDS-76. It therefore, infers that antibodies detected among poultry investigated from rural households and LBMs were as a result of natural infection of the poultry with the EDS-76 field virus. This is possible because of their free range system of rearing as suggested by Nawathe and Abegunde (1980). This free range system of rearing could further lead to unrestricted spread of the virus among local poultry.

There is considerable activity of EDS-76 virus in the poultry sampled from LBMs because of the demonstration of the presence of EDS-76 antibodies (52.8%). This could be due to multi-species, multi-age, multi-type of birds kept and sold in the LBMs, because of the way they are kept together there is possibility of lateral spread of EDS-76 virus from an infected virus shedding bird to a susceptible bird, although reported to be slow and intermittent, taking weeks to achieve as reported by Raj *et al*., (2007). This implies that poultry sourced from LBMs may serve as a reservoir of EDS-76 virus and a possible source of infection to susceptible flock.

Chickens had the highest number of positive cases compared to other type of poultry. It is of economic significance as a drop in egg production with poor egg quality are the usual clinical signs noticed in such birds (CFSPH, 2006). However, the sero-prevalence obtained from geese (33.3%) is of great significance to the epidemiology of EDS-76 virus in the study area because

geese are known to be reservoir host to EDS-76 virus. This signifies that they could serve as source of infection to commercial poultry or other poultry that are kept together or in close proximity. From this study, chickens had the highest prevalence. High EDS-76 sero-prevalence in chickens could be as a result of natural infection for poultry in rural households and vaccination for the commercial laying birds. This disagrees with the findings of Abubakar *et al.* (2008) who worked in North-eastern Nigeria, they recorded a higher sero-prevalence in turkeys (85.7%) and low in chickens (1.4%), this could be because they sampled only rural poultry which are rarely vaccinated against any poultry disease.

In this study high sero-prevalence was obtained in broiler and breeder stock and this agrees with the work of Badar *et al*. (2006) who recorded higher prevalence in broiler and layer breeders. Also in this study, high prevalence was detected in local poultry, this result does not correlate with the finding of Oyeduntan and Durojaiye (1999). Local chickens which are known to roam about, may have been infected by the field virus. A significant difference ($p < 0.0002$) was observed between local chickens and layers. This is of great concern as they could serve as a source of infection to commercial laying birds.

The high sero-prevalence of ND antibodies in poultry in this study further complement findings of previous studies on the endemicity of the disease in Nigeria and that it affects both local and exotic poultry (Abdu *et al*., 1985; Sa'idu *et al*., 1994; Baba *et al*., 1998; Mai *et al*., 2004) Serological evidence of ND antibodies by LGA shows a high mean antibody titre in poultry in Zaria LGA. Flocks in five different locations (Zango commercial farm, Wusasa, Graceland, Sakaru, Samaru commercial farm) in the study area had 100% prevalence, this could be as a result of vaccination of poultry with booster doses of either NDV La sota or NDV Komarov

which is a common practice by poultry farmers in Nigeria for exotic birds as reported by Emikpe *et al*. (2007)and Oluwole *et al*. (2012).

Giwa LGA had the lowest mean antibody titre ($3.55 \pm 0.32 \log_2$) with 41% sero-prevalence for ND. This might be because rural poultry from this area are rarely vaccinated against ND. The poultry with antibodies in this area could have been exposed to mild field NDV. Therefore, when these poultry are exposed to virulent strains of NDV, there is likely to be an outbreak in the birds around Giwa LGA, especially as they had higher percentage of poultry at risk to ND (84.8%). It is also important to note that poultry purchased from this area when carried to a flock or vicinity free of ND could infect susceptible birds when they are kept together. The lowest mean ND antibody titre recorded in Pan Hauya ($2.32 \pm 0.38 \log_2$), and also low percentage of poultry with protective ND antibody titre (14.5%), this may be as a result of exposure to mild strain of NDV which then circulates in the birds body system. Exposure to a virulent strain of NDV in this area may lead to ND outbreak which in-turn may cause high mortality, reduced income, drop in egg production and finally few replacement flocks. Based on sampling units, commercial farms had the highest mean ND antibody titre, this could be due to vaccination, or infection by NDV. It has been reported by Alexander, (1988) that infected chickens usually serve as a source of infection to other susceptible poultry. Also, the deep-litter management system used in most commercial farms could also lead to the multiplication and spread of the virus within a flock. Live bird market and rural households had higher percentage of poultry at risk of being infected with NDV, this may be attributed to the fact that most poultry in the LBM are transitory and are rarely vaccinated because they are kept for minimal period of time, those which are from commercial origin which are harboring the virus could serve as a source of infection to other susceptible birds in the LBMs. It therefore implies that poultry sourced from LBMs and taken to a

susceptible flock could serve as a source of exposure to NDV. Based on the specie of poultry sampled, the highest mean ND antibody titre observed in chickens in comparism to other specie of poultry, this could be attributed to the fact that chickens are the most routinely vaccinated against ND and most kept.Chickens are particularly susceptible to NDV, and may experience very high morbidity and mortality rates (CFSPH, 2008).

In commercial farms especially laying flock, routine vaccinations are done against many diseases, so as to prevent diseases. Based on the type of chickens tested high mean ND antibody titres were observed in layers and breeders compared to broilers and local chickens. This agrees with the findings of Abdu *et al*. (2005), and could be due to higher number of routine vaccinations carried out on these birds because of the time and purpose for which they are kept. Broilers on the other hand are kept for shorter periods, most farmers rarely vaccinate them more than two times, therefore it could be that the antibody titre of the birds was gradually waning, local chickens are rarely vaccinated, so the ND antibody titre observed could be due to exposure to the field NDV. Local chicken had the highest percentage of poultry at risk of being affected with NDV, this may be attributed to the poultry not frequently vaccinated and thus having low mean antibody titres or no detectable antibodies.

In this study, the sero-prevalence obtained for IBD showed activity of IBD in the study area. The sero-prevalence obtained from this study supports endemicity of IBD in Nigeria as reported by previous researchers (Onunkwo and Momoh, 1981; Nawathe and Lamorde, 1982) and also in Zaria as reported by earlier investigations(Abdu, 1988; Tong *et al*., 1993; Mbuko *et al*., 2010). The prevalence of 48.4% obtained in this study was higher than 8% reported in Gombe State by Bukar-Kolo *et al*.(2007) and slightly higher than 48.2% reported in Borno State by El-yuguda *et al*.(2009). The prevalence was lower than 98.1% reported in Zaria by Umoh *et al*.(1982) and the

68.0% reported in Ibadan by Adene *et al*. (1985) and 63% reported in Yobe State by Sule *et al*. (2013). High IBD antibody titre observed in Giwa LGA indicates high activity of the virus in that area, it was observed that all sera tested were from rural household areas which are rarely vaccinated against IBDV so there is a possibility that they got infected by an exposure to field IBDV. Management practices and poor biosecurity measures in rural household areas might have led to the high prevalence (60.7%) recorded, this is because IBDV could be transmitted via fecal droppings from infected birds, mechanically through people, equipment and vehicles, so this might have enhanced the spread of the virus in the rural household areas more and possible spread to commercial birds.

Live bird markets are usually very busy and without adequate human and poultry traffic control and sanitary measures, this could lead to the introduction and possible spread of IBDV within the market area and even out of the market. So there is possibility that the sero-prevalence obtained in LBMs could be due to exposure to field virus.

The prevalence obtained from sera of the specie of poultry showed a high sero-prevalence in chickens as they are the most affected at young age. Pigeons had 7.9% sero-prevalence, this is lower than the prevalence obtained by Fagbohun *et al*. (2000) who had a prevalence of 36.7%, this implies that pigeons could serve as reservoir of the virus to susceptible flock.

Local chickens had higher sero-prevalence of IBD compared to broilers and layers. This may be due to exposure to field virus of the disease and as local poultry are not vaccinated against IBD. It may also be attributed to vaccine virus which the birds may pick from the environment or fomites. The finding in this study agree with Okoye *et al*. (1999) who reported thatthe village chickens are highly susceptible to IBD. Local chickens that are not managed intensively are

susceptible to diseases and contribute significantly in the maintenance and spread of the diseases (Okwor *et al*., 2011), it therefore implies that these birds could maintain and spread this disease in a population.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study inferences made include that:

i. Antibodies to EDS-76, ND and IBD were detected in poultry in the study area with overall sero-prevalence of 69.2%, 72.1% and 48.4% respectively.

ii. The overall mean antibody titre determined for EDS-76 and ND was 4.73 ± 0.09 $\log_2$ and 6.08 ± 0.13 $\log_2$ respectively.

iii. The sero-prevalence of EDS-76 in Giwa, SabonGari and Zaria LGAs was 60.8%, 70.2% and 97.7% respectively.

iv. Commercial farm had the highest sero-prevalence of EDS-76 (95%) and the highest mean EDS-76 antibody titre ($5.61 \pm 0.14\log_2$), while LBM had the lowest sero-prevalence (52.4%) and the lowest mean EDS-76 antibody titre ($4.02 \pm 0.18\log_2$).

v. Geese had a sero-prevalence of EDS-76 (33.3%).

vi. Giwa LGA had mean ND antibody titre of $3.55 \pm 0.32\log_2$, with 84.8% of poultry at risk of being affected by ND.

vii. Commercial farms had the highest mean ND antibody titre of $8.48 \pm 0.12\log_2$ while rural households hadmean ND antibody titre of $4.45 \pm 0.23\log_2$.

viii. Local chicken had 73.0% of birds at risk of being affected by ND.

ix. Poultry from Giwa LGA had the highest sero-prevalencefor IBD (60.7%) while SabonGari LGA had the lowest (44.1%)**.**

x. Pigeons had a sero-prevalence of 7.9% for IBD.

6.1 Recommendations

From this study it is recommended that:

i. Researchers should conduct further studies to isolate EDS-76 virus in Zaria and environs, Kaduna State, Nigeria.

ii. Further studies on EDS-76 virus should be carried out in other locations in the state and the country at large.

iii. Clinicians should include EDS-76 as a differiential to cases with history of drop in egg production and where possible screen for antibodies to EDS-76.

iv. Further studies to investigate possible role of pigeons in the transmission of IBD should be carried out.

v. Awareness campaigns should be done in rural household areas by animal health workers on the importance of vaccinating poultry.

vi. Stakeholders in the poultry industry should ensure chickens in Giwa LGA are routinely vaccinated against ND and IBD.

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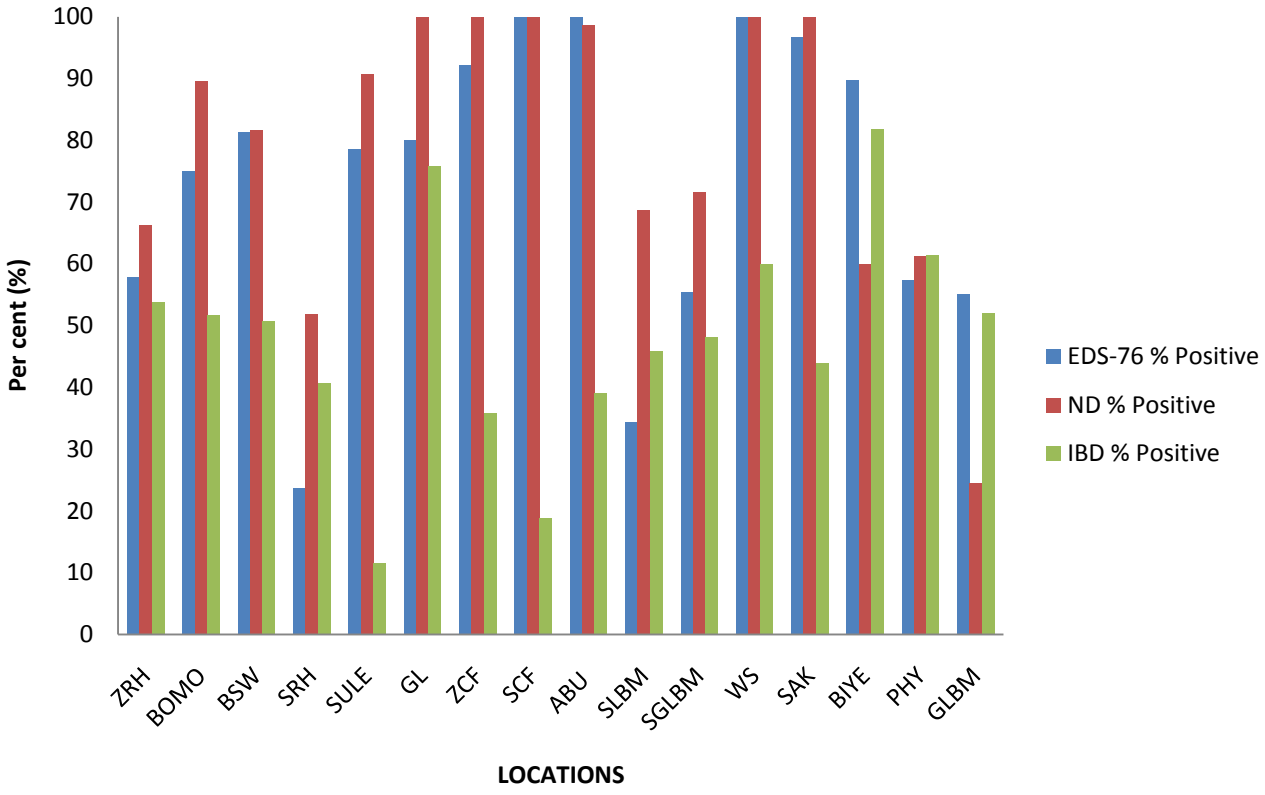
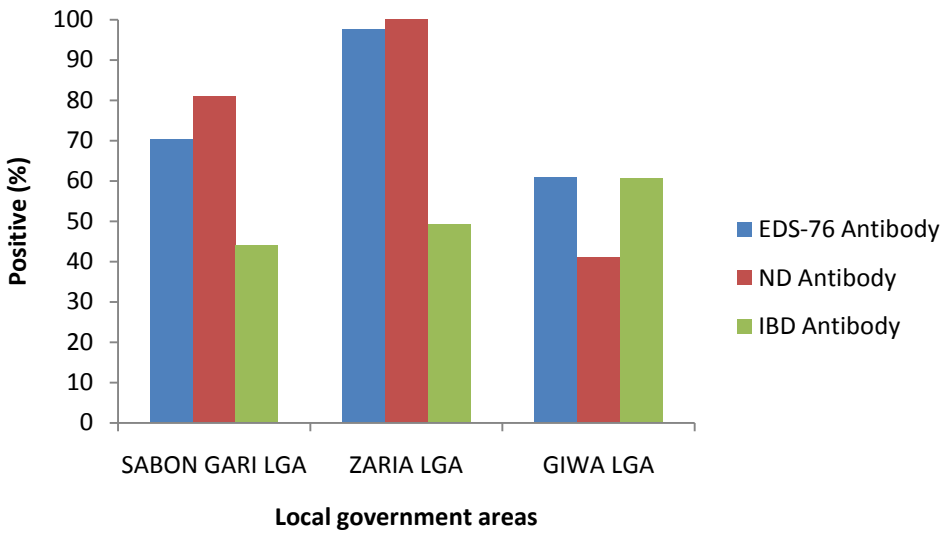
APPENDICES

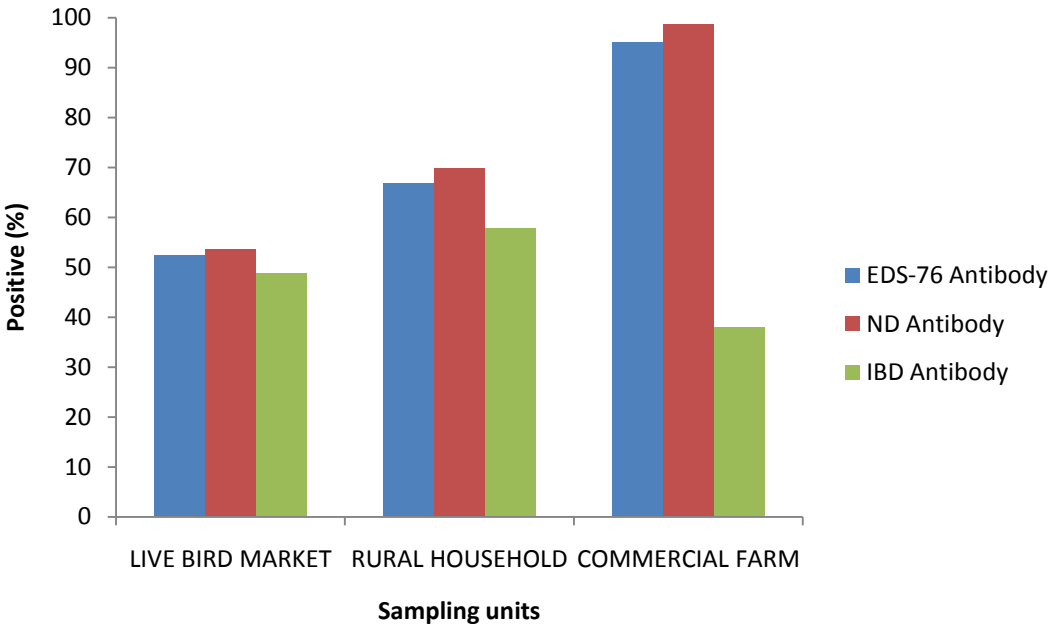


Appendix I: Distribution of egg drop syndrome-76, Newcastle disease and infectious bursal disease antibodies based onspecific locations sampled fromGiwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.

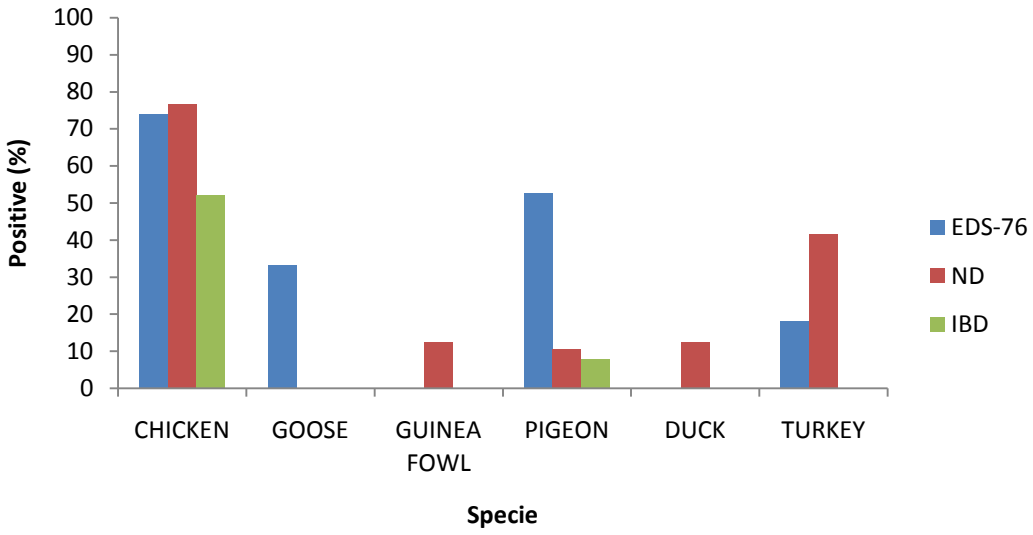
KEY: **ZRH**=ZANGO RURAL HOUSEHOLD; **BSW**=BASAWA; **SRH**=SAMARU RURAL HOUSEHOLD; **SULE**=SULEIMAN FARMS; **GL**=GRACELAND; **ZCF**=ZANGO COMMERCIAL FARM; **SCF**=SAMARU COMMERCIAL FARM; **ABU**=ABU QUARTERS (AREA A,C and G); **SLBM**=SAMARU LBM; **SGLBM**=SABON GARI LBM; **WS**=WUSASA; **SAK**=SAKARU; **PHY**=PAN HAUYA; **GLBM**=GIWA LBM.



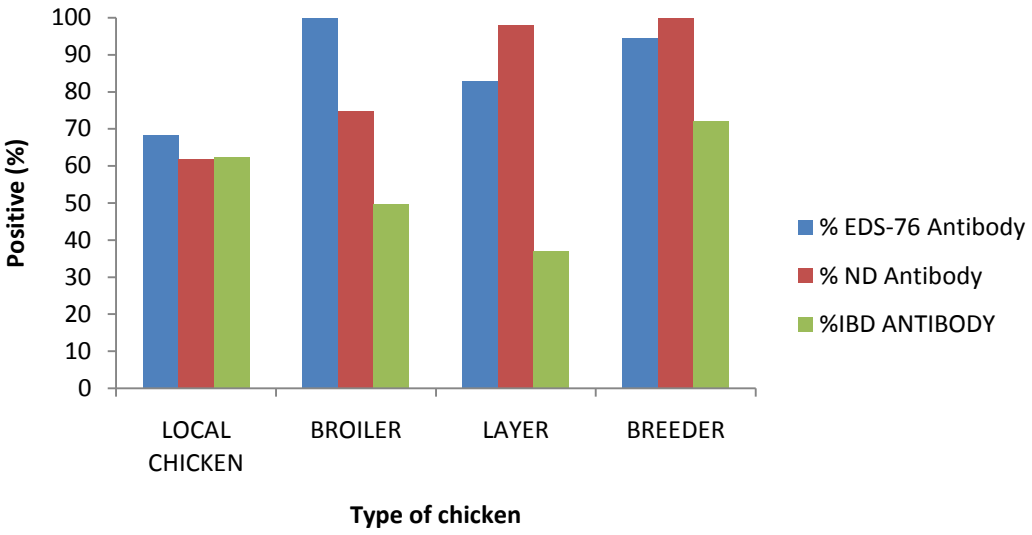
Appendix II: Distribution of egg drop syndrome-76, Newcastle disease and infectious bursal disease antibodies based on Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.



Appendix III: Distribution of egg drop syndrome-76, Newcastle disease and infectious bursal disease antibodies based on sampling units in Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.



Appendix IV: Distribution of egg drop syndrome-76, Newcastle disease and infectious bursal disease antibodies based on species of poultry in Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.



Appendix V: Distribution of egg drop syndrome-76, Newcastle disease and infectious bursal disease antibodies based on type of chickens in Giwa, SabonGari and Zaria Local Government Areas of Kaduna State, Nigeria.